Chinese Journal of Chemical Engineering 70 (2024) 73–81

Contents lists available at ScienceDirect

Chinese Journal of Chemical Engineering

journal homepage: www.elsevier.com/locate/CJChE

Full Length Article

Oil-in-water nanoemulsions loaded with lycopene extracts encapsulated by spray drying: Formulation, characterization and optimization

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ARTICLE INFO

Article history:
Received 24 October 2023
Received in revised form
8 March 2024
Accepted 11 March 2024
Available online 20 March 2024

Keywords:
Emulsions
Powders
Stability
Isopropyl myristate
Lycopene
Spray-drying

ABSTRACT

Lycopene is very susceptible to degradation once released from the protective chromoplast environment. In this study, oil-in-water (O/W) nanoemulsions coupled with spray drying technology were applied for the encapsulation and stabilization of lycopene extracted from tomato waste. Tomato extract was obtained by ultrasound-assisted extraction. Nanoemulsions were prepared by a high-speed rotor stator using isopropyl myristate as the oil phase and Pluronic F-127 as the emulsifier for the aqueous external phase. The effect of emulsification process parameters was investigated. Spray drying of the produced emulsions was attempted to obtain a stabilized dry powder after the addition of a coating agent. The effect of different coating agents (maltodextrin, inulin, gum arabic, pectin, whey and polyvinylpyrrolidone), drying temperature (120–170 °C), and feed flow rate (3–9 ml·min^{-1}) on the obtained particles was evaluated. Results revealed that the emulsion formulation of 20/80 (O/W) with 1.5% (mass fraction) of Pluronic F-127 as stabilizer in the aqueous phase resulted in a stable nanoemulsion with droplet sizes in the range of 259–276 nm with a unimodal and sharp size distribution. The extract in the nanoemulsion was well protected at room temperature with a degradation rate of lycopene of about 50% during a month of storage time. The most stable emulsions were then processed by spray drying to obtain a dry powder. Spray drying was particularly successful when using maltodextrin as a coating agent, obtaining dried spherical particles with mean diameters of (4.87±0.17) μm with a smooth surface. The possibility of dissolving the spray dried powder in order to repristinate. The original emulsion was also successfully verified.

1. Introduction

A large amount of tomato waste is generated due to the characteristics of tomato industrial processing technology. Therefore, the efficient and convenient recovery of active compounds from tomato waste has become a key research goal. In particular, tomato waste extract is characterized by antioxidant activity due to compounds largely contained in tomatoes, like carotenoids and polyphenols. Lycopene constitutes more than 85% of the total carotenoids in tomatoes, this lipophilic red pigment is one of the most powerful antioxidants [1].

However, natural lycopene has very low solubility in a number of common solvents, *e.g.* water, dimethyl sulfoxide, and ethanol. Its extremely low solubility limits its bioavailability [2,3]. Due to the special chemical structure of lycopene, the molecule is unstable in the presence of light, oxygen, and heat [4]. These problems limited the use of natural tomato waste extract as a source of lycopene. In order to overcome these drawbacks, encapsulation techniques can be employed. Indeed, encapsulation of bioactive substances (flavors, drugs, enzymes, vitamins, essential oils and carotenoids) is used to protect them from external denaturizing agents, control their release, and improve their bioavailability [5].

There are many encapsulation techniques for bioactive substances, such as vacuum freeze drying [6–8], spray drying [7,9,10] and nanoemulsions [11–13]. Among them, nanoemulsions have been widely used in the food industry [14,15] due to their various advantages, for

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https://doi.org/10.1016/j.cjche.2024.03.002

example, the potential to improve the solubility and bioavailability of many food active compounds. Nanoemulsions have been applied as carriers for lipid active compounds and pharmaceuticals using the ability to dissolve nonpolar active compounds for drug delivery, *etc*. [16–18]. In cosmetics, nanoemulsions can be used in the synthesis of skin and hair care products [19], as well as in petroleum [20], paints and coatings [21,22], *etc*.

Emulsions consist in a nonequilibrium system formed by a mixture of two or more liquids stabilized by surfactants. They can be of two types: single oil-in-water (O/W) or double water-in-oil-in-water (W/O/W) emulsions [23]. Homogeneous mixtures in which droplets are formed in the size range of 10–500 nm are called nanoemulsions [21]. Compared with other traditional emulsions, nanoemulsions have ultra-low interfacial tension due to the continuous Brownian motion of the droplets [24], which slows down the emulsion destabilization processes, including coalescence, flocculation, Ostwald ripening, *etc*. However, although with the improved thermodynamic stability, nanoemulsions are still sensitive to environmental stresses such as heat, freezing and thawing, and altering pH, which would further destabilize the encapsulated compounds.

Lycopene encapsulation into nanoemulsion-based systems showed problems of precipitation and separation under prolonged storage, thus losing their protective effect on the active substance. For example, Kim *et al.* [25] showed that the retention of lycopene extract in nanoemulsions varied between 2.58% and 52.35% after 16 weeks of storage away from light, and the oxidized lycopene exceeded 50%. Also, Zhao *et al.* [12] investigated the stability of nanoemulsions of lycopene at different temperatures and showed that the rate of lycopene degradation was much lower at 4 °C storage than at 37 °C. Therefore, the lycopene literature demonstrated that further manipulation is needed for more effective protection of active compounds. The production of microcapsules from O/W emulsions by spray drying can increase the physicochemical stability of loaded bioactive compounds, as recently demonstrated by Rehman *et al.* [26] for the stabilization of nanoemulsions loaded with curcumin, resveratrol, and borage seed oil by spray drying postprocessing. The O/W emulsion spray drying also allows the encapsulation of lipophilic compounds into more hydrophilic carriers, which is otherwise difficult by the spray drying technique.

Therefore, in this study, the stabilization of a natural lycopene extract obtained from tomato waste was attempted. The work was divided into two parts, in the first part, the optimization of tomato waste extract encapsulation in O/W nanoemulsions was performed in order to obtain a good control of droplet size distribution (DSD) and emulsion stability. Then, in the second part of the work, dry powers were produced by spray drying of nanoemulsions after the addition of coating agents. O/W nanoemulsions were produced using isopropyl myristate as the oil phase and Pluronic F-127 as the surfactant. Emulsions were produced by a rotor-stator mixer, and the effect of different operative parameters on DSD and extract encapsulation was evaluated. Nanoemulsion stability tests were also conducted to determine the optimal formulation and storage conditions of the obtained nanoemulsions. Oil-in-water-loaded extract nanoemulsions were further converted into solid powders by spray drying technology. The effect of different coating agents on product yield was investigated. Finally, the possibility of restoring the original emulsions was attempted by re-dissolving the spray-dried particles.

2. Materials and Methods

2.1. Chemicals and sample preparation

2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (Sigma–Aldrich, Milan, Italy); citrus pectin (Thermo Scientific, USA); gum arabic (Carlo Erba, France); inulin (Thermo scientific, China); polyvinylpyrrolidone (Sigma–Aldrich, China); maltodextrin (Sigma–Aldrich, USA); potassium persulfate, isopropanol, acetonitrile, methanol (Carlo Erba, Cornaredo, Italy); isopropyl tetradecanoate, 98% (Alfa Aesar, Germany); Pluronic F-127 (Sigma–Aldrich, USA); whey from bovine milk (Sigma–Aldrich, USA); all-trans-lycopene and standards (Sigma–Aldrich, Milan, Italy) employed in this study were of analytical grade and used without further purification. The water was obtained with a Milli-Q system (Merck, Darmstadt, Germany). Tomato peels and seeds were kindly provided by a producer of tomato sauce in northern Italy. The tomato waste was stored at −20 °C for characterization of biomass and partially dried at 60 °C up to a constant mass for extraction experiments.

2.2. Apparatuses

The complete layout of the process proposed in this work is schematized in Fig. 1. As shown, the extraction technology used in this study was an ultrasound-assisted process. The extract obtained was then mixed with the oil phase for the preparation of the emulsion by the ultrasound probe, and then O/W nanoemulsion was prepared by a high-speed rotor stator. In the second part of the work, selected nanoemulsions were then encapsulated using a coating agent by a spray drying process. Furthermore, in the final stage of this study, the dissolution of the produced microparticles in water was verified.

2.2.1. Extraction of biocomponents from tomato waste

The enriched lycopene extract was obtained with pure ethanol by ultrasound-assisted extraction. Specific details about the process and the optimal extraction conditions were studied in a previous study reported by Li *et al.* [27].

The extraction was conducted using 3 g of tomato waste at a temperature of 65 °C, with an extraction time of 20 min and a liquid-to-solvent ratio L/S of 72 $ml \cdot g^{-1}$, ultrasound amplitude was set at 65% of the power (Sonicator Vibra Cell 75115, 750 W, Bioblock Scientific Co., Strasbourg, France) with a pulsed mode (on 33 s and off 30 s). The extraction vessel used has an internal volume of 90 ml.

The total content of lycopene in the obtained extract was evaluated by a UV–vis spectrophotometer (Lambda 25, PerkinElmer, Wellesley, MA, USA) at 470 nm. The sample absorbance is converted to concentration (expressed as milligrams of equivalent lycopene per milliliter of extract ($mg \cdot ml^{-1}$)) by the calibration curve reported in Eq. (1), which is made using Trolox standard ethanol solution [28]:

$$\text{Concentration} = 7.2884 \times \text{ABS}_{470\text{nm}} \quad (1)$$

where $\text{ABS}_{470\text{nm}}$ is absorbance at 470nm.

2.2.2. Preparation of oil-in-water emulsions and characterizations

For the preparation of the emulsion, isopropyl myristate was used as oil phase, whereas the water phase was prepared by dissolving Pluronic F-127 at a concentration of 1.5% (mass ratio). The mass ratio between the water phase and the oil phase varied between 10/90 and 20/80. Briefly, a known amount of isopropyl myristate was mixed with a fixed amount of lycopene-enriched extract using an ultrasonic probe (Sonicator Vibra Cell 75115, 750 W, Bioblock Scientific Co., Strasbourg, France) at 40% amplitude, on/off = 30/30 $s \cdot s^{-1}$ for 1 min. Then 90 g of the Pluronic F-127 water solution was stabilized at 7000 $r \cdot min^{-1}$ for 1 min using an L5M-A rotor-stator type emulsifier (Silverson Machines Ltd., Chesham, England). Then, the mixed oil/extract phase was slowly

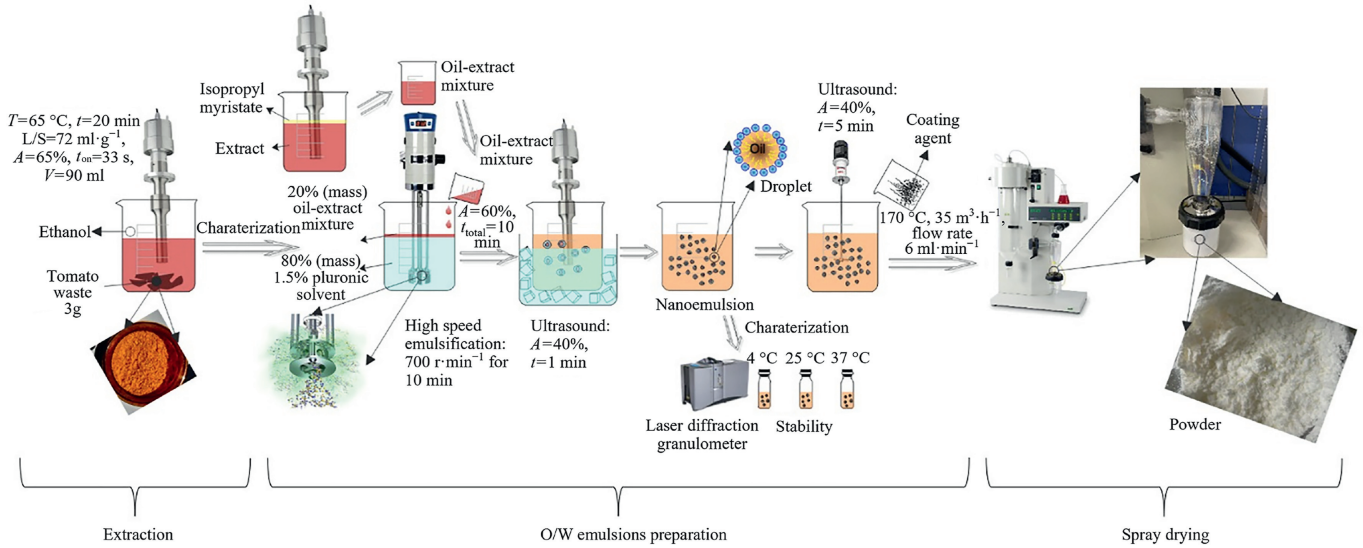

Fig. 1. Schematization of the entire process adopted in this work, from extraction to nanoemulsions to powders for the encapsulation of natural lycopene-enriched extract.

added to the water phase and emulsified for 9 min. Empty emulsions were produced using ethanol in place of the extract. The produced emulsions were analyzed using Mastersizer-3000 (Malvern Instruments Ltd., Malvern, UK) for droplet size distribution (DSD) using the methods described in Ref. [29] with slight modifications. The emulsions were dispersed in deionized water up to a laser obscuration between 10% and 12% using a circulating pump speed of 1800 r·min^{-1}. The refractive index and absorption of the aqueous phase used were 1.344 (measured by the Abbe refractometer) and 0.1, respectively; droplet size data are presented as volume fractions.

The Sauter mean diameter ($d_{3,2}$) was calculated using Eq. (2) and used to represent the droplet size [30]:

$$d_{3,2} = \frac{\sum\left(n_i \cdot d_i^3\right)}{\sum\left(n_i \cdot d_i^2\right)'} \tag{2}$$

where n_i is the number of droplets with a size of d_i.

The uniformity, a measure of the absolute deviation from the median value, was calculated using Eq. (3) and used to represent the symmetry of DSD:

$$\text{Uniformity} = \frac{\sum X_i \left| d_{(x,0.5)} - d_i \right|}{d_{(x,0.5)} \sum X_i'} \tag{3}$$

where $d_{(x,0.5)}$ is the size of the droplet below 50% of the samples and X_i is the volume of the number of droplets with a diameter existing between the two consecutive diameters [31].

The emulsions obtained were then ultrasonicated at 70% amplitude with a probe for 5 min (on/off = 30 s/30 s). At the end of the process, emulsions were again analyzed for DSD. All the processes were conducted in an ice bath. Emulsions were also characterized using an optical microscope (Nikon, ECLIPSE LV 100D, Japan) with a 50-fold mirror. The images were collected using a microscopic camera (Delta Pix, Invenio 6EIII S/N. 26656, Denmark) with an interface (Delta Pix, inSight 6.4).

The total lycopene amount and free lycopene in nanoemulsions were determined using the procedure adapted from Zhao *et al.* [12]. For the total lycopene amount, 1 ml of emulsions were dissolved in 4 ml of acetone and vortexed for 1 min to release the lycopene from the oil phase. The emulsions without lycopene were used as a control. The absorbance of the total lycopene amount was measured using a UV–vis spectrophotometer (Lambda 25, PerkinElmer, Wellesley, MA, USA) at 470 nm using a calibration curve [12]. For free lycopene content, 5 ml of emulsions were dissolved in 5 ml of *n*-hexane, shacked for 1 min, then left for 30 min to allow solvent separation. After this time, the organic liquid separated on top was taken and centrifuged at 5000 r·min^{-1} for 10 min to obtain the *n*-hexane phase. This operation was repeated twice. The absorbance of the free lycopene amount in *n*-hexane was measured using a UV–vis spectrophotometer at 470 nm.

The encapsulation rate (%) was obtained by the following Eq. (4):

$$\text{Encapsulation rate} = \frac{m_{\text{total}} - m_{\text{free}}}{m_{\text{initial}}} \times 100\% \tag{4}$$

where m_{total} is the total amount of lycopene in the nanoemulsions, m_{free} is the free lycopene in the nanoemulsions, and m_{initial} the initial amount of lycopene contained in the extract loaded into the nanoemulsions.

Stability studies on empty and loaded lycopene nanoemulsions were performed by keeping the sample at 4, 25 and 37 °C. These studies were performed for periods of 1 and 3 months. The DSDs were determined by Malvern Mastersizer-3000 every week. For chemical stability, the total lycopene content of the samples stored at the different temperatures was determined every week using the methods described previously.

2.2.3. Microparticles production by spray drying, experimental design and particles characterizations

The encapsulation of O/W nanoemulsions was carried out in a BÜCHI Mini Spray Dryer B-290 (BÜCHI Labortechnik AG, Flawil, Switzerland), working in a co-current configuration and equipped with a two-fluid nozzle (DM = 0.7 mm) for the dispersion of the liquid feed into fine droplets. A detailed description of the apparatus is reported elsewere [32–34]. Several operative parameters were investigated, such as air inlet temperature (135, 140, 170 °C), outlet temperature (7, 81, 96 °C), and feed flow rate (3, 6, 9 ml·min^{-1}), whereas aspiration rate (35 m^3·h^{-1}) and mass of the nanoemulsions processed (80 g) were kept constant. The coating agent was dissolved directly inside the nanoemulsion until

complete dissolution before spray-drying. Six different coating agents with a coating/emulsion mass ratio of 2/5 were tested: maltodextrin, inulin, polyvinylpyrrolidone (PVP), citrus pectin, whey from bovine milk and arabic gum.

The influence of the process operating conditions was evaluated on total lycopene content, product recovery and particle morphology.

After the spray drying process, the obtained microparticles were collected, weighed, and stored in water-proof containers under dark conditions at room temperature for further analysis.

The product recovery (PR) was calculated according to Eq. (5) as the ratio between the total solids recovered in the collection vessel of the spray dryer and the total solids fed to the process:

$$\mathrm{PR} = \frac{\text{Mass of dry particles}}{\text{Mass of coating agent}} \tag{5}$$

The mass of nanoemulsion fed was determined by drying 10 ml of the nanoemulsion at 110 °C to a constant mass.

Particle size distributions (PSD) of spray-dried particles were carried out using a Malvern Mastersizer-3000 granulometer. The powders (1.00 g) were suspended in isopropanol (10 ml), vortexed for one minute, and each sample was poured into the small volume cell to obtain a laser obscuration between 10% and 12% and a circulating pump speed of 1800 $\mathrm{r\cdot min^{-1}}$. The refractive index and absorption of the alcohol phase were 1.673 (measured by the Abbe refractometer) and 0.1, respectively. PSD data were presented as volume fractions. The morphology of dry samples was observed by field emission scanning electron microscopy (FESEM, LEO 1525, Carl Zeiss SMT AG, Oberkochen, Germany). Samples were placed on a carbon tab previously fixed to an aluminum stub (Agar Scientific, UK) and then coated with gold-palladium (layer thickness 25 nm) using a sputtering coater (108 A, Agar Scientific, Stansted, UK). Several FESEM images at different magnifications were taken for each sample.

For the determination of the concentration of the lycopene present in the spray dried microparticles, the method described by Rocha *et al.* [35] was used with some modifications. A mass of 10 mg of microparticles was dissolved in 1 ml of water in order to break the microcapsules. Then 4 ml of acetone were added, and the samples were vortexed for 1 min and left in the dark for about 2 h for the separation of the coating material. The concentration of lycopene in the liquid phase (supernatant) was quantified by UV–vis essay at the visible wavelength of 470 nm using a standard curve. Encapsulation efficiency was calculated as the quantity of lycopene present in the capsules compared to the lycopene initially loaded in the starting emulsions.

In spray-dried emulsions, the content of nonencapsulated oil is a key parameter in determining product quality. It has been shown that in fat-containing emulsions, the surface is almost completely covered by a thin layer of fat, and this surface fat determines the flow and wettability of spray-dried emulsions [36,37]. In addition, unencapsulated fat is subject to oxidation, which can lead to the formation of off-flavors. According to the method described by Drush and Berg [38] and Kim *et al.* [39], oil on the surface of microcapsules was determined with a few modifications. Briefly, 2 g of powder were placed on filter paper and washed with 4 × 10 ml of hexane. The filtrate was evaporated under nitrogen and weighed.

According to the method described by Anderson [40], the water solubility index and water absorption index were determined. The sample (0.5 g of dry powder) and 6 ml of water were vigorously mixed using Vortex. Then the mixture was incubated in a water bath at 30 °C for 30 min and centrifuged at 3000 *g* for 15 min (centrifuge MF-20-R, Alliance Bio Expertise, Guipry, France). The supernatant was collected in a capsule, and the residues were weighted after drying for three days at 110 °C. The water solubility index (WSI) was calculated by Eq. (6):

$$\mathrm{WSI} = \frac{\text{Mass of solid in the dried surnatant}}{\text{Mass of initial dried sample}} \tag{6}$$

The water absorption index (WAI) was calculated by Eq. (7):

$$\mathrm{WAI} = \frac{\text{Mass of pellet}}{\text{Mass of initial dried sample}} \tag{7}$$

According to the method described by Lai and Cheng [41], swelling capacity (SWC) was determined by Eq. (8):

$$\mathrm{SWC} = \frac{\text{Mass of pellet}}{\text{Mass of initial dried sample} \times (1 - \mathrm{WSI})} \tag{8}$$

where WSI is the water solubility index, calculated as shown in the previous paragraph.

2.2.4. Statistical analysis

All experiments were repeated at least three times, unless otherwise stated. All data were given as mean ± standard deviation. The statistical analysis was performed using Origin Pro. 8.5 (Origin Lab Co., USA), followed by a one-way ANOVA with a Tukey test at the $p < 0.05$ significance level.

3. Results and Discussion

3.1. Emulsion production

Empty and loaded nanoemulsions were produced using the high-speed rotor-stator mixer and ultrasonication. The extract produced was characterized by total carotenoids of (1408 ± 14) $\mu\mathrm{g\cdot g^{-1}}$ lycopene, lycopene yield of (1536 ± 53) $\mu\mathrm{g\cdot g^{-1}}$, antiradical power of (36.1 ± 0.9) $\mu\mathrm{g\cdot g^{-1}}$ Trolox, in agreement with what was found in a previous related article [27]. Emulsions were produced at an O/W ratio of 10/90 and 20/80. Extract was loaded in different quantities between 1 and 14 g (added in the oil phase up to 20 g). Empty emulsions were produced for comparison purposes; in this case, pure ethanol was added to the oil phase in the same quantity used for the extract-loaded emulsions.

Table 1 reports the experimental tests conducted and the results in terms of the droplets diameter of the produced nanoemulsions. It is possible to observe that empty emulsions were characterized by mean diameters of 410 nm, whereas loaded emulsions were characterized by mean diameters between 259 and 632 nm. In all conditions tested, well-formed nanoemulsions were formed.

Fig. 2(a) shows the macroscopic aspect of nanoemulsions. The nanoemulsion with the addition of lycopene extract was characterized by a noticeable change in color. Optical microscopy observations indicated that there were small differences in the microstructure of the emulsions loaded with lycopene, ascribable to an increase in droplet size (Fig. 2(b) and (c)).

Fig. 3 shows the results in terms of the mean diameters of the different nanoemulsions produced, comparing the empty and loaded emulsions at the same process conditions. It can be seen that for the simple O/W emulsions at a 10/90 ratio (samples E_0 and L_0) and at a 20/80 ratio (samples E_4 and L_4), the droplet mean diameter is larger than that of the nanoemulsion loaded with ethanol or lycopene-enriched extract. This result is consistent with the findings of Ferreira *et al.* [42], who observed that a certain level of ethanol can decrease the size of the nanoemulsion droplets, *i.e.* ethanol can act as a coemulsifier. It is also known that the O/W ratio could have an effect on the nanoemulsion droplet diameter. Indeed,

Table 1
Emulsions production conditions and results in terms of granulometric data. (–) None or not measured

Name	Ethanol/g	O/W (mass ratio)	$d_{3,2}$/nm	Uniformity	Name	Extract/g	O/W (mass ratio)	$d_{3,2}$/nm	Uniformity	Theoretical lycopene content/μg·ml^{-1}	Total lycopene content/μg·ml^{-1}	Lycopene encapsulation rate/%
E_0	0	10/90	410	0.477	L_0	0	10/90	410	0.477	(–)	(–)	(–)
E_1	1	10/90	312	0.353	L_1	1	10/90	345	0.741	0.071	0.68 ± 0.012	89
E_2	3	10/90	327	0.353	L_2	3	10/90	303	0.570	0.21	2.1 ± 0.025	95
E_3	5	10/90	413	0.645	L_3	5	10/90	331	0.334	0.35	3.3 ± 0.06	97
E_4	0	20/80	489	0.432	L_4	0	20/80	489	0.432	(–)	(–)	(–)
E_5	10	20/80	323	0.487	L_5	10	20/80	272	0.615	0.71	6.5 ± 0.015	98
E_6	14	20/80	306	0.717	L_6	14	20/80	259	0.370	0.99	9.6 ± 0.02	99

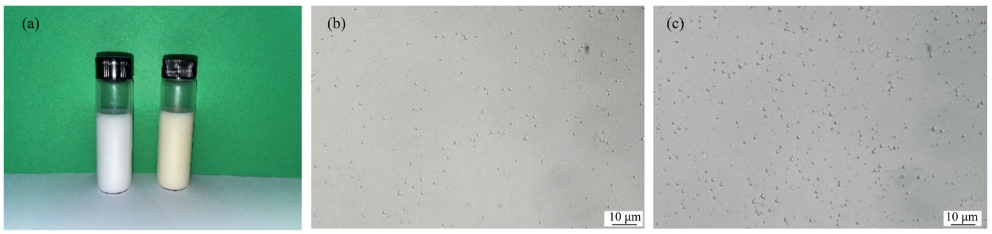

Fig. 2. Macro- and micro-level photos of empty and loaded nanoemulsions: (a) the empty nanoemulsion (on the left) and the loaded nanoemulsion (on the right); (b) and (c) optical microscope images of empty and loaded nanoemulsions.

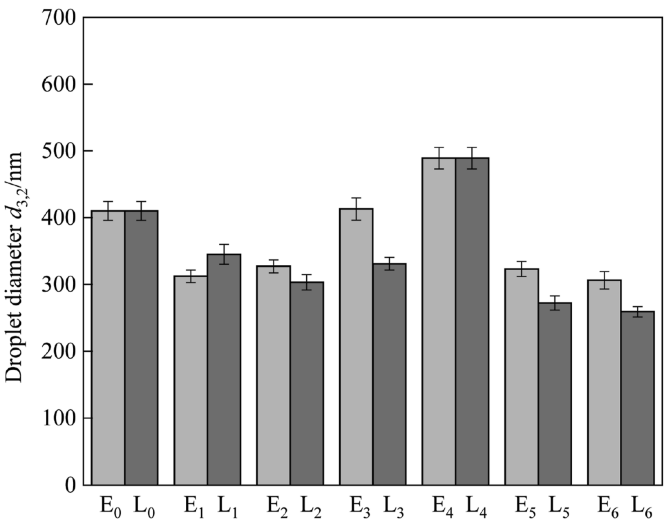

Fig. 3. Droplet diameters of empty and loaded nanoemulsions at different conditions.

observing the results obtained for the simple O/W emulsions (sample E_0, L_0 and samples E_4 and L_4), it can be observed that the droplet mean diameter was slightly smaller at O/W 10/90 (mass ratio) than at O/W 20/80 (mass ratio). Looking at the data of empty and loaded emulsions, another consideration can be made: loaded emulsions showed smaller diameters if compared to empty emulsions produced under the same conditions, probably due to the presence of natural extract compounds that favor the emulsification process. The addition of the extract did not modify the emulsions produced. This demonstrates the feasibility of using the oil-in-water nanoemulsion technique for lycopene-enriched extract containment and protection.

The stability of empty nanoemulsions was evaluated for three months at different storage temperatures at 4 °C, 25 °C and 37 °C. The results are reported in Fig. 4.

From the stability tests conducted, it was noted that the stability of the nanoemulsions decreases with increasing temperature; this fact is probably due to the molecular activity being more intense with increasing temperature. In addition, the stability of the nanoemulsions decreased at the higher O/W ratio of 20/80 due to

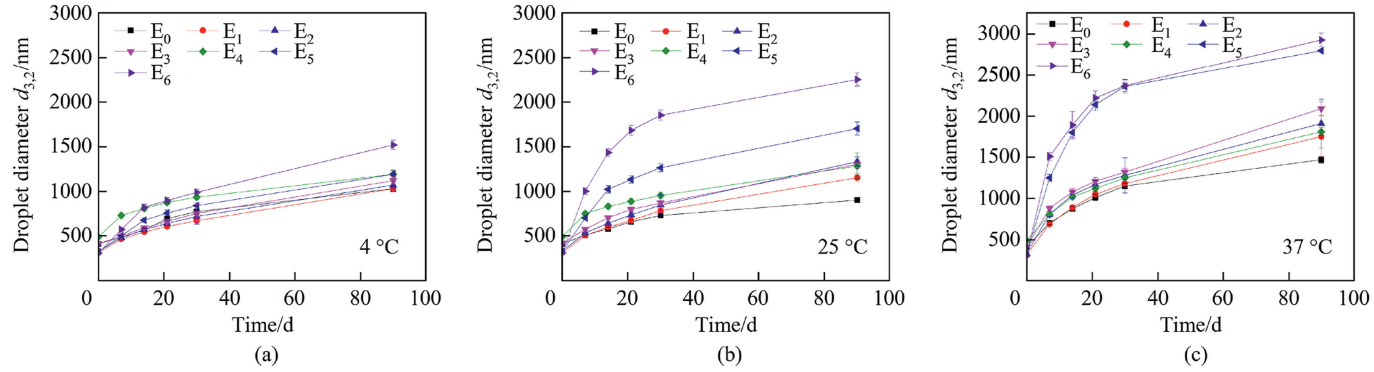

Fig. 4. Empty emulsions physical stability was evaluated at (a) 4 °C, (b) 25 °C and (c) 37 °C for 90 d.

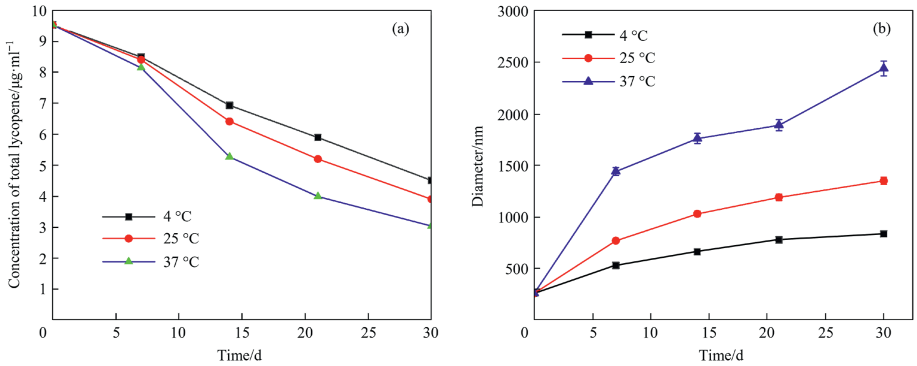

Fig. 5. (a) Stability of lycopene content during time in sample L_6 at different temperatures; (b) physical stability on sample L_6 loaded in nanoemulsion during time at different temperatures.

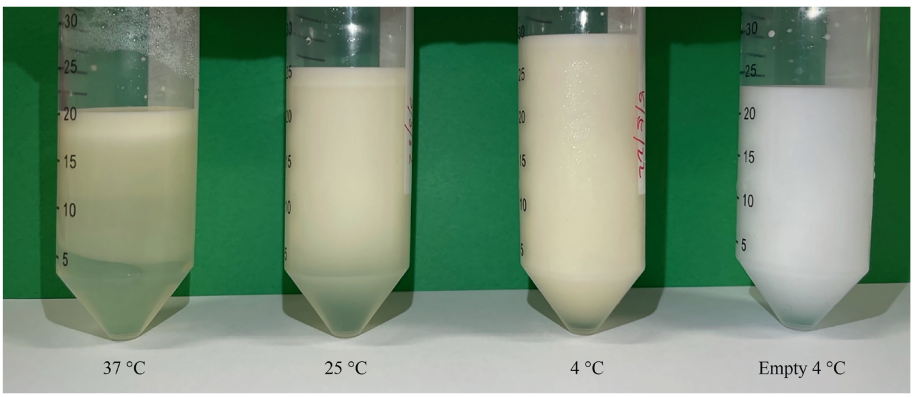

Fig. 6. Visual appearance of empty nanoemulsion and loaded nanoemulsion (sample L_6) after 3 months at 4 °C, 25 °C and 37 °C of storage.

the high oil droplet concentration, which favored the droplet condensation induced by temperature.

The chemical stability of loaded nanoemulsions was also studied by monitoring changes in total lycopene throughout a 30-day storage period at different temperatures, *i.e.*, 4 °C, 25 °C and 37 °C. Also, the physical stability of loaded emulsions was studied.

From the results obtained, it was noted that the encapsulated lycopene showed the highest lycopene degradation under high temperature conditions (Fig. 5(a)). This can be attributed to the fact that the high temperatures can accelerate lycopene degradation by breaking the oil-in-water system and exposing the lycopene to degradation, resulting in a reduction of its stability [43]. Looking at Fig. 5, it can be seen that after a month, the nanoemulsions still contain about 47.27% of lycopene at a temperature of 4 °C, 40.91% of lycopene at a temperature of 25 °C, and 31.82% of lycopene at a temperature of 37 °C. This result is in agreement with Kim *et al.* [25], who reported an increase in stability of encapsulated lycopene in the emulsions over the nonencapsulated form at 4 °C. In addition, it can also be observed from Fig. 5(b) that the diameter of the droplets changed significantly at higher temperatures during storage, confirming the results observed for the empty emulsion stability tests.

The visual appearance of samples after three months of storage at the different temperatures investigated is reported in Fig. 6.

Comparing the lycopene nanoemulsions stored at different temperatures, it can be concluded that at a low temperature of 4 °C, the natural extracted loaded nanoemulsion appears well conserved with only a small amount of delamination at the bottom of the test tube, like the blank control group. Moreover, the color of the nanoemulsion is relatively uniform. As the temperature increases, significant delamination of the loaded nanoemulsions can be observed after three months of storage.

These storage stability tests for loaded emulsions confirmed that the lower the storage temperature, the more stable the system is, both from a physical and chemical point of view. For this reason, for a good storage of loaded nanoemulsions refrigeration is suggested, otherwise spray drying of nanoemulsions can be attempted in order to obtain a long stability product.

3.2. Encapsulation of nanoemulsion with different coating agent by spray drying

In order to provide an alternative to refrigerated storage of the natural extract encapsulated product, nanoemulsions loaded with tomato extract were dried by spray drying, and a comprehensive data analysis of the powdered product was carried out.

First of all, empty nanoemulsions were subjected to spray drying using maltodextrin as a coating agent. For this study, nanoemulsion (sample E_6) produced at the following operative conditions was used: O/W 20/80, 14 g of ethanol and 6 g of isopropyl myristate mixed by ultrasonic ($A = 40\%$ and 1 min), 80 g of pluronic solution (1.5% mass fraction). Indeed, this emulsion showed good results in terms of DSD and stability over time. Before the spray-drying experiment, the coating agent was dissolved in a fixed amount directly in the prepared nanoemulsion until complete dissolution.

Spray drying operative conditions such as temperature, feed flow rate, and the concentration of coating agent were varied in order to find the condition at which a good powder recovery was reached. In Table 2 a summary of the preliminary tests conducted is reported.

Table 2
Preliminary spray drying tests of empty nanoemulsions (sample E_6) using maltodextrin as a coating agent

Sample	Process conditions	Coating agent/%	Nanoemulsions mean diameter, $d_{3,2}$/nm	Product recovery/%	Powder mean diameter, $d_{3,2}$/μm
D_1	135 °C, 35 $m^3 \cdot h^{-1}$, 3 $ml \cdot min^{-1}$	10	267 ± 16	7.4	4.21 ± 0.16
D_2	135 °C, 35 $m^3 \cdot h^{-1}$, 6 $ml \cdot min^{-1}$	10	235 ± 21	3.7	6.27 ± 0.22
D_3	140 °C, 35 $m^3 \cdot h^{-1}$, 6 $ml \cdot min^{-1}$	20	249 ± 19	16.6	10.4 ± 0.37
D_4	140 °C, 35 $m^3 \cdot h^{-1}$, 6 $ml \cdot min^{-1}$	40	246 ± 17	47.7	6.14 ± 0.23
D_5	140 °C, 35 $m^3 \cdot h^{-1}$, 9 $ml \cdot min^{-1}$	40	256 ± 16	34.6	4.57 ± 0.19
D_6	170 °C, 35 $m^3 \cdot h^{-1}$, 3 $ml \cdot min^{-1}$	40	252 ± 13	10.7	3.44 ± 0.15
D_7	170 °C, 35 $m^3 \cdot h^{-1}$, 6 $ml \cdot min^{-1}$	40	236 ± 24	80.3	4.87 ± 0.17
D_8	170 °C, 35 $m^3 \cdot h^{-1}$, 9 $ml \cdot min^{-1}$	40	251 ± 15	14.5	10.1 ± 0.36
D_9	170 °C, 35 $m^3 \cdot h^{-1}$, 6 $ml \cdot min^{-1}$	20	253 ± 16	38.4	8.51 ± 0.34

The operating conditions at which spray drying reached the highest recovery were the input temperature of 170 °C, the gas flow rate of 35 $m^3 \cdot h^{-1}$, the nanoemulsion flow rate of 6 $ml \cdot min^{-1}$, and the coating agent at 40%. In this condition, the recovery of the product reaches 80.3%.

Field emission scanning electron microscopy (FESEM) images of maltodextrin particles obtained at the best conditions (experiment D_7) are reported in Fig. 7(a). Particles are spherical and have a regular shape, with a mean diameter of (4.87 ± 0.17) μm.

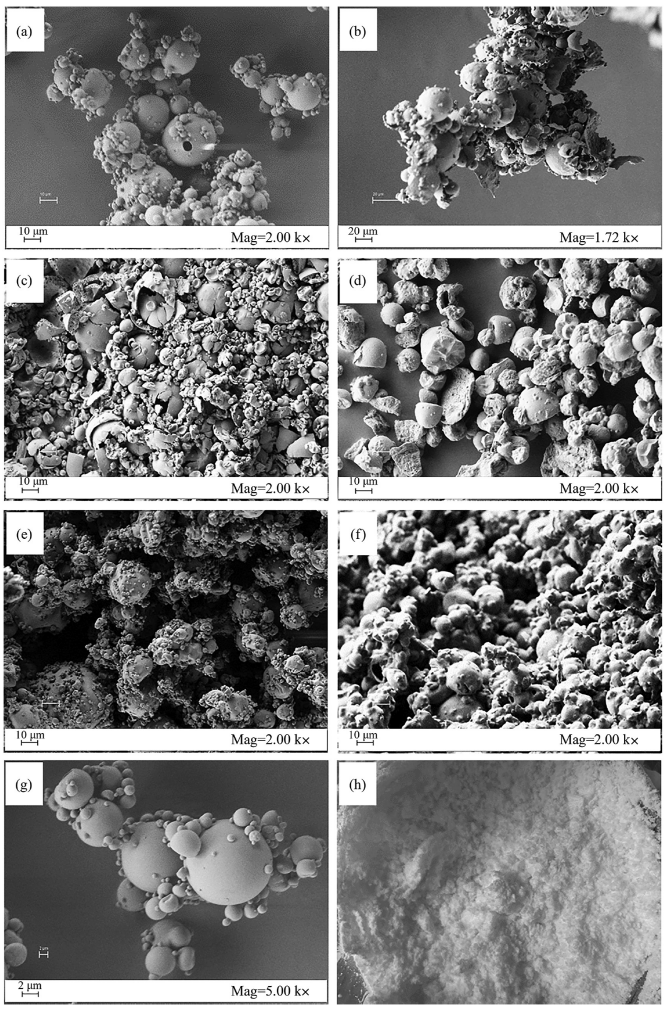

Fig. 7. FESEM images of spray drying nanoemulsions produced with different coating agents: (a) maltodextrin; (b) Inulin; (c) gum arabic; (d) pectin; (e) PVP; (f) whey, (g) maltodextrin, (h) dried powder for lycopene-loaded nanoemulsions with maltodextrin.

Considering the good results obtained with maltodextrin, other coating agents were also considered. Spray drying experiments were performed at the operative conditions optimized in the feasibility study. For this part of the study, loaded nanoemulsions produced at the condition of sample L_6 were used, and the different coating agents were added at 40% with respect to the emulsion mass. Table 3 represents the experiments performed and the results obtained in terms of product recovery and particle mean diameters. The data showed that the mean diameter of the starting nanoemulsion was modified by the addition of the different coating agents. Inulin had a weak effect on the emulsion droplet diameter after being fully dissolved, while Pectin Citrus had the most significant effect on the droplet diameter, causing an increase in droplet mean diameter up to 1670 nm. The coating agent dissolved in the water phase can have an effect on the hydrodynamic diameter, especially for substances that show an affinity with emulsion droplets and thus tend to deposit on the droplet surface. The highest product recovery was found for maltodextrin, 80.88%, while the lowest product recovery was found for PVP, with only about 16.28% of power recovered.

Table 3 also shows the results of the water absorption index, water solubility index, and swelling capacity for encapsulated lycopene. Different coating agents have different WSI indexes after encapsulation. Higher WSI may be due to the greater swelling capacity of coating agents. However, a lower WAI and SWC were found for the higher WSI of maltodextrin used. These differences can be attributed to differences in particle structure (see Fig. 7). In short, a high WSI means that a high-concentration solution can be prepared, and a low WAI means that the powder is not able to absorb a large amount of water and becomes wet when exposed to the air.

Fig. 7 shows SEM images of the powder produced with different coating agents used. There were almost four types of particles observed: (1) smooth spherical (Fig. 7(g)), (2) wrinkled (Fig. 7(d) and (e)); (3) spherical with small particles (Fig. 7(a) and (b)), (4) broken flakes and collapsed particles (Fig. 7(c), (f)). From the morphology point of view, inulin and PVP reveals themselves to be good coating agents, but considering the data about recovery, maltodextrin confirmed to be the best coating agent. The dried powders appear creamy-colored, as shown in Fig. 7(h).

The amount of nonencapsulated oil on the surface of microcapsules was also evaluated, and it resulted in approximately 1.9%. The surface free-fat content was very low, similar to the findings of Kim *et al.* [39]. This also confirmed why spray drying of emulsions requires a larger amount of coating agent and a higher temperature for the drying process than direct drying of extracts [27].

From the comparison of powder particle size, particle shape, surface smoothness, *etc.*, maltodextrin can be considered the best coating agent. The average particle size of the powder obtained is (4.87 ± 0.17) μm (Fig. 8(b)), with a very high encapsulation efficiency of 99%.

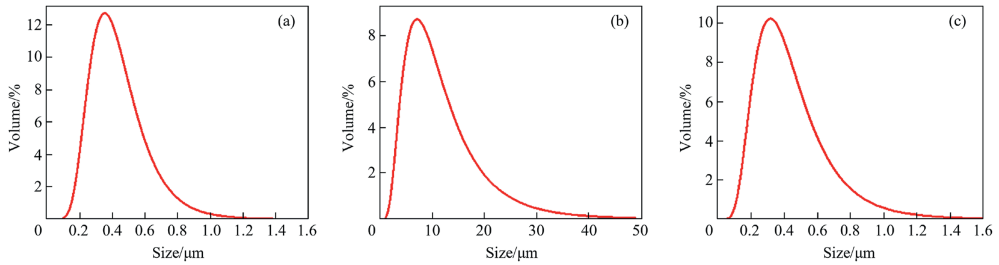

Fig. 8. Granulometric data of (a) lycopene-loaded nanoemulsions, (b) spray drying products, and (c) emulsions after rehydration.

Table 3
Characteristics of nanoemulsion and product recovery from spray drying with different coating agents. (–) None or not measured

		Nanoemulsion mean diameter, $d_{3,2}$/nm	Product recovery/%	Moisture/%	Water activity	Water solubility	Water absorption	Swelling capacity
Emu	Emulsion	195 ± 6	(–)	(–)	(–)	(–)	(–)	(–)
I	Emulsion + inulin	386 ± 14	51.02	5.4	0.362	0.5623	0.0586	0.1338
M	Emulsion + maltodextrin	418 ± 16	80.88	9.7	0.328	0.7871	0.0013	0.0062
PVP	Emulsion + PVP	466 ± 17	16.28	6.1	0.348	0.8014	0.6751	3.3987
GA	Emulsion + gum arabic	477 ± 61	59.86	10.5	0.204	0.8064	0.4680	2.4177
W	Emulsion + whey	1100 ± 83	40.69	7.6	0.287	0.6503	0.1249	0.3571
PC	Emulsion + pectin citrus	1670 ± 96	36.35	7.8	0.350	0.1880	0.2950	0.3634

A final experiment was conducted to assess the possibility of dissolving the powder obtained by spray drying and restoring the original emulsion. To perform this proof of concept, the powder obtained using maltodextrin as a coating agent and lycopene-loaded nanoemulsion product under optimized conditions (T = 65 °C, t = 20 min, L/S = 72 ml·g^{-1}, A = 65%, t_{on} = 33 s, V = 90 ml) was used.

In Fig. 9, photos of the single step of this proof of concept are reported: (a) preparation of the emulsion that contains the coating agent; (b) powder production using spray drying; (c) powder addition in water; (d) emulsion restoration. The powder was dissolved in water, fixing a 10/1 liquid/solid mass ratio. From Fig. 9, it is possible to see the similarity of steps (a) and step (d) in terms of their visual appearance, indicating that, with a good probability, the emulsion has been repristinated after powder dissolution. In terms of particle size distribution (PSD), the system was analyzed at each step. Granulometric data are reported in Fig. 8.

Emulsion after production is characterized by a mean diameter of 276 nm. A small enlargement of the DSD was observed after maltodextrin addition (Fig. 8(a)). The spray drying of emulsion + maltodextrin led to the formation of particles of 4.87 μm in mean diameter and micrometric PSD (Fig. 8(b)). After the dissolution of the powder in water, an opaque liquid system was obtained again with a mean diameter of 394 nm (Fig. 8(c)), *i.e.* very similar to the DSD of Fig. 8(a) (the starting curve). These results constituted a confirmation of the possibility of drying nanoemulsions using spray drying and restoring the original emulsions by simple water dissolution of the powder.

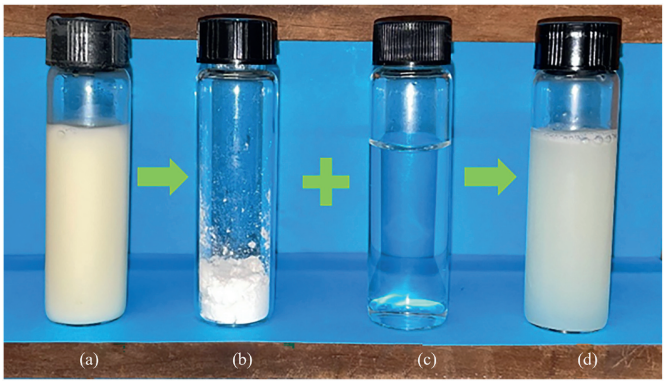

Fig. 9. From emulsion to powder and back to emulsion steps: (a) loaded nanoemulsion with 40% (mass ratio) of maltodextrin; (b) dried powder; (c) water; (d) nanoemulsion rehydration.

4. Conclusions

An efficient method for natural lycopene encapsulation and its preservation in oil-in-water nanoemulsions was proposed. Nanoemulsions were found stable for up to three months at the optimal storage temperature, as no significant agglomeration was observed. The addition of an extracted solution was found to significantly reduce the droplet size and increase the stability of nanoemulsions. However, a degradation phenomenon of encapsulated lycopene was observed even in refrigerated conditions. For this reason, the drying of the emulsions was attempted. The spray drying technology was found efficient for transferring the nanoemulsion to a solid powder. Under the optimal conditions, the powder obtained using maltodextrin as a coating agent presented a better microstructure property and high lycopene encapsulation efficiency. The obtained microparticles have a good dissolution in water, and the reconstituted emulsions maintain nanosize.

CRediT Authorship Contribution Statement

Junyang Li: Methodology, Formal analysis, Validation, Writing – original draft, Data curation. Roberta Campardelli: Conceptualization, Methodology, Supervision, Review & editing. Giuseppe Firpo: Methodology, Data curation. Jingtao Zhang: Review & editing. Patrizia Perego: Project administration, Supervision.

Data Availability

Data will be made available on request.

Declaration of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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