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Optimization of a method for preparing solid complexes of essential clove oil with β-cyclodextrins

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ABSTRACT

BACKGROUND

Clove oil (CO) is and aromatic oily liquid used in food, cosmetic and pharmaceutical industries due to their functional properties. However its disadvantages as pungent taste, volatility, light sensitivity and poor water solubility can be solved by applying microencapsulation or complexation techniques.

RESULTS

Essential CO was successfully solubilized in aqueous solution by forming inclusion complexes with β-cyclodextrins (β-CDs). Moreover, phase solubility studies demonstrated that essential CO also forms insoluble complexes with β-CDs. Based on these results, essential CO-β-CD solid complexes were prepared by the novel approach of microwave irradiation (MWI) followed by three different drying methods: vacuum oven drying (VO), freeze drying (FD) or spray drying (SD). Quantification of the solid complexes formed pointed to the treatment not involving heat, FD, as the best drying method, followed by VO and SD, which led to significantly lower amounts of encapsulated essential CO.

CONCLUSION.

MWI can be used efficiently to prepare essential CO-β-CDs complexes with good yields on an industrial scale.

Keywords

Complexation. Cyclodextrin. Essential clove oil. Eugenol. Spray drying. Freeze drying.

INTRODUCTION

Essentials oils (EOs), also called volatile or ethereal oils, are aromatic oily liquids obtained from plant material (flower, bud, seeds, leaves, twigs, bark, herbs, wood, fruit and roots). [1] The greatest use of EOs is in food (as flavourings), perfumes (fragrances) and pharmaceuticals (due to their functional properties). [2] Individual components of EOs, either extracted from plant material or synthetically manufactured, are also used as food flavourings. [3]

Essential clove oil (CO) (*Eugenia caryophyllata,* Myrtaceae) has received attention as an ideal fish anesthetic [4-6] as fragrant and flavouring agent in a variety of cosmetic products and food [7] as flavor ingredient replacing mustard in classical formulation of mayonnaise, [8] in meat protection. [9-12] The properties of essential CO are mainly due to its principal component eugenol (EG) (4-allyl-2-methoxyphenol). This phenolic compound has demonstrated several biological activities as an anti-inflammatory agent by inhibiting the enzyme ciclooxygenase II, [13] as an analgesic due to its selective binding at the capsaicin receptor, [14] and as an anti-oxidant [15] and anti-bacterial agent against both gram positive and gram negative microorganisms. [16,17]

However, irritation towards the mucosa and skin, its pungent taste, volatility, light sensitivity and poor water solubility, hinder the use of essential CO and EG in industry, problems that can be solved by applying microencapsulation or complexation techniques.

The complexation of volatile compounds with β-CDs has been used as a technique to protect them against oxidation, heat and light degradation, evaporation and moisture. Such protection is possible because the flavor molecules are tightly held within the hydrophobic cavity of β-CDs. [18]

The complexation of flavor molecules by β-CDs can be achieved in various ways. CDs and flavors can be stirred in aqueous solution, a method that has been applied to the complexation of aromatic compounds such as *d*-limonene, eugenol and *Menta x Villosa*. [19-21] Complexation can also be achieved by bubbling the flavors in vapor form through a solution of CDs, or mixing with a CDs paste. [18] The co-precipitation method has been used with garlic oil, *Menta x Villosa* and cinnamon leaf oil. [21,22] Bhandari and col. [23,24] compared several methods for complexating essential lemon oil with β-CDs, namely ethanol precipitation and kneading to form a paste, followed by spray or vacuum-drying. The selection of the most appropriate method depends on several factors, including yield, rapidity, simplicity of scaling up, low cost and characteristics of the final product. [25]

Microwave irradiation (MWI) is one method that could bypass the disadvantages associated with traditional complexation techniques, resulting in shorter reaction times and higher yields. [26,27] The main advantage of MWI compared with traditional methods is the absence of residues derived from the use of large volumes of organic solvents. Complexation with CDs using MWI irradiation has proved effective in improving the solubility of poorly soluble drugs. [28,29] In the pharmaceutical industry, MWI has been used because of its thermal effect, shortening the length of the drying process (granules or crystals), and also for sterilising sanitary tools. [30,31]

One of the main advantages of the using CDs for flavour microencapsulation is the possibility to obtain complexes in dry powder form, which makes their industrial manipulation easier. This kind of complexation involves the drying of solid complexes after their preparation, for which purpose several different drying methods can be used. Among these, spray drying is a very fast drying method, although it presents certain disadvantages, such as the high processing temperature involved (about 200 ºC, which

100 can cause the loss of volatile compounds) and the fact that it is limited to water soluble
101 matrices. The use of vacuum oven drying means that a lower temperature can be used
102 than in spray drying, but the exposure time is increased.

103 Freeze drying has been demonstrated to be a useful method for improving the
104 shelf life of dehydrated products. As the name suggests, drying is carried at low
105 temperature and the absence of air prevents or minimizes product deterioration in the
106 form of decomposition, or changes in the structure, texture, appearance and flavor as a
107 result of oxidation or chemical modifications.

108 Many studies have focused on the complexation of essential CO, but none has
109 considered the effect of the drying method on the final quantity and properties of the
110 solid complexes obtained. Each drying method offers advantages and disadvantages that
111 should be taken into account due to the influence on the quantity of essential CO finally
112 retained.

113 The aim of the present work was to optimize a method for preparing solid
114 essential CO-β-CDs complexes. For this purpose, two studies were performed: a
115 comparison of the use of ultrasound and MWI as energy source for essential CO-β-CDs
116 complexes formation, and the influence of the drying method used on the final essential
117 clove oil concentration: Vacuum oven drying (VO), spray drying (SD) and freeze
118 drying (FD).

119

120 **EXPERIMENTALS**

121 **Materials**

122 β-CDs were purchased from TCI Europe NV (Zwijndrecht, Belgium). Essential
123 CO was kindly supplied by Lidervet, SA (Tarragona, Spain). EG was obtained from

Sigma-Aldrich Química SL (Tres Cantos, Madrid, Spain). All the other chemicals used were of analytical grade.

Preparation of complexes of essential CO with β-CDs

Preparation of essential CO-β-CDs complexes involves the addition of an excess of essential CO (0.01g) to 70 mL of β-CDs solutions (0, 13, 30, 50, 75 or 100 mmol L^{-1}). Two methods with different energy sources (ultrasound or MWI) were compared. In both cases, soluble and solid essential-CO-β-CDs complexes were obtained.

Ultrasound Method (U)

Increasing β-CDs solutions (70 mL from 0 to 100 mmol L^{-1}) were kept at 50 ºC in an ultrasound bath for 2 hours. After that, an excess of essential CO was added to the suspension. Again, samples were kept at 50 ºC in an ultrasound bath (P-Selecta Ultrasounds, Barcelona, Spain) for 2 hour for the CO and β-CD complexation process to be completed. At this point, samples were divided in two groups. The first one was centrifuged at 14,800 g at 25 ºC for 60 min at 25 ºC in a centrifuge Heraeus Biofuge Stratos (Hanau, Germany) to separate the solid complexes (1 cycle of ultrasound: 1C-U). The second group was kept overnight in sealed vials to repeat the ultrasound process 12 hours later before centrifugation at 14,800 g at 25 ºC for 60 min (2 cycles of ultrasound: 2C-U).

Centrifugation divided samples into two phases: (i) the supernatant phase, containing free dissolved essential CO, soluble essential CO-β-CDs complexes and the excess of non-complexed, undissolved essential CO and (ii) the pellet, containing solid essential CO- β-CDs complexes and non-dissolved β-CDs

148 The supernatants were filtered through 0.2 μm nylon membrane filter to remove
149 the excess of non-complexed undissolved essential CO, and the dissolved essential CO
150 and soluble essential CO complexes were obtained from the filtrate. To quantify the
151 total essential CO present in the filtrate the samples were diluted in 80% ethanol and
152 analyzed by GC-MS.
153 The solid complexes formed retained in the nylon membrane filter were dried by
154 vacuum oven (Fistreem International Limited, Leicestershire, United Kingdom) at 40
155 ºC. Dry solid complexes were dissolved in 100% ethanol and analyzed by GC-MS.
156
157 **Microwave irradiation method (MWI)**
158 Solid essential CO-β-CDs complexes were formed using MWI as energy source
159 as described by Souto, [32] with some modifications. Solutions of β-CDs (70 mL, from 0
160 to 100 mmol L^{-1}) were irradiated in a microwave oven (LG Grill Wavedom, LG
161 Electronics España, Las Rozas, Madrid, Spain) at 700 W for 30 s at 10 s intervals to
162 reach 70 ºC. This process increases the aqueous solubility of β-CDs and facilitates
163 essential CO complexation. An excess of essential CO was added to each β-CDs
164 solutions, which were again irradiated for 30 s at 10 s intervals to reach 70 ºC. Then, the
165 samples were stirred and kept overnight in sealed vials in darkness at 25 ºC before being
166 divided in two groups. The first one was centrifuged at 14,800 g at 25 ºC for 60 min (1
167 cycle of microwave, 1C-MWI), while the second group was subjected to the same
168 process 12 hours later (MWI up to 70 ºC, 12 h in darkness and centrifugation) (2 cycles
169 of microwave, 2C-MWI).
170 The supernatants were filtered through 0.2 μm nylon membrane filter to remove
171 the excess of non-complexed undissolved essential CO, and the dissolved essential CO
172 and soluble essential CO complexes were obtained from the filtrate. To quantify the

173 total essential CO present in the filtrate the samples were diluted in 80% ethanol and
174 analyzed by GC-MS.
175 The solid complexes formed retained in the nylon membrane filter were dried by
176 vacuum oven at 40 ºC. Dry solid complexes were dissolved in 100% ethanol and
177 analyzed by GC-MS.
178
179 **Methods for drying the solid essential CO-β-CDS complexes**
180 To evaluate the effect of the drying method on the CO concentration in the solid
181 complexes obtained, three different methods were assayed: vacuum oven drying (VO),
182 spray drying (SD) and freeze drying (FD).
183 **Vacuum Oven (VO)**. Solid complexes were kept in a vacuum oven (Fistreem
184 International Limited, Leicestershire, United Kingdom) at 40 ºC until a constant mass.
185 The recovered powder was stored in an airtight glass container prior to analysis.
186 **Freeze Drying (FD).** The precipitated material obtained by vacuum filtration was
187 frozen at -80 ºC for 3 hours. Later, samples were placed in a Christ Alpha 1-2 LD Plus
188 freeze dryer (Osterode am Harz, Germany). During the drying process, the ice
189 condenser was set at -50 ºC for 3 hours and the pressure was held at around 0.1 mbar.
190 Freeze dried powder was stored in an airtight glass container prior to analysis.
191 **Spray Drying (SD).** To obtain dried solid complexes by this method, precipitates
192 obtained after centrifugation were not subjected to vacuum filtration. Instead, they were
193 suspended in water and fed through a Buchi B-290 spray dryer (Flawil, Switzerland).
194 The operational conditions of the spray drier were as follows: inlet air temperature 140
195 ºC, outlet air temperature 60 ºC, rotational speed of atomizer 30,000 rpm. The recovered
196 powder was stored in an airtight glass container prior to analysis.
197

Quantification of essential CO by GC-MS analysis

The quantification of essential CO was carried out on the basis on its main compound, Eugenol (EG). To obtain the signal for the analyte in the mass spectrometer, a control sample of essential CO was spiked. The main compound of essential CO is EG, [33] which was used to prepare a calibration curve (Figure 1). Three replications were made for each measurement and the standard error obtained was not higher than 5 %.

The GC used was a Shimadzu GC-QP 2010 (Kyoto, Japan) coupled to a mass spectrometer. Helium was used as carrier gas at an average flow rate of 0.5 mL min^{-1}. The capillary column was a ω-WAX 250 fused silica supelco (30 m x 0.25 mm x 0.25 µm thickness). For individual analyte identification and quantification, the temperature was as follows: 3 min at 40 ºC, raised to 47 ºC at 2ºC min^{-1}, held at 47 ºC for 2 min, raised to 52 ºC at 2 ºC min^{-1}, from 52 ºC to 110 ºC at 5 ºC min^{-1}, ramped at 25 ºC min^{-1} up to 200ºC and maintained finally at 200 ºC for 5 min. The peak area of each sample was used for essential CO quantification.

Field Emission Scanning Electron Microscope (FESEM) images

Uncoated samples were examined under Field Emission Scanning Electron Microscopy (FESEM) using MERLIN™ VP COMPACT (Carl Zeiss Microscopy SL, Germany). Images detailing morphology were taken using an SE2 detector under an accelerating voltage of 1 kV.

Statistical analysis

Data were analysed by using the statistical analysis software SPSS (v.21). Values represent means of triplicate determinations and error bars in figures represent standard deviation.

RESULTS AND DISCUSSION

Effect of encapsulation method on essential CO and β-CDs complex formation

Figure 2 shows the effect of the encapsulation method (U or MWI) on the total essential CO retained in soluble complexes, expressed as eugenol concentration. Encapsulation was significantly more effective when MWI was used as energy source rather than ultrasound. The differences between both methods were significant above a β-CDs concentration of 20 mmol L^{-1}, and continued to increase as the β-CDs concentration increased.

The maximum essential CO concentration encapsulated with one cycle of ultrasound (1C-U, Fig. 2, □) was 5 mmol L^{-1} with a β-CDs concentration above 13 mmol L^{-1}, at which point saturation could be observed while further addition of β-CDs did not improve the encapsulation of essential CO in the form of soluble complexes.

The application of one cycle of MWI (1C-MWI) yielded to encapsulate a maximum of 16 mmol L^{-1} of essential CO (Fig. 2, ○). This represented an increase of 200 % with respect to essential CO encapsulated with one cycle of ultrasound (Fig. 2, □). Even though encapsulation of essential CO was maximal at the maximum β-CDs concentration used (100 mmol L^{-1}), concentrations above 40 mmol L^{-1} β-CDs did not produce any marked improvement in encapsulation.

The influence of the number of cycles on essential CO complexation was also shown in Figure 2. In both methods, the application of 2 energy cycles increased the amount of encapsulated essential CO in soluble complexes, reaching maximum values of 12.5 and 33 mmol L^{-1}, respectively, of essential CO for ultrasounds (Fig. 2, ■) and MWI (Fig. 2, ●), respectively. When 2 cycles by using MWI were applied, the essential CO concentration increased linearly until 80 mmol L^{-1} for β-CDs, remaining constant after that β-CDs concentration.

After analyzing the soluble complexes, the effect of the complexation method on the formation of solid complexes was studied.

The analysis of the solid essential CO-β-CDs complexes formed by ultrasounds and MWI is shown in Figure 3. The behavior of encapsulated essential CO in solid complexes was similar to that observed in the case of soluble ones. The essential CO encapsulated was higher when MWI was used as energy source (Fig. 3, ●, ○) compared with ultrasounds (Fig. 3, ■, □), regardless of the β-CDs concentration. The results clearly pointed to an increase in encapsulated essential CO when two ultrasonic or MWI cycles were applied. This effect was even more evident in the case of MWI, in which case the essential CO concentration reached with 2 cycles was 48.5 mg g^{-1} of solid complexes compared with the 20 mg g^{-1} of solid complexes obtained with one cycle.

An increase in the β-CDs concentration visibly increased the essential CO retained in the solid complexes. In the same way as was found for soluble complexes, β-CDs concentrations above 50 mmol L^{-1} did not mean any significant increase in the essential CO retained in solid complexes.

On the basis of the results obtained, the optimum method to prepare the essential CO-β-CDs solid complexes was 2C-MWI. More than simply increasing the effectiveness of the process, MWI also provides technological and economic advantages for the industrial scaling up of the process. [26,27]

These results agree with those obtained by Mohitm and col., [34] who studied the effect of the complexation method on cefdinir-β-CDs complex formation and who suggested that MWI leads to a higher rate of dissolution compared with the complexes prepared by kneading or by co-evaporation.

Others authors have studied and compared MWI and kneading to form inclusion complexes of loratidine, [35] and it was found that the results were very similar by using

273 both preparation methods. However, they described the MWI method as being more
274 convenient for the following reasons: the drying time is substantially shorter, industrial
275 scale up is simpler for handling the greater quantities involved, and the method speed up
276 complex preparation in the case of poorly water-soluble drugs and CDs.
277
278 **Influence of the drying method on essential CO-β-CDs solid complexes**
279 The influence of the drying method on the final essential CO concentration in
280 the solid complexes was studied using MWI with a double treatment (2C-MWI). The
281 objective of this study was to optimize the final step in the process to obtain solid and
282 dry essential CO-β-CDs complexes. Three drying methods were evaluated: vacuum
283 oven drying at 40 ºC, spray drying and freeze drying (Fig. 4).
284 When solid complexes were dried at 40 ºC in a vacuum oven until constant
285 mass, the highest value of essential CO retained was 48.5 mg g^{-1} of solid complexes by
286 using 100 mmol L^{-1} β-CDs (Fig. 4, ●).
287 Figure 4 (○) shows the results obtained for spray drying. As can be seen,
288 increasing the β-CDs concentration led to higher amounts of essential CO being
289 encapsulated up to a maximum 28 mg g^{-1} of solid complexes by using 100 mmol L^{-1} β-
290 CDs.
291 Both vacuum oven and spray drying involve high temperatures that can affect
292 flavors. In the case of VO (Fig. 4, ●), despite the fact that the temperature was quite
293 moderate (40 ºC), the exposure time was longer than in the case of spray drying (Fig. 4,
294 ○), in which the inlet atomizer temperature was 160 ºC.
295 Figure 4 (■) shows the essential CO retained in solid complexes when they were
296 dried by freeze dryer. The maximum value of essential CO retained was obtained using
297 100 mmol L^{-1} β-CDs. The amount of essential CO retained using a freeze dryer was

much higher (180 mg g^{-1} of solid complexes) than when a vacuum oven (48.5 mg g^{-1} of solid complexes) or spray dryer (28 mg g^{-1} of solid complexes) were used.

Assuming that freeze drying is the most respectful method for the encapsulated essential CO and given that the amount of essential CO retained was maximum with this method (180 mg g^{-1} = 100%), the use of VO would imply a loss of 73% CO during treatment, and a loss of 84% in the case of spray drying, the most aggressive method, (Figure 5).

These results showed that not only the drying method, but also temperature are important factors for the preparation of CO-β-CDs solid complexes. In a recent study, Anwar and Kunz [36] compared the stability of microcapsules prepared by using different drying methods, spray granulation, spray drying and freeze drying, finding that spray granulation was the best for producing stable microcapsules. Sahin and col. [37] observed that air temperature increases above 155 ºC could provoke losses of 1,8-cineole encapsulates by spry drying. Although freeze drying does not use heat, the authors demonstrated that the final particle morphology is a limiting factor in relation to oxygen diffusivity and that the porous structure of the freeze drying powder accelerates oxidation due to an easy oxygen access into matrices. In contrast, Heinzelmann and Franke [38] described the FD technique as an opportunity to produce microencapsulating fish oil (PUFA) with good oxidation stability.

Influence of the encapsulation and drying methods on CO-β-CDs solid complexes macrostructure

Physical properties of solid complexes can determine technical aspects such as density and solubility. Therefore, it is important to analyze the structure, shape and size

that different types of encapsulation and drying methods can confer to solid complexes obtained.

Particle structure and size of the solid complexes obtained by using different encapsulation and drying methods are shown in Figure 6. Encapsulation method appears to be decisive for the particle size of the final solid complexes resulting in a higher particle size when encapsulation procedure was made by ultrasounds (Fig. 6 A and B). The largest particle size and compactness of crystals was observed by using ultrasound encapsulation with vacuum oven as drying process (Fig. 6.A).

With respect to drying methods, freeze drying produced a more homogeneous size and shape of solid particles (Figure 6.B and D). Spray dry method (Fig. 6.E) produced solid complexes with an important variety of size and shape of particle. There are large and compact crystalline structures with rounded and small structures (Fig. 6. F).

CONCLUSION

The use of MWI could be an alternative for the aroma industry for preparing soluble and insoluble essential CO-β-CDs complexes since, it reduces the preparation time and the energy used, resulting in economic benefits.

Quantification of the solid complexes formed after applying different drying methods clearly pointed to freeze drying as the best method for drying the solids, followed by vacuum oven and spray drying, both of which resulted in significant reductions in the amount of essential CO encapsulated.

Based on these results MWI and freeze drying could be efficiently used to prepare essential CO-β-CDs complexes with good yields.

346 Encapsulation and drying methods are be decisive in the final solid complexes
347 structure.
348
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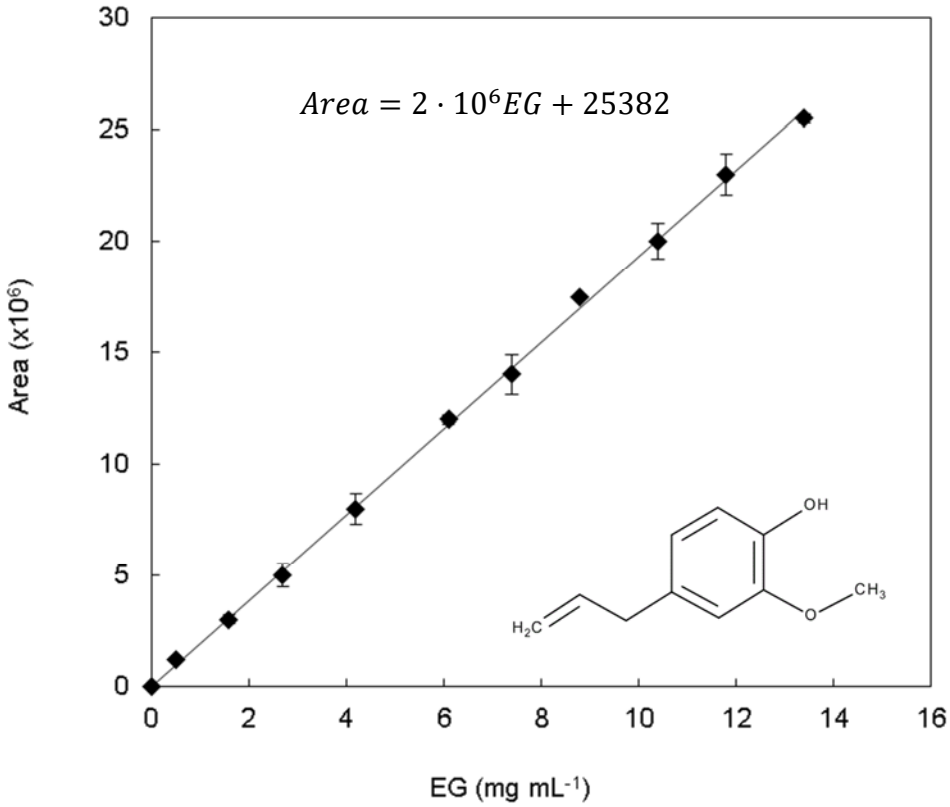

Figure 1. EG calibration curve obtained by GC-MS: Inset Chemical structure of eugenol (EG). Values represent means of triplicate determination.

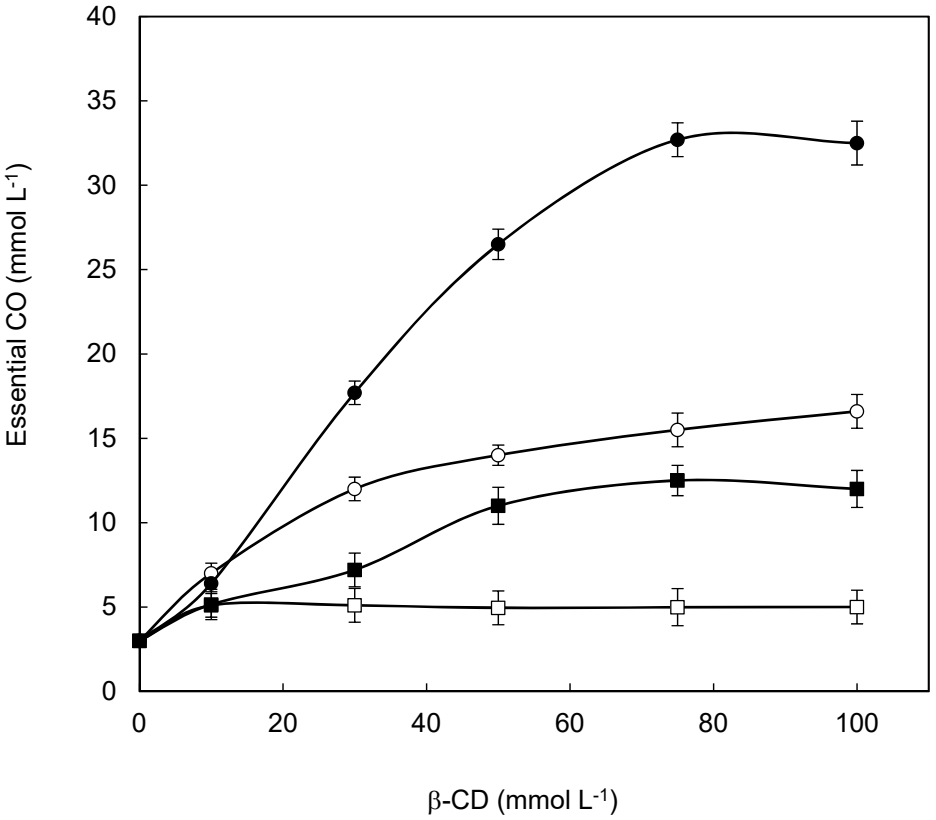

Figure 2. Influence of the preparation method (MWI or ultrasound) on the formation of soluble essential CO-β-CDs complexes (based on its main component, EG) with increasing β-CDs concentration (0-100 mmol L^{-1}). (□) 1 cycle of ultrasound (1C-U). (■) 2 cycles of ultrasound (2C-U). (○) 1 cycle of microwave (1C-MWI). (●) 2 cycles of microwave (2C-MWI). Values represent means of triplicate determination.

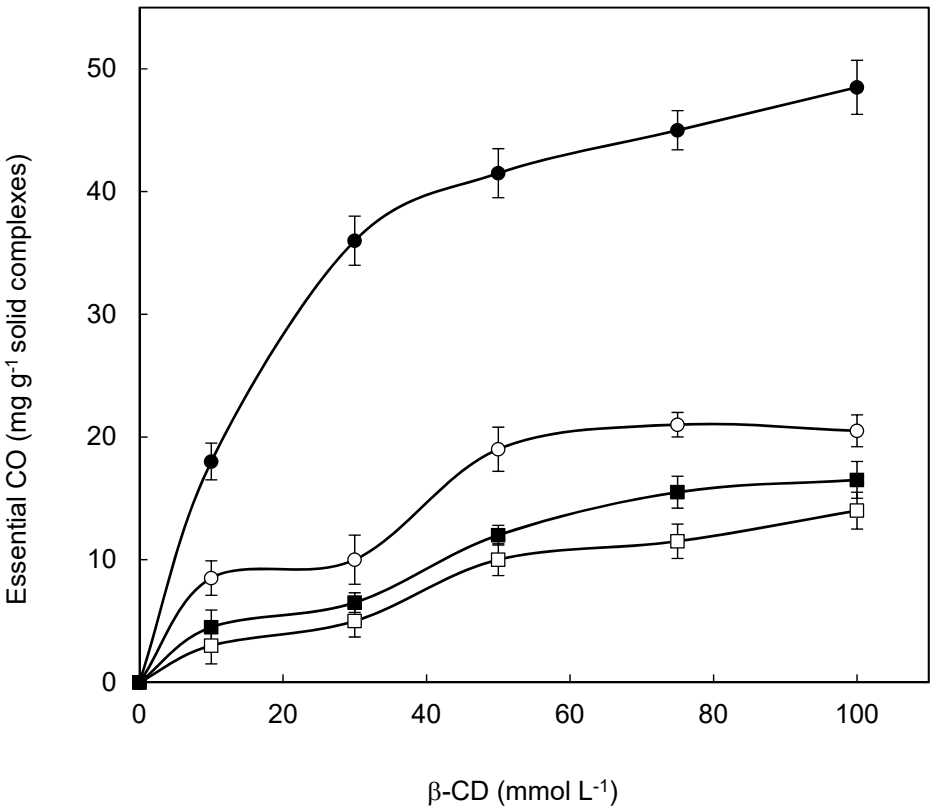

Figure 3. Influence of the preparation method (MWI or ultrasound) on the solid essential CO-β-CDs complexes formation (based on its main component, EG) with increasing β-CDs concentration (0-100 mmol L^{-1}). (□) 1 cycle of ultrasound (1C-U). (■) 2 cycles of ultrasound (2C-U). (○) 1 cycle of microwave (1C-MWI). (●) 2 cycles of microwave (2C-MWI). Values represent means of triplicate determination.

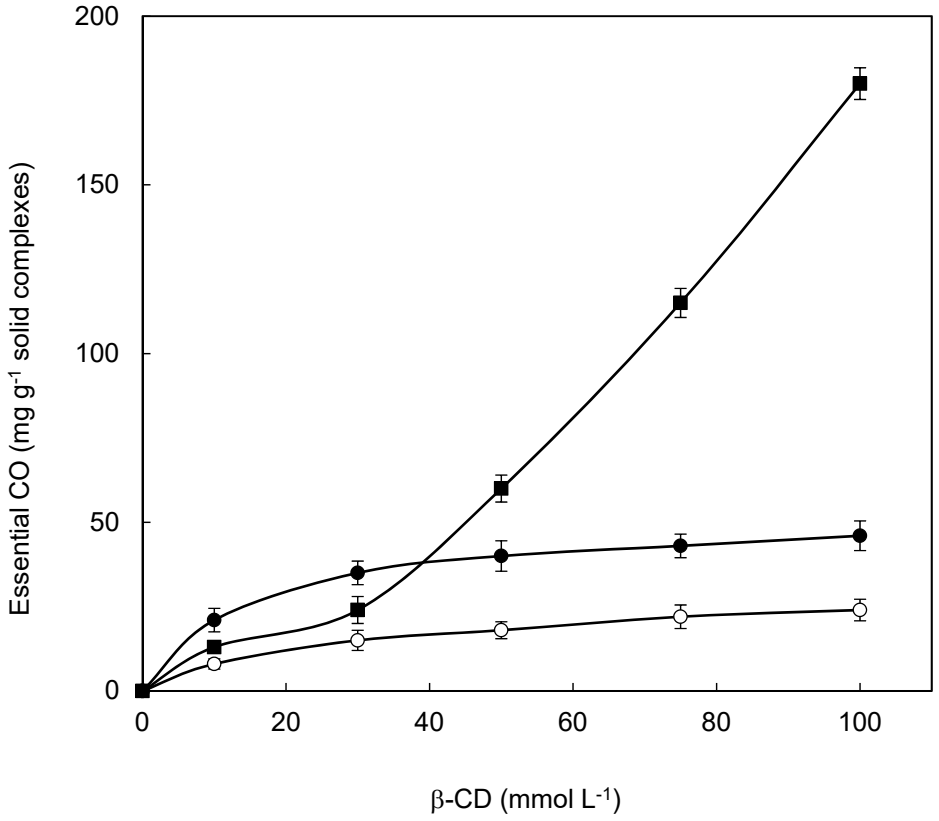

Figure 4. Essential CO content in solid essential CO-β-CDs complexes (on the basis of its main component, EG) with increasing β-CDs concentration (0-100 mmol L^{-1}) and using different drying systems. (●) Vacuum oven. (○) Spray drying. (■) Freeze drying. Values represent means of triplicate determination.

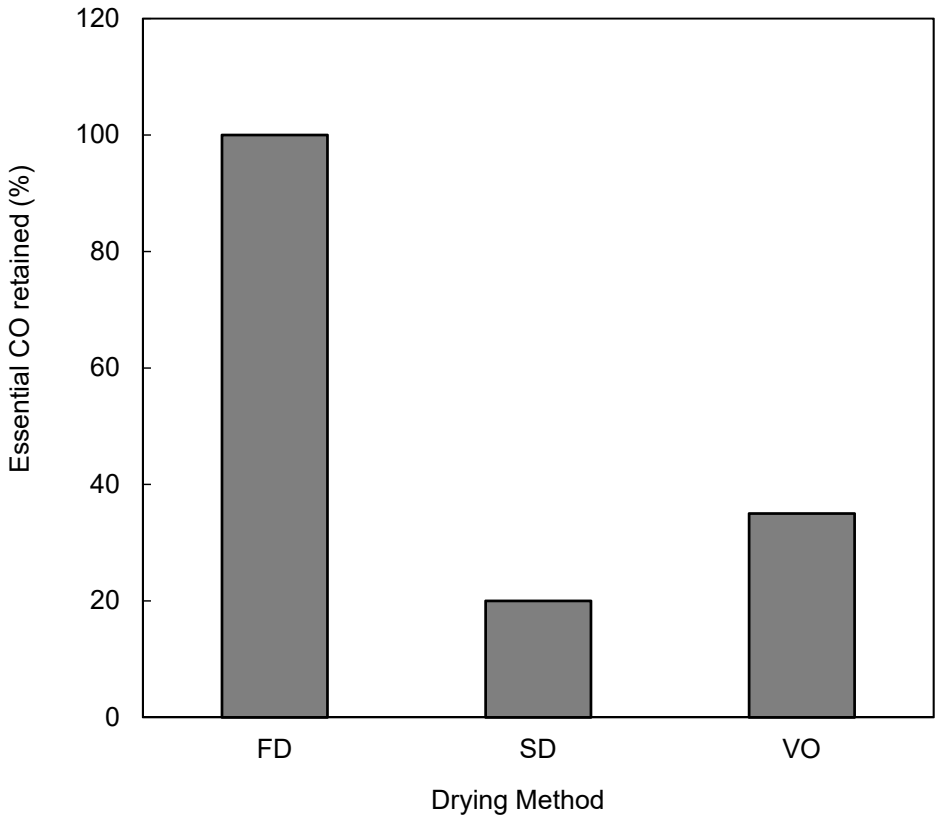

Figure 5. Maximum essential CO concentration retained (%) after the application of different drying methods in essential-CO-β-CDs complexes. Freeze drying (FD). Spray drying (SD). Vacuum oven (VO).

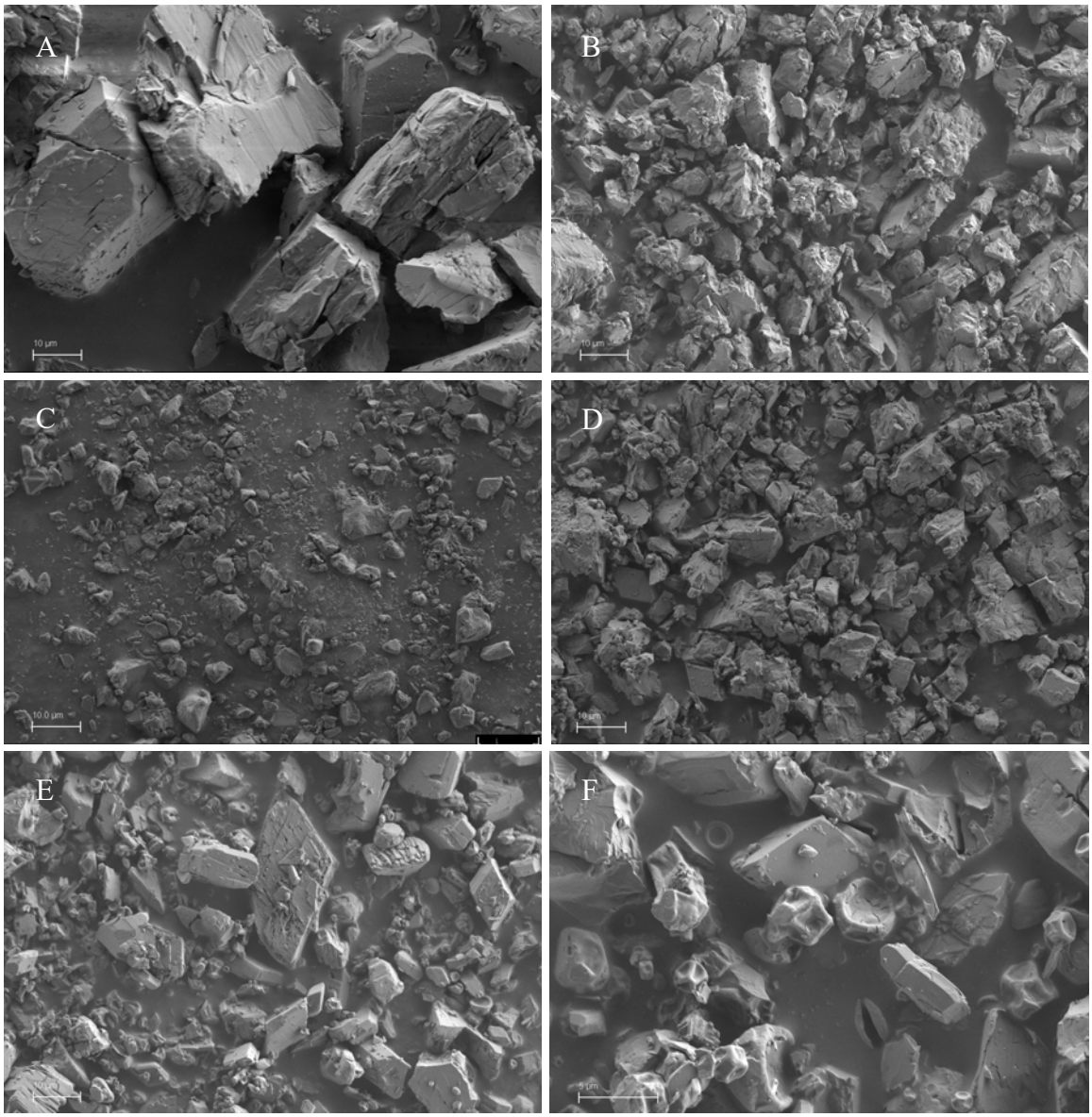

Figure 6. Field Emission Scanning Electron Microscope (FESEM) images of encapsulation – drying methods. A. Ultrasound – Oven. B. Ultrasound – Freeze dry. C. Microwave – Oven. D. Microwave – Freeze dry. E. Microwave – Spray dry. F. Microwave – Spray dry detail