EFFICIENT PRODUCTION OF SOY-BEAN LECITHIN – PLURONIC L64® ENCAPSULATED QUERCETIN PARTICLES IN NANOMETRIC SCALE USING SFEE AND PGSS DRYING PROCESSES

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Quercetin is an antioxidant compound, and it is a highly promising material against a wide variety of diseases, including cancer. A major limitation for the clinical application of quercetin is its low bioavailability, due to its low solubility in water. One way to increase the bioavailability of quercetin is to precipitate it in sub-micrometric scale, encapsulated by a surfactant material, using Supercritical Fluid Extraction of Emulsions (SFEE) and/or Particles from Gas Saturated Solutions (PGSS) drying technology. In this work the efficiency of SFEE- and PGSS drying processes, in producing of quercetin loaded soy-bean lecithin – Pluronic L64® particles in sub-micrometric scale is studied. Robustness study of a batch SFEE process is done, moreover a scaled-up, semi-continuous SFEE process is developed, in order to increase the efficiency of the process, and to decrease the energy consumption. SFEE produced aqueous suspensions are further treated by PGSS drying and by lyophilization, in order to produce solid encapsulated quercetin particles, which are available for long term storage. Encapsulation efficiency and antioxidant activity of with PGSS drying and with lyophilization prepared dried products are measured and compared with each other.

INTRODUCTION

Quercetin is a bioflavonoid and it occurs in various fruits, vegetables and oils. According to preliminary studies, it has strong antioxidant-, antiviral-, antibacterial-, antihistaminic-, and anti-inflammatory effect. Due to these properties, quercetin is a highly promising material against a wide variety of diseases, including cancer. A major limitation for the clinical application of quercetin is its low bioavailability, that makes it necessary to administrate in high doses (50 mg/kg) [1]. One way to increase the bioavailability of quercetin is to produce encapsulated quercetin particles in nanometric scale, SFEE and/or PGSS drying technology.

SFEE technology is used for producing aqueous suspensions, containing encapsulated valuable material (drug, bioactive compound etc.) in sub-micrometric scale. In SFEE process, an initially prepared oil in water (o/w) emulsion (Fig 2 A) (material of interest is dissolved in the organic phase) is contacted with a supercritical fluid, in order to extract the organic phase from the emulsion. Due to the rapid supersaturation of the dissolution medium by the active compound, it is precipitating in sub-micrometric scale. Supercritical fluid must be chosen to have high affinity for the organic solvent, meanwhile negligible affinity for the active compound. Principles of SFEE technology is shown on Fig 1.

[1]C. Somsuta, S. Sami, D. Nirmalendu, T. C. Somsubhra, G. Swarpupa, S. Snehasitka, The use of nano-quercetin to arrest mitochondrial damage and MMP-9 upregulation during prevention of gastric inflammation induced by ethanol in rat, Biomaterials Vol. 33, p. 2991, 2012

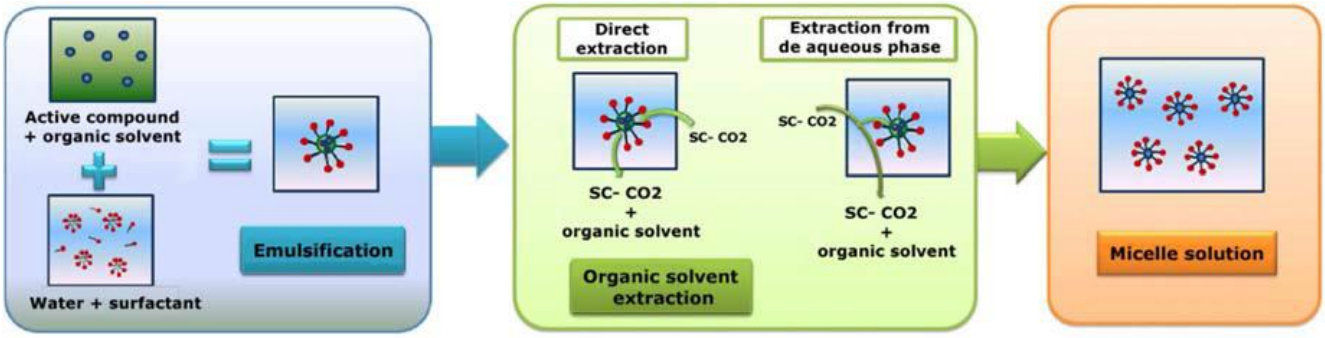

Fig 1: Working methodology of supercritical fluid extraction of emulsion (SFEE) technology

MATERIAL AND METHODS

In this work SFEE, PGSS drying and lyophilization techniques were used to produce quercetin loaded lecithin – Pluronic L64® particles in sub-micrometric scale, and antioxidant activity, quercetin encapsulation efficiency in products were studied. Particle size distribution of SFEE produced aqueous suspensions was measured by light scattering, encapsulated quercetin content of SFEE produced aqueous suspensions, and with PGSS drying and with lyophilization prepared dried products, was analysed by HPLC – UV – VIS. Moreover antioxidant activity of aqueous suspension and of dried products was measured by Oxygen radical absorbance capacity (ORAC) measurement. Remained organic solvent concentration in SFEE produced aqueous suspensions were measured by Head Space Gas Chromatography.

Initially, SFEE experiments were carried out in a batch apparatus – presented in Fig 3 –, consisting of an extractor vessel with a volume of 85 mL, and a buffer vessel with a volume of 100 mL. The vessels are located in a tempered oven, and they are separable from each other by two valves. Closing these valves, the $scCO_2$ content of the buffer vessel is renewable, without any pressure or temperature change in the extractor vessel. According to preliminary experiments, five cycles proved to be optimum by decreasing the residual organic content under the restriction of the FDA [2], without significant degradation or agglomeration effect of the encapsulated quercetin particles. Between two cycles, approximately 60 V/V% of the $scCO_2$ content of the whole system was changed, by increasing this way the organic solvent capacity of the $scCO_2$ from cycle to cycle, as described in previous work [3].

RESULTS

Applying SFEE technology as a batch process, a multivesicular soy-ben lecithin system is obtained, containing the encapsulated quercetin in sub-micrometric scale (Fig 2 B). We managed to increase the concentration of encapsulated quercetin up to 310 mg/L, equal with an encapsulation efficiency of 90%, and a concentration significantly higher than the solubility of quercetin in pure water: 0.01 g/L [4]. Encapsulated quercetin is stable up to two weeks, with a mean diameter size of vesicles around 100 nm, and with a residual organic content less than 350 ppm. According to experimental plan results, no significant factors were found regarding vesicular size distribution and quercetin encapsulation efficiency, which means, using SFEE as a batch process, robust, repeatable results can be obtained.

[2] Guidance for Industry Q3C — Tables and List, U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER), ICH, Revision 2, 2012
[3] Lévai Gy. Martín Á., Paz E., Rodríguez R. S., Cocero M. J., Production of stabilized quercetin aqueous suspensions bysupercritical fluid extraction of emulsions, Journal of Supercritical Fluids 100, p.:34-45, 2015
[4] L. Chebil, C. Humeau, J. Anthoni, F. Dehez, J. M. Engasser, M. Ghoul, Solubility of flavonoids in organic solvents. J. Chem. Eng. Data 52, p. 1552– 1556, 2007

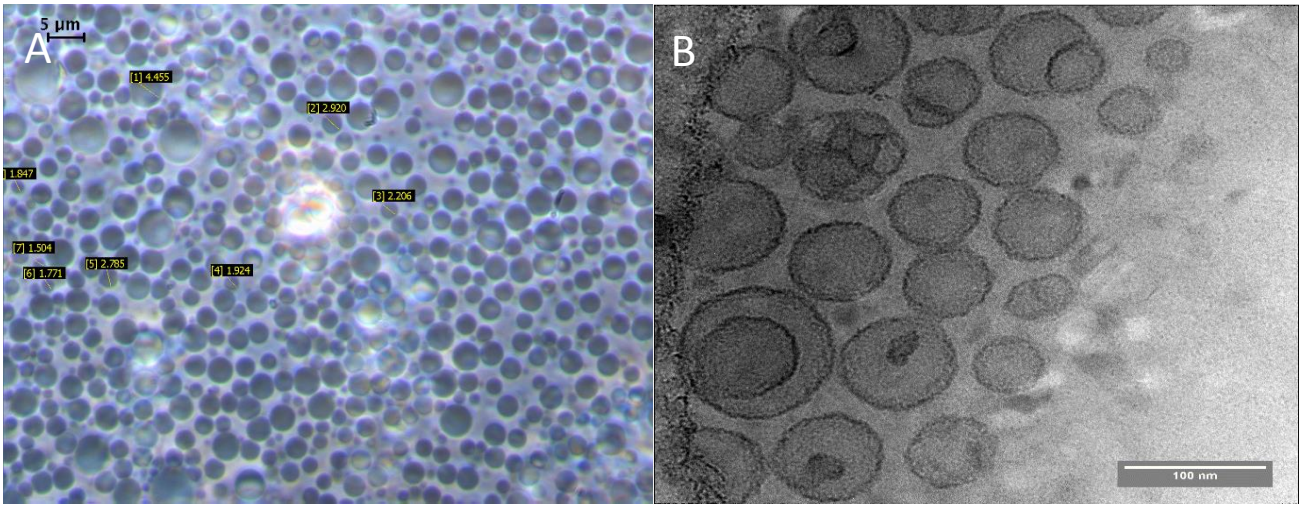

Fig 2: A: Optical microscopy picture of the initial o/w emulsion: prepared with EtAc – water – quercetin – lecithin; B:TEM picture of the SFEE produced aqueous suspension

As robustness of batch SFEE process was proved, a scaled-up, semi-continuous SFEE process was designed (Fig 4), in order to extract the organic solvent from o/w emulsion using continuous $scCO_2$ flow. Scaled-up SFEE equipment contains only an increased dimensions, electrically tempered extractor vessel with a micrometric valve on its output, in order to maintain a controlled continuous $scCO_2$ flow. This process is only semi-continuous, due to emulsion flow is not taking place during the process, only the extracted organic part is moving out with the $scCO_2$ from the extractor vessel.

Semi-continuous SFEE process is much faster than batch SFEE process, and due to shorter processing time, degradation effect of quercetin is decreased. Moreover by using semi-continuous SFEE process, energy consumption is efficiently decreased, as comparing to batch SFEE process, an around six times higher volume of aqueous suspension is produced, with an around six times shorter process duration. Using the scaled-up, semi-continuous SFEE process, – similarly to the batch SFEE process –, a multivesicular aqueous product is obtained, with a main particle size distribution around 100 nm, and a quercetin encapsulation efficiency above 80% in all experimental runs. Residual organic content was below 400 ppm in all products, obtained by scaled up SFEE equipment.

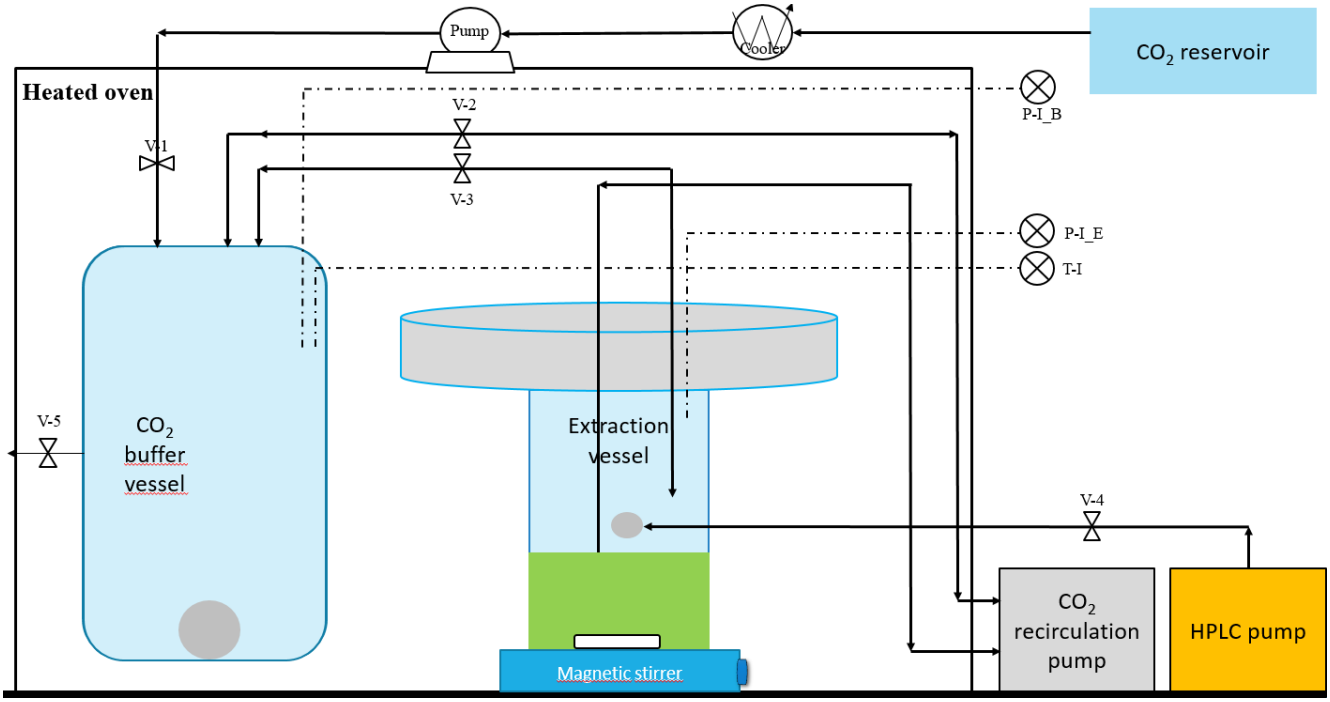

Fig 3: SFEE batch process

SFEE process obtained aqueous suspensions were treated forward by PGSS drying, in order to obtain solid product, by extracting the water content of them. Using PGSS drying method, products become available for long-term storage, without significant degradation effect of quercetin, which is rapidly taking place in aqueous medium [5]. Moreover solid product could be easily convertible back to aqueous suspension by adding water to it. Best process conditions in terms of less residual water content and high efficiency of quercetin encapsulation during PGSS drying process were studied already in a previous work [6], and only obtained best settings were used in all experiments in this work. By PGSS drying, residual water content was decreased below 8 w/w%, meanwhile degradation of quercetin was less than 30%, with an average quercetin encapsulation efficiency of 8.3 mg quercetin / g of sample. A part of aqueous suspensions was treated by lyophilisation instead of PGSS drying, in order to compare quercetin degradation during the processes, and antioxidant activity the solid products obtained by lyophilisation and by PGSS drying. Residual quercetin concentration and antioxidant activity value do not show significant deviation between the two processes, means PGSS drying is an efficient method to produce solid quercetin particles in nanometric scale, encapsulated and protected by soy-bean lecithin as can be seen on Fig 5.

[5] Althans D., Schrader P., Enders S., Solubilisation of quercetin: Comparison of hyperbranched polymer and hydrogel, Journal of Molecular Liquids 196, p.: 86–93, 2014

[6]Varona S., Martín A., Cocero M., Liposomal Incorporation of Lavandin Essential Oil by a Thin-Film Hydration Method and by Particles from Gas-Saturated Solutions, Industrial & Engineering Chemistry 50, p.: 2088–2097, 2011

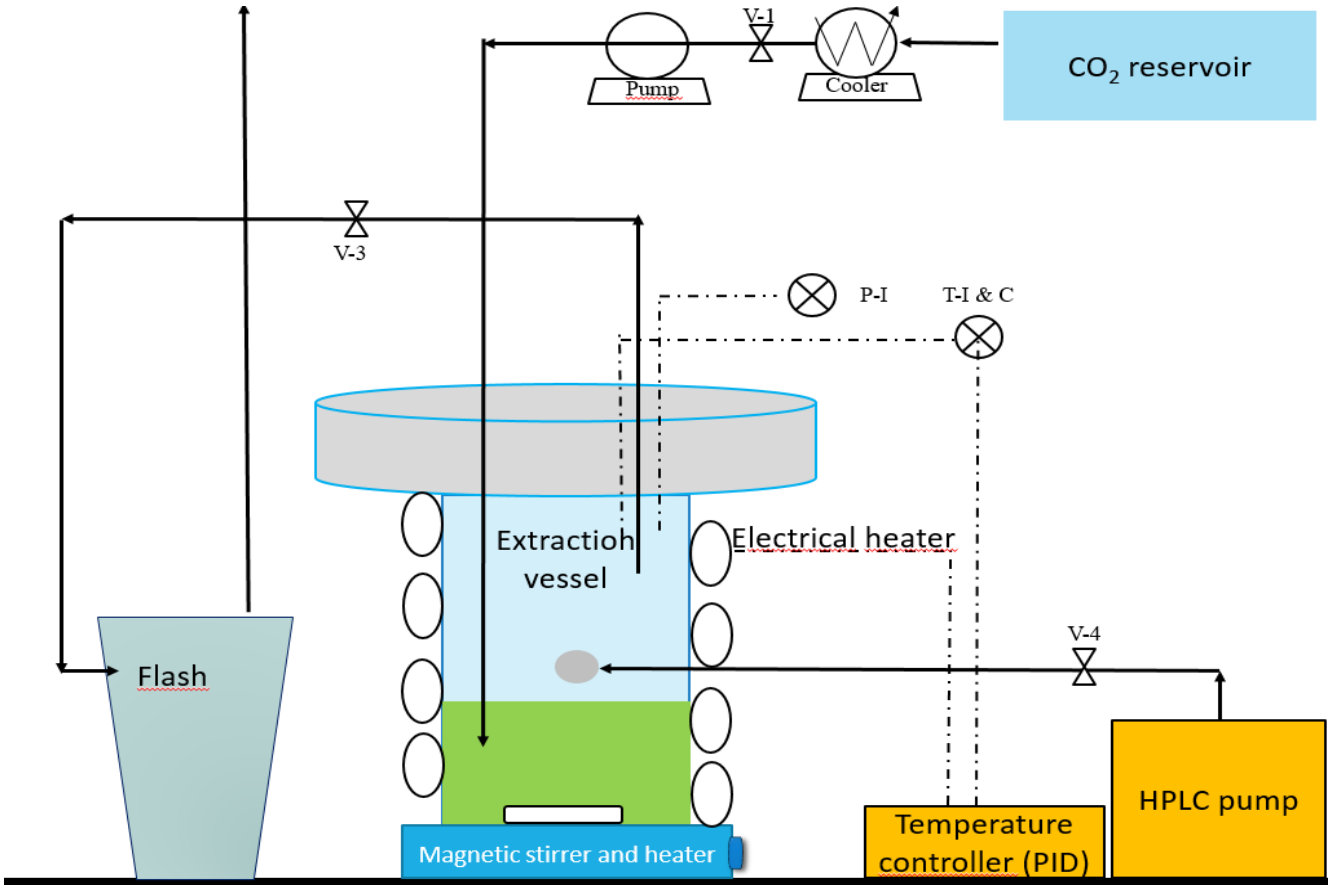

Fig 4: Scaled-up SFEE semi-continuous process

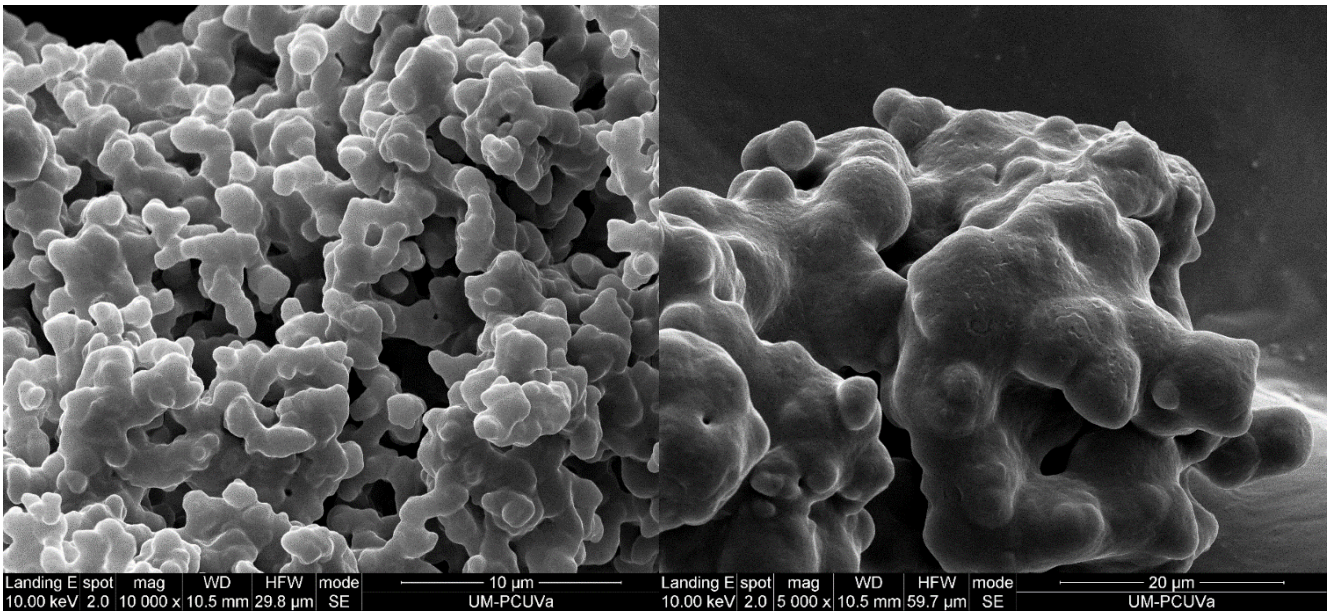

Fig 5: SEM picture about solid lecithin – Pluronic L64® encapsulated quercetin particles obtained by PGSS drying

CONCLUSION

In this work with soy-bean lecithin – Pluronic L64® encapsulated quercetin particles were produced in nanometric scale, using SFEE and PGSS drying processes. Using SFEE technology, multivesicular aqueous suspensions were obtained, which were treated forward by PGSS drying, in order to extract water, and increase long-term stability of product. Moreover PGSS dried solid product is easily convertible back to aqueous suspension, by adding water to it. A scaled up, semi-continuous SFEE process is successfully developed, in order to increase efficiency-, and hence decrease energy consumption of process. Robustness of scaled-up process is proved, in all experimental runs minimum 80% of the initially added quercetin was stably encapsulated in the obtained aqueous suspensions, with a residual organic content less than 400 ppm. Antioxidant activity and quercetin degradation in case of with PGSS drying prepared solid products were as less as in case of with lyophilization prepared solid products.