Supercritical assisted process for the encapsulation of olive pomace extract into liposomes

P. Trucillo [a], R. Campardelli *, [a], B. Aliakbarian [b], P. Perego [b], E. Reverchon [a]

[a] Department of Industrial Engineering, University of Salerno, Via Giovanni Paolo II, 132, 84084, Fisciano (SA), Italy

[b] Department of Civil, Chemical and Environmental Engineering (DICCA), University of Genoa, Via Opera Pia 15, 16145 Genova, Italy

*rcampardelli@unisa.it

www.supercriticalfluidgroup.unisa.it

Abstract

Polyphenols are part of a family of compounds occurring in nature that can have great applications in food industry, but also in the prevention of cardiac and aging-related illness. Since they are particularly sensible to light, heat and oxygen exposure, it is well recognized the necessity to entrap them into drug carriers, such as liposomes.

In this work, SuperLip was used for the encapsulation of a polyphenol-rich aqueous extract from olive pomace. The effect on liposome morphology and encapsulation efficiency of different operative parameters, such as working pressure, atomization nozzle diameter and the content of polyphenols in the feed, was studied. Liposomes were produced with mean diameters smaller than 265 nm at 130 bar and down to 168 nm for 170 bar processed liposomes. Narrower liposome distribution curves were also obtained changing the nozzle diameter for the atomization of water. Encapsulation efficiencies up to 58 % were obtained that are about six times larger than using conventional methods. Nanoliposomes rich in polyphenols produced in this work can be used in the formulation of cosmetics and nutraceutical products.

Keywords: liposomes, supercritical fluids, olive pomace, nutraceutical.

1. Introduction

Polyphenols are compounds largely available in nature; for example, vegetables, cereals and fruit have a high content in polyphenols (Pandey and Rizvi, 2009). These compounds are generally produced by the secondary metabolism of plants (Beckman, 2000). In general, they are found in different quantities in cellular and sub-cellular plant tissues; in particular, water soluble phenolic compounds generally occur in cell vacuoles (Wink, 1997). Phenolics can be divided in flavonoids (Terahara, 2015), phenolic acids (Vinayagam et al., 2016), stilbenes (Likhitwitayawuid, 2008) and lignans (Adlercreutz and Mazur, 1997).

Food industry uses phenolic compounds as additives to cover bitterness, to add color, flavor and also to protect the product against oxidative stress (Papuc et al., 2010; Reverchon et al., 1994). Moreover, polyphenols are frequently added to human diet (Manach et al., 2004) because they can have beneficial bioactive properties for human beings (Cevallos-Casals and Cisneros-Zevallos, 2010). Indeed, they are used to prevent cardiovascular and heart diseases (Scalbert and Mazur, 2002), cancer (Sancho and Mach, 2015), type 1 and type 2 diabetes (Dragan et al., 2015; Solayman et al., 2016), osteoporosis (Hagiwara et al., 2011) and neuronal illnesses (Scarmeas et al., 2006). Anti-aging (Biesalski, 2002; Harman, 2006), anti-viral (Eichhorn et al., 1985) and antimicrobial (Daglia, 2012) activities are also recognized for many polyphenols, according to their antioxidant power (Vissers et al., 2004), that it helps to inhibit degenerative body processes (Mantovani et al., 2008). Olive and olive oil by-products are well-known resources of natural phenolic compounds. In particular, olive pomace has been identified as an inexpensive source of phenolic compounds (Aliakbarian et al., 2011; Aliakbarian et al., 2012; Palmieri et al., 2012).

However, polyphenols are extremely volatile, unstable, and sensible to light (Barth et al., 1994; Gellerstedt, 1975), heat (Sauvage et al., 2010) and oxygen (De Leonardis et al., 2013; Volf et al., 2014). The antioxidant property of polyphenols (Fang and Bhandari, 2010) can be preserved, by enhancing their stability (Volf et al., 2014), bioactivity (Taamalli et al., 2012) and bioavailability (Williamson and Manach, 2005) using polymer carriers.

Technologies commonly used to perform polyphenols encapsulation are spray drying (Desai and Park, 2005; Paini et al., 2015a), coacervation (Gouin, 2004), co-crystallization (Deladino et al., 2007), yeast encapsulation (Blanquet et al., 2005), and microcapsules and membranes entrapment (De Marco et al., 2017; Paini et al., 2015b). However, these conventional processes suffer of some drawbacks related to high process temperatures, low polyphenols encapsulation efficiencies and difficult control of particle size distribution.

Liposomes are vesicles formed by an external lipid double layer and an internal aqueous core (Nakayama, 2006). They are powerful drug carriers for their similarity to cell wall membranes. Drugs can be encapsulated into liposomes in the lipophilic and in the hydrophilic compartment, depending on their affinity with water molecules or with the lipidic membrane. Conventional processes, such as thin layer hydration method, have been used to encapsulate polyphenols into liposomes; for example, apigenin was loaded into nanometric liposomes with a relatively high entrapment efficiency (Paini et al., 2015c).

Recently, a supercritical assisted process has been developed for liposome production, named SuperLip (Supercritical Assisted Liposome Formation) . The key of this process consists in creating

first the droplet of water and, then, to surround it by one or more double layers of phospholipids. Using this technique, it was possible to produce vesicles with mean diameters from 100 to 300 nm, with encapsulation efficiencies up to 99 % for fluorescein (Campardelli et al., 2016b), 94 % for bovine serum albumin (Campardelli et al., 2016a), 98 % for theophylline (Trucillo, 2017), and 93 % for eugenol (Trucillo et al., 2017).

The aim of the present work is to apply Superlip to the encapsulation of aqueous phenolic compounds extracted from olive pomace, for nutraceutical purposes. The advantage of SuperLip in the encapsulation of this extract is linked to the possibility to work at mild operative conditions, crucial in the preservation of these temperature sensitive compounds. Furthermore, this process allows the continuous production of stabilized water liposome suspensions with a good control of liposome dimension, also at nanometrical level. The effects of some process parameters such as operative pressure, injector diameter, phenolic compounds loading on the encapsulation efficiency, liposomes morphology and diameters were studied. Conventional thin layer hydration method was also used to encapsulate phenolic compounds from olive pomace and the results were compared with those obtained using SuperLip.

2. Materials and methods

2.1 Chemicals and reagents

L-α-Phosphatidylcholine (> 60 % purity, lyophilized powder), Ethanol (> 99.8 %, liquid) and Folin & Ciocalteu's phenol were purchased from Sigma Aldrich (Milan, Italy). Carbon dioxide (> 99.4 % purity, gas-liquid equilibrium) was provided by Morlando group (Naples, Italy). Olive pomace extract was produced in the Department of Civil, Chemical and Environmental Engineering of the University of Genoa, Italy. All the materials and reagents were used as received.

2.2 Olive pomace extract

Taggiasca cultivar olive pomace from a three phase oil extraction decanter was supplied by an Italian olive oil producer located at Imperia (Liguria, Italy) and stored at -20 °C prior to analysis. A high-pressure high-temperature (HPHT) stirred reactor model 4560 (Parr Instruments Company, Moline, IL, USA) was used for the extraction. In this way the simultaneous effect of high extraction temperature and high pressure were achieved: solvent viscosity decreased, mass transfer enhanced, and better penetration of solvents in matrix particles was obtained. The reactor was kept at constant temperature of 180 °C for 90 minutes. the sample was prepared for the experiment diluting 40 g of dried olive in 400 mL of deionized water. The extraction parameters were based on the previous optimization study performed by Aliakbarian et al. (Aliakbarian et al., 2011). The aqueous extract was then centrifuged by a PK131 centrifuge (ALC, Alberta, Canada) at 6000×g for 10 minutes. The supernatant was, then, subjected to quantitative analyses for total phenolic compounds calculation. The solution was kept at 4 °C prior to the encapsulation process.

2.3 Liposome production

2.3.1. SuperLip

SuperLip apparatus is schematized in **Figure 1**. Briefly, phospholipids were dissolved in ethanol; then, SC-CO_2 and ethanol solution were fed separately in a thermally heated saturator, working a temperature of 40 °C. An expanded liquid (EL) was obtained due to the large internal surface. The saturator (S) worked at pressures in the range of 130 bar and 170 bar. Then, the EL was fed to a high pressure stainless steel Formation Vessel (FV) that worked at the same pressure. Aqueous solution was atomized in the FV to generate water droplets using nozzles of different diameters (60 µm and 80 µm). In this study, aqueous extract of olive pomace was used as the water solution. The extracts were diluted with deionized water to obtain fixed total phenolic compounds (TP) to the phosphatidylcholine (PC) ratios from 5 % to 20 % (TP/PC). Carbon Dioxide was pumped using an Ecoflow pump (mod. LDC-M-2, Lewa, Germany); aqueous and ethanol solutions were pumped separately using high pressure precision pumps (Model 305, Gilson, France). At the top of the FV the expanded liquid was delivered to a stainless-steel separator (Sep) operated at 10 bar and 25 °C. The liposome suspension was recovered at the bottom of the formation vessel using an on –off valve.

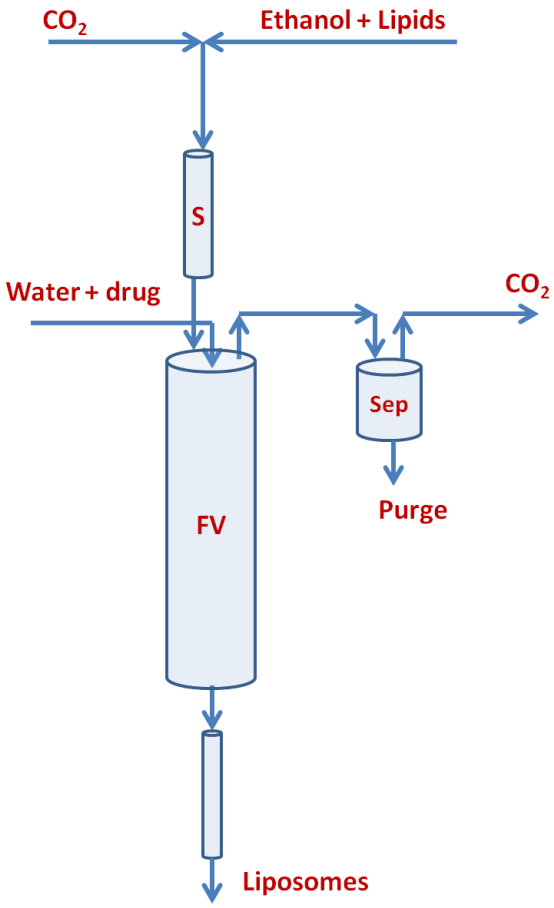

Figure 1 SuperLip process scheme

2.3.2 Conventional liposome production process (Bangham)

Thin Layer Hydration method is the conventional method developed by Bangham (Bangham, 1983). It was used for comparison purpose. First, lipids were dissolved into an ethanol solution; olive oil pomace hydrophilic extract was dissolved instead into an aqueous bulk. The organic solvent was evaporated under vacuum and a thin lipid layer was formed on the walls of the glass flask. The lipidic layer was hydrated using the aqueous solution under gentle agitation (250 rpm). After 1 hour agitation, liposome suspension was recovered and analyzed.

2.4 Liposomes dimension

Liposome suspensions were characterized using Dynamic Light Scattering (DLS) with Zetasizer Nano S (Worcestershire, UK). Liposomes mean diameters (MD) were measured, as well as polydispersion index (PDI) and standard deviation (SD) of the samples obtained. DLS instrument works at 25 °C and is equipped with a 5.0 mW He-Ne laser operating at 633 nm with a scattering angle of 173°. Every sample was measured 3 times in order to calculate the mean value of the measurements.

2.5. Liposomes morphology

Liposomes morphology was analyzed using a Field Emission-Scanning Electron Microscope (FE-SEM mod. LEO 1525; Carl Zeiss SMT AG, Oberkochen, Germany). The samples were centrifuged at 6500 rpm for 50 minutes at -4 °C (Thermo Scientific, mod. IEC CL30R). After that, the supernatant was separated and the vesicles were re-suspended in distilled water. This operation was repeated twice. Then, a drop of the suspension was spread over an adhesive carbon tab placed on an aluminum stub and dried at air for 2 days. Then, the dried samples were coated with a gold layer using a sputter coater (thickness 250 Å, model B7341; Agar Scientific, Stansted, UK), before FE-SEM observation.

Liposomes suspensions produced using thin layer hydration method were also observed using an optical microscope (OM, mod. BX 50 Olympus, Tokyo, Japan), equipped with a phase contrast condenser.

2.6 Encapsulation efficiency

Encapsulation efficiency (EE) of liposomes was measured using the supernatant method (Otake et al., 2006). Liposomes suspensions were centrifuged at 6500 rpm for 50 minutes at -4 °C using the same centrifuge described above and supernatant was collected. The amount of phenolic compounds in the supernatant was measured using the method described in the following.

Total polyphenol (TP) concentration was measured following the colorimetric Folin–Ciocalteau assay (Swain, 1959) using a Micro-volume UV-Vis spectrophotometer (BioSpec-nano, Shimadzu Scientific Instruments, Columbia, USA) at the wavelength of 725 nm. TP concentration, expressed

as milligram of equivalent caffeic acid per milliliter (mg_{CAE}/mL), was calibrated (R^2 = 0.9945) using standard solutions of caffeic acid (50–1000 µg/mL) following the **Eq.1**:

$ABS(_{725nm}) = 2.0442*Concentration$ (1)

The Encapsulation Efficiency was calculated as the complement to 100 of the percentage of phenolic content in the supernatant, using the following equation **Eq.2:**

$$\mathrm{EE}\ [\%] = 100 * \left(1 - \frac{\mathrm{ppm_{supern}}}{\mathrm{ppm_{loaded}}}\right) \quad (2)$$

3. Results and Discussion

Olive pomace water extract is a mixture of polyphenols showing different water solubilities. For this reason, its encapsulation, in carriers such as liposomes, needs to be optimized finding the best process conditions for a good EE of the compounds and liposome size distribution. Water extract of olive pomace, obtained by the HPHT extractor, have a TP concentration of 2.6 ± 0.1 mg_{CAE}/mL. Loadings of 5 %, 10 %, 15 % and 20 % w/w of total polyphenols contained in olive pomace/phosphatidylcholine (TP/PC) were chosen for the first set of experiments. Phosphatidylcholine was dissolved in ethanol at the concentration of 5 mg/mL for all the experiments.

The operative pressure was set at 130 bar and temperature was fixed at 40 °C in the saturator for the formation of the expanded liquid and at 40 °C in the formation vessel. A 60 µm injector diameter was used for water atomization. The flow rate of the ethanol solution was set at 3.5 mL/min, and CO_2 was fed to the saturator at 6.5 g/min, together with ethanol solution. Mass based Gas to Liquid Ratio (GLR) among ethanol and CO_2 was equal to 2.4 for all the experiments. Water flow rate was fixed to 5 mL/min for all the experiments. Mean diameter (MD), standard deviation (SD) and polydispersion index (PDI) of liposomes are listed in **Table 1.**

Table 1 Mean diameter (MD), standard deviation (SD) and polydispersion index (PDI) of liposomes produced using SuperLip and thin layer hydration method, used for comparison purpose, loaded with 5 %, 10 %, 15 % 20 % mg olive pomace total polyphenols per mg of phosphatidylcholine (TP/PC).

SuperLip				
Pressure [bar]	**Injector diameter [µm]**	**TP/PC [%, w/w]**	**MD [nm ± SD]**	**PDI**
130	60	5	134 ± 50	0.37
		10	148 ± 59	0.40
		15	210 ± 84	0.40
		20	250 ± 125	0.50
130	80	5	245 ± 76	0.31
		10	260 ± 91	0.35
		15	264 ± 98	0.37
		20	265 ± 101	0.38
170	80	5	165 ± 26	0.16
		10	171 ± 31	0.18
		15	185 ± 46	0.25
		20	199 ± 52	0.26
Thin layer hydration		**TP/PC [%, w/w]**	**MD [µm ± SD]**	**PDI**
		15	50 ± 8	0.18

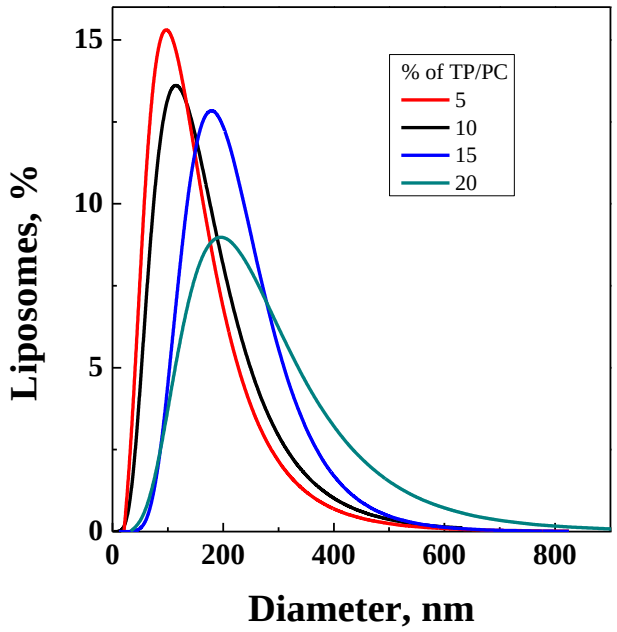

Figure 2 Frequency distribution curves of olive pomace loaded liposomes at 130 bar and using 60 µm injection diameter

Nanometric liposomes loaded with olive pomace extract were obtained. In the experiments presented in **Table 1**, liposomes showed mean dimensions between a minimum of 134 ± 76 nm and a maximum of 250 ± 125 nm; mean size of the vesicles increased with the increase of the theoretical loading of olive pomace extract. PDIs ranged between a minimum of 0.37 to a maximum of 0.50, increasing drug theoretical loading. This means that at higher bioactive compounds loading of liposomes larger dispersions were obtained, as also shown in **Figure 2**.

FE-SEM was used to confirm mean size and morphology of vesicles produced with SuperLip. As an example, a 5 % TP/PC loaded liposomes FE-SEM image is reported in **Figure 3**.

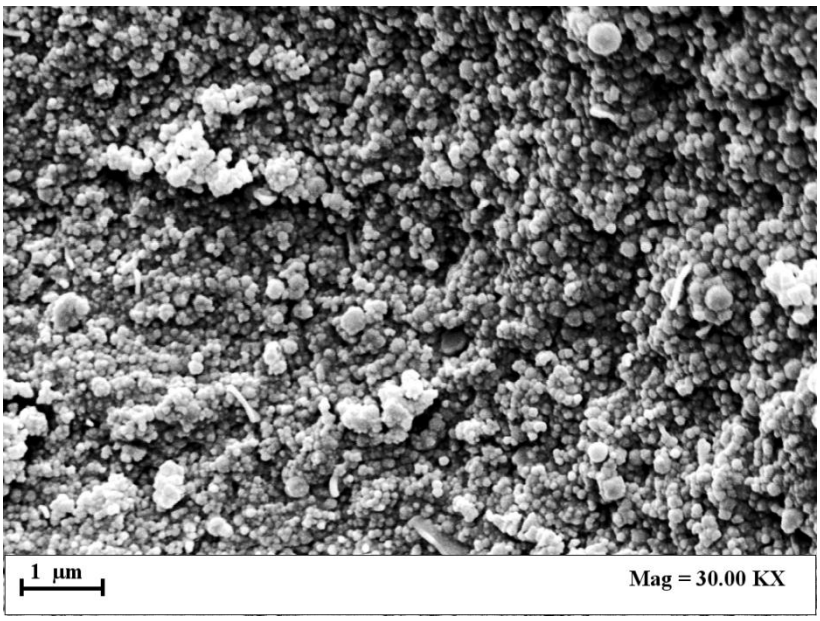

Figure 3 FE-SEM image of liposomes loaded with 5 % mg total polyphenols from olive pomace per mg of phosphatidylcholine (TP/PC), produced at 130 bar using a 60 µm injector.

As shown in **Figure 3**, liposomes are characterized by spherical shape and a slightly smooth surface. Mean dimensions measured using specialized software on SEM image, confirmed the values reported in **Table 1.**

A second set of experiments was performed using 80 µm nozzle diameter, all the other process parameters were maintained constant. As shown in **Table 1** the increase of the injector diameter produced liposomes with larger average diameters. Indeed, mean diameters ranged between a minimum of 245 ± 76 nm to a maximum of 265 ± 101 nm. As observed for the liposomes produced with the smaller nozzle, also in this case polydispersion index slightly increased from a minimum of 0.31 to a maximum of 0.38 when TP/PC increased from 5 to 20 %. In this case, mean dimensions of liposomes were practically constant; however, the distribution curves are narrower than 60 µm produced samples.

Characterization of size and morphology of liposomes produced with SuperLip using 80 µm injector was also performed using FE-SEM. An example of vesicle image is reported in **Figure 4** for the sample produced using a nozzle of 80 µm for 5 % TP/PC ratio.

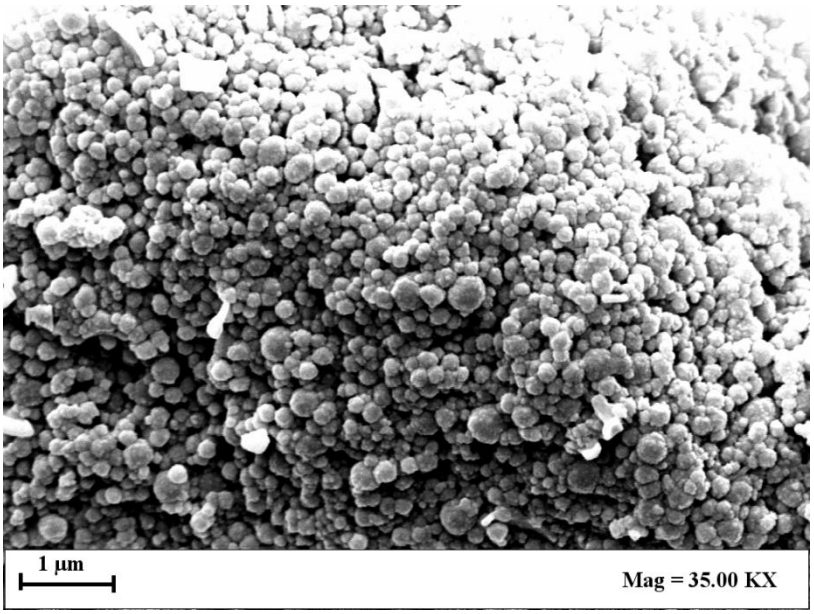

Figure 4 FE-SEM image of liposomes loaded with 5 % mg total polyphenols from olive pomace per mg of phosphatidylcholine (TP/PC), produced at the pressure of 130 bar and 80 µm injection diameter

Another set of experiment was performed increasing only the formation vessel pressure to 170 bar, all the other operative parameters remained unchanged.

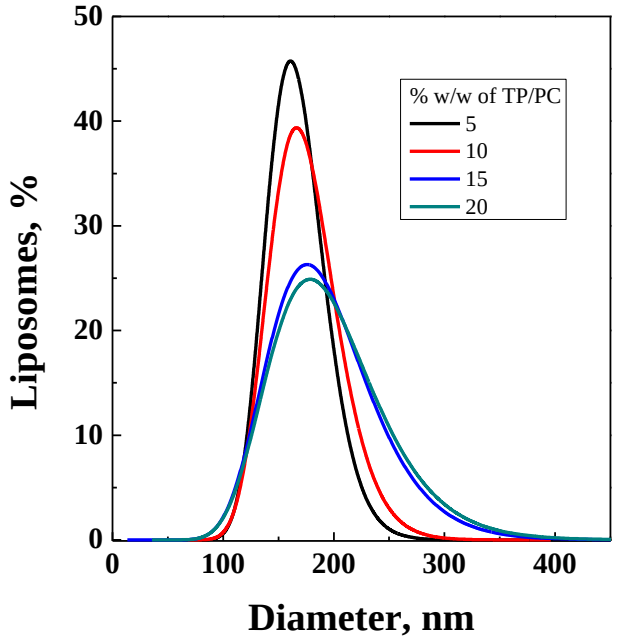

Figure 5 Frequency distributions curves of olive pomace loaded liposomes at 170 bar and 80 µm injection diameter

As shown in **Table 1**, liposomes with mean diameters in the range from 165 ± 26 nm and 199 ± 52 nm with PDI from 0.16 to 0.26 were obtained. In particular, looking at results for different TP/PC % as reported in **Figure 5**, increasing the theoretical loading of TP, the mean diameter, increased from a minimum of 165 ± 26 nm for 5 % w/w TP/PC to a maximum of 199 ± 52 nm for 20 % w/w TP/PC. Also, polydispersion indexes increased from a minimum of 0.16 for the lowest TP/PC ratio

to 0.26 for the highest ratio. Summarizing these results, the increase of operative pressure produced smaller liposomes with a narrow PSD. In this case, there is a double positive effect of pressure and water injector nozzle on vesicles formation. The increase of the pressure produces a significant effect on the decrease of mean diameter and control of PSD. The simultaneous positive effects of pressure increase (Espirito Santo et al., 2015) and of the use of a larger nozzle allowed a better control of water droplets atomization and, as a consequence, of the PSD of liposomes loaded with olive pomace extract (Espirito Santo et al., 2014).

A FE-SEM image of liposomes produced at 170 bar is reported in **Figure 6** and allows to qualitatively compare these vesicles with those produced at lower pressure.

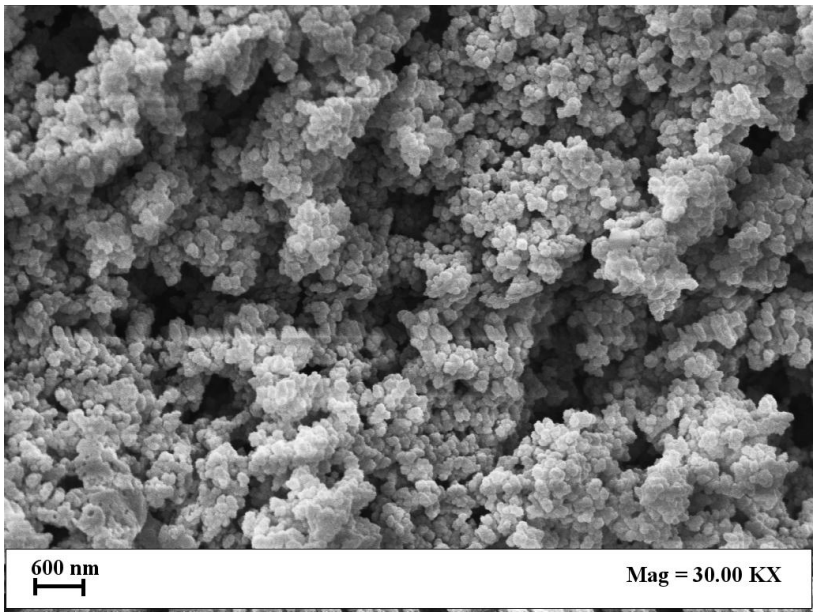

Figure 6 FE-SEM image of liposomes loaded with 5 % mg total polyphenols from olive pomace per mg of phosphatidylcholine (TP/PC), produced at the pressure of 170 bar

FE-SEM image confirms that mean diameter of liposomes is smaller when vesicles are produced at lower pressure.

The samples prepared with SuperLip at the bioactive compounds loading of 15 % w/w were compared with liposomes produced using the thin layer hydration. Olive pomace extract was diluted in water to obtain a theoretical olive pomace loading of 15% with respect to the lipid content. Results are shown in **Table 1**. The general problem, related to the use of a conventional method, is the production of vesicles generally larger than 10 µm if post-processing steps such as sonication or extrusion are not added. In this particular case, the experiment at 15 % TP/PC loaded liposomes resulted in the production of vesicles with a diameter of 50 ± 8 µm with a PDI of 018; i.e. more than 350 times larger than liposomes produced using SuperLip at the same theoretical loading. These, larger liposomes did not maintain their stability over time, due to vesicles aggregation; therefore, mean diameter modified with time and drug leakage was obtained during storage (Nedovic et al., 2011). Optical images of the samples were also obtained. As shown in **Figure 7**, liposomes, produced using thin layer hydration method, are spherical and characterized by a double lipidic layer.

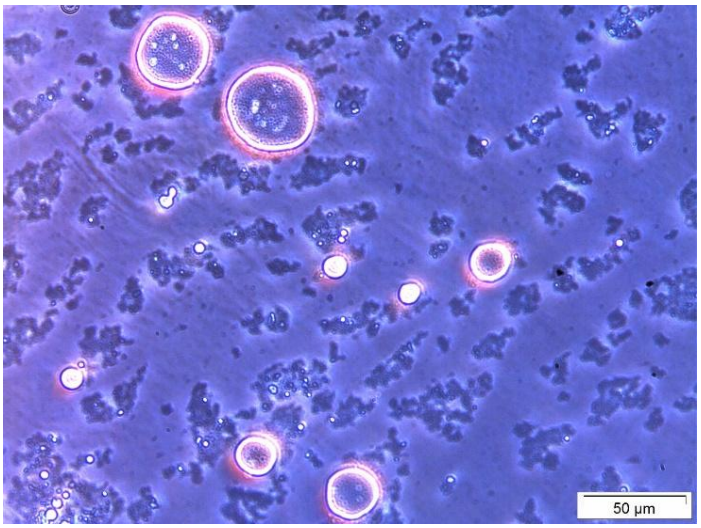

Figure 7 Optical image of liposomes loaded with 15 % mg total polyphenols from olive pomace per mg of phosphatidylcholine (TP/PC), produced with Bangham method

According to the dimension bar of **Figure 7**, the mean size confirmed the dimension indicated in **Table 1.** Looking at **Figure 7**, it is possible to see that material is present in the external bulk of the giant liposomes, implying that the encapsulation efficiency of total polyphenols was not successful.

The total polyphenols concentration entrapped into liposomes as a function of the injector diameter, pressure and the ratio of TP/PC, is listed in **Table 2**.

Table 2 Total polyphenols content measurement of liposomes produced

Injector Diameter [µm]	Pressure [bar]	TP/PC [%, w/w]	Encapsulation Efficiency [%]
60	130	5	44.9
		10	47.9
		15	49.0
		20	50.3
80	130	5	34.2
		10	42.7
		15	56.7
		20	58.1
80	170	5	25.4
		10	40.9
		15	44.9
		20	45.5
Bangham		15	10.1

Liposomes produced using the 60 µm nozzle working at 130 bar showed a slight increase of EE percentage by increasing the ratio of TP/PC. TP encapsulation efficiencies ranged between 44.9 % and 50.3 % for this set of experiments. Using 80 µm nozzle and working at the same pressure, the

trend is confirmed, by increasing the bioactive compounds loading. But, in this case, the range of TP encapsulation efficiency is wider, from a minimum of 34.2 % to a maximum of 58.1 %. Increasing the pressure to 170 bar, TP content entrapped by liposomes also increased from a minimum of 25.4 % to a maximum of 45.5 %. A general effect of pressure or injector diameter on EE was not observed. The only remarkable effect on TP encapsulation efficiency was due to TP/PC total loading. As a final comparison, conventional loaded samples (see **Table 2**) had an EE of 10.1 % of TP, much smaller than vesicles produced directly using SuperLip (Campardelli et al., 2016b).

To perform a vesicles stability study, the mean diameter of 15 % w/w TP/PC loaded vesicles was monitored over a period of 120 days, storing the vesicles at a temperature of 4 °C. Measurements were performed at fixed time intervals using DSL. Results are reported in **Figure 8**.

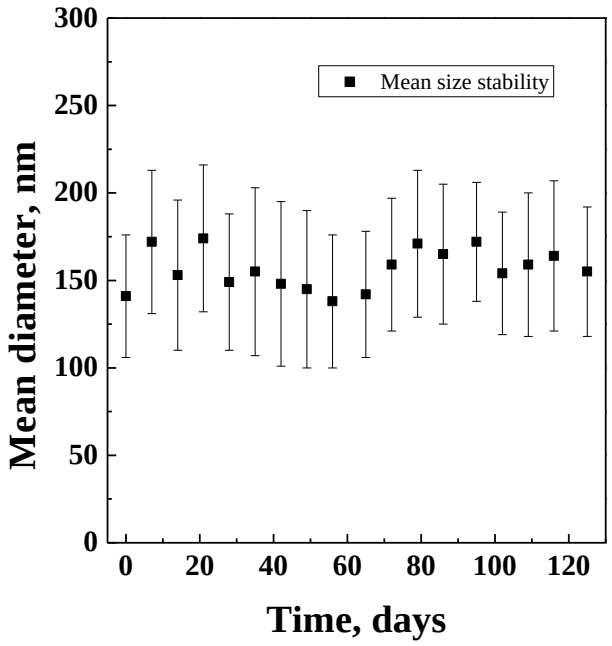

Figure 8 Mean Diameter stability test of liposomes loaded with 15 % w/w TP/PC

The mean diameter of SuperLip produced liposomes remained constant over a period of more than 4 months. Error bars are also reported to describe phenomena of aggregation and disaggregation of lipids, typical of the vesicles stored in an aqueous medium.

Conclusions

In this study it was demonstrated that SuperLip can be successfully used for the production of liposomes entrapping polyphenolic compounds extracted from olive pomace. A good compromise between particle size and encapsulation efficiency was achieved. Liposomes of 265 ± 101 nm mean diameter entrapped up to 58.1 %. The systematic study of SuperLip operative parameters a showed simultaneous effect of a larger nozzle diameter and higher working pressure on the control of PSDs.

A consideration has to be done concerning the encapsulation efficiency: it is not high as generally obtained using SuperLip. The encapsulation of polyphenol contained in olive pomace remains extremely difficult because the natural extract contains a lot of different compounds. However, encapsulation results obtained in this study are largely better than the ones reported in the literature.

Acknowledgements

The authors want to acknowledge Luca Vitale for his help in this work during his bachelor internship period.

References

Adlercreutz, H., Mazur, W., (1997). Phyto-oestrogens and Western diseases. Annals of Medicine 29(2), 95-120.

Aliakbarian, B., Casazza, A.A., Perego, P., (2011). Valorization of olive oil solid waste using high pressure-high temperature reactor. Food Chemistry 128(3), 704-710.

Aliakbarian, B., Palmieri, D., Casazza, A.A., Palombo, D., Perego, P., (2012). Antioxidant activity and biological evaluation of olive pomace extract. Natural Product Research 26(24), 2280-2290.

Bangham, A.D., (1983). Liposomes in Nuce. Biology of the Cell 47(1), 1-9.

Barth, D., Chouchi, D., Della Porta, G., Reverchon, E., Perrut, M., (1994). Desorption of lemon peel oil by supercritical carbon dioxide: deterpenation and psoralens elimination. The Journal of Supercritical Fluids 7(3), 177-183.

Beckman, C.H., (2000). Phenolic-storing cells: keys to programmed cell death and periderm formation in wilt disease resistance and in general defence responses in plants? Physiological and Molecular Plant Pathology 57(3), 101-110.

Biesalski, H.K., (2002). Free radical theory of aging. Current Opinion in Clinical Nutrition and Metabolic Care 5(1), 5-10.

Blanquet, S., Garrait, G., Beyssac, E., Perrier, C., Denis, S., Hebrard, G., Alric, M., (2005). Effects of cryoprotectants on the viability and activity of freeze dried recombinant yeasts as novel oral drug delivery systems assessed by an artificial digestive system. European Journal of Pharmaceutics and Biopharmaceutics 61(1-2), 32-39.

Campardelli, R., Espirito Santo, I., Albuquerque, E.C., Vieira de Melo, S., Della Porta, G., Reverchon, E., (2016a). Efficient encapsulation of proteins in submicro liposomes using a supercritical fluid assisted continuous process. Journal of Supercritical Fluids 107, 163-169.

Campardelli, R., Trucillo, P., Reverchon, E., (2016b). A Supercritical Fluid-Based Process for the Production of Fluorescein-Loaded Liposomes. Industrial & Engineering Chemistry Research 55(18), 5359-5365.

Cevallos-Casals, B.A., Cisneros-Zevallos, L., (2010). Impact of germination on phenolic content and antioxidant activity of 13 edible seed species. Food Chemistry 119(4), 1485-1490.

Daglia, M., (2012). Polyphenols as antimicrobial agents. Current Opinion in Biotechnology 23(2), 174-181.

De Leonardis, A., Angelico, R., Macciola, V., Ceglie, A., (2013). Effects of polyphenol enzymatic-oxidation on the oxidative stability of virgin olive oil. Food Research International 54(2), 2001-2007.

De Marco, I., Iannone, R., Miranda, S., Riemma, S., (2017). An environmental study on starch aerogel for drug delivery applications: effect of plant scale-up. The International Journal of Life Cycle Assessment, 1-12.

Deladino, L., Anbinder, P.S., Navarro, A.S., Martino, M.N., (2007). Co-crystallization of yerba mate extract (Ilex paraguariensis) and mineral salts within a sucrose matrix. Journal of Food Engineering 80(2), 573-580.

Desai, K.G.H., Park, H.J., (2005). Recent developments in microencapsulation of food ingredients. Drying Technology 23(7), 1361-1394.

Dragan, S., Andrica, F., Serban, M.C., Timar, R., (2015). Polyphenols-Rich Natural Products for Treatment of Diabetes. Current Medicinal Chemistry 22(1), 14-22.

Eichhorn, U., Helbig, B., Klocking, R., Schweizer, H., Sprossig, M., (1985). Comparison of the Antiviral Activity of Enzymatically and Non-Enzymatically Produced Phenol Substance Polymerides (Pkp) by Means of Cr-51 Release Test in Coxsackie-Virus A9-Infected Fl-Cells. Pharmazie 40(4), 282-282.

Espirito Santo, I., Campardelli, R., Albuquerque, E.C., Vieira de Melo, S., Della Porta, G., Reverchon, E., (2014). Liposomes preparation using a supercritical fluid assisted continuous process. Chemical Engineering Journal 249, 153-159.

Espirito Santo, I., Campardelli, R., Cabral Albuquerque, E., Vieira De Melo, S.A.B., Reverchon, E., Della Porta, G., (2015). Liposomes Size Engineering by Combination of Ethanol Injection and Supercritical Processing. Journal of Pharmaceutical Sciences 104(11), 3842-3850.

Fang, Z.X., Bhandari, B., (2010). Encapsulation of polyphenols - a review. Trends in Food Science & Technology 21(10), 510-523.

Gellerstedt, G., Petterson, E. L., (1975). Light-induced Oxidation of The Behaviour of Structural Units Containing a Ring-conjugated Double Bond. Acta Chem. Scand. B 29(10), 1005-1010.

Gouin, S., (2004). Microencapsulation: industrial appraisal of existing technologies and trends. Trends in Food Science & Technology 15(7-8), 330-347.
Hagiwara, K., Goto, T., Araki, M., Miyazaki, H., Hagiwara, H., (2011). Olive polyphenol hydroxytyrosol prevents bone loss. European Journal of Pharmacology 662(1-3), 78-84.
Harman, D., (2006). Free Radical Theory of Aging: An Update Increasing the Functional Life Span. Understanding and Modulating Aging 1067, 10-21.
Likhitwitayawuid, K., (2008). Stilbenes with tyrosinase inhibitory activity. Current Science 94(1), 44-52.
Manach, C., Scalbert, A., Morand, C., Remesy, C., Jimenez, L., (2004). Polyphenols: food sources and bioavailability. American Journal of Clinical Nutrition 79(5), 727-747.
Mantovani, A., Allavena, P., Sica, A., Balkwill, F., (2008). Cancer-related inflammation. Nature 454(7203), 436-444.
Nakayama, T., Kajiya, K., Kumazawa, S., (2006). Interaction of plant polyphenols with liposomes. Advances in Planar Lipid Bilayers and Liposomes 4, 107-133.
Nedovic, V., Kalusevic, A., Manojlovic, V., Levic, S., Bugarski, B., (2011). An overview of encapsulation technologies for food applications. 11th International Congress on Engineering and Food (Icef11) 1, 1806-1815.
Otake, K., Shimomura, T., Goto, T., Imura, T., Furuya, T., Yoda, S., Takebayashi, Y., Sakai, H., Abe, M., (2006). Preparation of liposomes using an improved supercritical reverse phase evaporation method. Langmuir 22(6), 2543-2550.
Paini, M., Aliakbarian, B., Casazza, A.A., Lagazzo, A., Botter, R., Perego, P., (2015a). Microencapsulation of phenolic compounds from olive pomace using spray drying: A study of operative parameters. Lwt-Food Science and Technology 62(1), 177-186.
Paini, M., Aliakbarian, B., Casazza, A.A., Perego, P., Ruggiero, C., Pastorino, L., (2015b). Chitosan/dextran multilayer microcapsules for polyphenol co-delivery. Materials Science & Engineering C-Materials for Biological Applications 46, 374-380.
Paini, M., Daly, S.R., Aliakbarian, B., Fathi, A., Tehrany, E.A., Perego, P., Dehghani, F., Valtchev, P., (2015c). An efficient liposome based method for antioxidants encapsulation. Colloids and Surfaces B-Biointerfaces 136, 1067-1072.
Palmieri, D., Aliakbarian, B., Casazza, A.A., Ferrari, N., Spinella, G., Pane, B., Cafueri, G., Perego, P., Palombo, D., (2012). Effects of polyphenol extract from olive pomace on anoxia-induced endothelial dysfunction. Microvascular Research 83(3), 281-289.
Pandey, K.B., Rizvi, S.I., (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity 2(5), 270-278.
Papuc, C., Criste, R., Durdun, N., Untea, A., Nicorescu, V., (2010). The Effect of Some Mineral and Phytogenic Additives Rich in Polyphenols on Lipid Peroxidation Process. Revista De Chimie 61(10), 920-924.
Reverchon, E., Ambruosi, A., Senatore, F., (1994). Isolation of peppermint oil using supercritical CO2 extraction. Flavour and Fragrance Journal 9(1), 19-23.
Sancho, M., Mach, N., (2015). Effects of Wine Polyphenols on Cancer Prevention. Nutricion Hospitalaria 31(2), 535-551.
Sauvage, F.X., Bach, B., Moutounet, M., Vernhet, A., (2010). Proteins in white wines: Thermo-sensitivity and differential adsorbtion by bentonite. Food Chemistry 118(1), 26-34.
Scalbert, A., Mazur, A., (2002). Dietary polyphenols and the prevention of cardiovascular diseases - State of the art. Cardiovascular Dieseases 2002, 351-357.
Scarmeas, N., Stern, Y., Tang, M.X., Mayeux, R., Luchsinger, J.A., (2006). Mediterranean diet and risk for Alzheimer's disease. Annals of Neurology 59(6), 912-921.
Solayman, M., Ali, Y., Alam, F., Islam, M.A., Alam, N., Khalil, M.I., Gan, S.H., (2016). Polyphenols: Potential Future Arsenals in the Treatment of Diabetes. Current Pharmaceutical Design 22(5), 549-565.
Swain, T., Hillis, W. E., (1959). The phenolic constituents of Prunus domestica. The quantitative analysis of phenolic constituents. Journal of Science of Food and Agriculture 10, 63-68.
Taamalli, A., Arraez-Roman, D., Zarrouk, M., Valverde, J., Segura-Carretero, A., Fernandez-Gutierrez, A., (2012). The Occurrence and Bioactivity of Polyphenols in Tunisian Olive Products and by-Products: A Review. Journal of Food Science 77(4), R83-R92.

Terahara, N., (2015). Flavonoids in Foods: A Review. Natural Product Communications 10(3), 521-528.
Trucillo, P., Campardelli, R., Reverchon, E., (2017). Encapsulation of Hydrophilic and Lipophilic Compounds in Nanosomes Produced with a Supercritical Based Process, *Advances in Bionanomaterials: Selected Papers from the 2nd Workshop in Bionanomaterials, BIONAM 2016, October 4-7, 2016, Salerno, Italy*. Springer, p. 23.
Trucillo, P., Campardelli, R., Reverchon, E., (2017). Supercritical assisted liposomes formation: optimization of the lipidic layer for an efficient hydrophilic drug loading. Journal of CO2 Utilization.
Vinayagam, R., Jayachandran, M., Xu, B.J., (2016). Antidiabetic Effects of Simple Phenolic Acids: A Comprehensive Review. Phytotherapy Research 30(2), 184-199.
Vissers, M.N., Zock, P.L., Katan, M.B., (2004). Bioavailability and antioxidant effects of olive oil phenols in humans: a review. European Journal of Clinical Nutrition 58(6), 955-965.
Volf, I., Ignat, I., Neamtu, M., Popa, V.I., (2014). Thermal stability, antioxidant activity, and photo-oxidation of natural polyphenols. Chemical Papers 68(1), 121-129.
Williamson, G., Manach, C., (2005). Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. American Journal of Clinical Nutrition 81(1), 243s-255s.
Wink, M., (1997). Compartmentation of secondary metabolites and xenobiotics in plant vacuoles. Advances in Botanical Research Incorporating Advances in Plant Pathology, Vol 25 25, 141-169.