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Complexation between oleanolic and maslinic acids with native and modified cyclodextrins

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Keywords

Oleanolic acid, maslinic acid, cyclodextrin, complexation, efficiency, thermodynamic parameters, complexation constant

Abstract

Oleanolic (OA) and maslinic (MA) acids are two natural triterpenoids with a wide range of beneficial effects for human health. However, their low solubility and permeability make their application in the food or pharmaceutical industry difficult. The complexation of OA and MA with α- β-, γ-, HP-α-, HP-β- and HP-γ-CDs under different pH and temperature conditions has been studied. Neither α- nor HP-α-CDs formed inclusion complexes, while β-, HP-β- and HP-γ-CDs provided A_L type and γ-CDs B_S phase solubility diagrams. Complexation was shown to be more stable in the case of MA but complexation efficiency was greater for OA. Increasing the pH and temperature of the complexation media tended to improve the complexation process with triterpenic acids.

1. Introduction

Oleanolic acid (OA) and maslinic acid (MA) are two natural pentacyclic triterpenoids widely distributed in nature (Fig. 1 Ai and Fig. 2 Ai). Many previous studies indicate that these compounds are naturally present, in both free and glycosylated forms, in hundreds of plants species (Somova, Nadar, Rammanan & Shode, 2003; Fai & Tao, 2009; Gao, Tang & Tong, 2012; Lin, Yan & Yin, 2014), as well as in other organisms such as bacteria, fungi and yeasts (Parra et al, 2014). One of the plant species in which these compounds are particularly plentiful is the olive tree, and significant quantities are found in the by-products resulting from the olive oil extraction. Chouaïb et al. (2015) studied the concentration of OA and MA in olive pomace from different olive tree varieties, finding values of between 0.19 and 3.40 mg/g DW, and between 0.29 and 8.50 mg/g DW for OA and MA, respectively.

The biological and chemical properties of natural triterpenes, as well as their derivatives, have been widely studied in recent years. Around 40 % of all scientific publications related to the biology and chemistry of these compounds were published between 2010 and 2015 (Sommerwerk, Heller, Kuhfs & Csuk, 2016), with an upward

55 trend between these dates (Tasca & Baggio, 2017). The huge interest in OA and MA lies
56 in their broad spectrum of biological properties. Many studies highlight their role as
57 hepatoprotectives (Chen, Liu, Yang, Zhao & Hu, 2005; Yan, Yang, Lee & Yin, 2014),
58 inhibitors of rheumatoid arthritis (Choi et al., 2016), and their antimicrobial (Kurek,
59 Nadkowska, Pliszka & Wolska, 2012; Chouaïb et al., 2015), anticarcinogenic (Lin et al.,
60 2014; Chouaïb et al., 2016), anti-inflammatory (Chouaïb et al., 2016), antidiabetic
61 (Castellano, Guinda, Rada, Delgado & Cayuela, 2013) and antiviral (anti-HIV) (Parra et
62 al., 2014) actions.

63 The main disadvantage of these compounds for application in food or
64 pharmaceutical industry as functional ingredients is their low aqueous solubility and
65 permeability. A variety of values for the water solubility of OA have been reported by
66 different authors. Jäger, Winkler, Pfüller and Scheffler (2007) established the water
67 solubility of OA as 0.02 μg/mL, and Li, Quan, Liu, Wei, Zhang and Xu (2009) concluded
68 that its aqueous solubility was lower than the detection limit of their methodology used
69 (<0.1 μg/mL). However, other authors indicated higher aqueous solubility values,
70 including 4.61 μg/mL at 20 ºC (Gao et al., 2012). No values for the aqueous solubility of
71 MA have been found in the literature. The permeability value of OA (Papp = 1.1-1.3 x
72 10^{-6} cm/s in the apical-to-basolateral direction at 10 and 20 μM) has been mentioned by
73 some authors (Jeong et al., 2007). This combination of low solubility and permeability
74 means that these triterpenoids are of low bioavailability. Another disadvantage associated
75 with poor aqueous solubility is the need to use organic solvents such as cyclohexane,
76 ethyl acetate, methanol, acetonitrile or acetone for their extraction from plant material
77 (Bernatoniene, Cizauskaite, Ivanauskas, Jakstas, Kalveniene & Kopustinskiene, 2016;
78 Tasca & Baggio, 2017).

Several efforts have focused on improving the bioavailability and extractability of OA and MA by increasing their aqueous solubility. Chen, Zhong, Tan, Wang and Wnag (2011) determined that solid-liquid nanoparticles improve the bioavailability of the OA. An increase in pH has also been proposed for increasing the solubility of these compounds. For example, Jäger et al. (2007) increased the water solubility of OA to 77.2 μg/mL by increasing the water pH to 11.5. Jiang, Yang, Du, Zhang and Zhang (2016) significantly increased the solubility of OA by the formation of solidified phospholipid complexes with hydroxyapatite. Other techniques studied to increase the aqueous solubility of these compounds have been the particle size reduction, solid dispersion, cosolvency, salt formation and the combination of any of them (Loftsson, Jarho, Másson & Järvinen, 2005).

Complexation with cyclodextrins (CDs) has been widely used to increase the aqueous solubility of different compounds (Lucas-Abellán, Fortea, Gabaldón & Núñez-Delicado, 2008; Mercader-Ros, Lucas-Abellán, Fortea, Gabaldón and Núñez-Delicado, 2010a; Mercader-Ros, Lucas-Abellán, Gabaldón, Fortea, Martínez-Cachá, Núñez-Delicado, 2010b), and to improve the extraction of compounds with a poor aqueous solubility from food by-products (Lopez-Miranda et al., 2016). Chen, Wu, Li and Cheng (2010) improved the extraction of OA from leaves of *Chaenomeles speciosa* by using HP-β-CDs. Li et al. (2009) observed that the concentration of extracted OA increased from 0.08 mmol/L to 2.15 mmol/L when the concentration of HP-β-CDs increased from 5 mmol/L to 60 mmol/L. In a study developed by Quan, Liu, Li, Zhang, Qian and Xu (2009), it was observed that the combination of HP-β-CDs with water soluble polymers (HPMC and PVP) improved the aqueous solubility of OA and its isomer ursolic acid.

CDs may be regarded as suitable tools for improving the aqueous solubility of triterpenic acids, especially OA and MA. However, previous studies have mainly focused

104 on OA and its interaction with HP-β-CDs, and there is no information about other CDs
105 types or different complexation conditions. For this reason, it was thought necessary to
106 improve our knowledge on the complexation mechanism between OA and MA with
107 different types of CDs, as well as different complexation conditions such as pH or
108 temperature.

109 The aim of this work was to study the complexation behavior of OA and MA with
110 natives α- β- or γ-CDs as well as their modified HP-α-, HP-β- or HP-γ-CDs, and the
111 effect of pH and temperature on the complexation process.

112

113 **2. Material and Methods**

114 *2.1. Reagents and standards*

115 OA (94% purity) and MA (83.4 % purity) were commercial extracts provided by
116 Nutrafur S.A. (Murcia, Spain). Acetonitrile and water of HPLC grade were purchased
117 from JT Baker (The Netherlands). The α- β-, γ-, HP-α-, HP-β- and HP-γ-CDs were
118 purchased form Winplus International Limited (China). Reagent grade acetic and boric
119 acids were purchase from Sharlau (Tarragona, Spain), and potassium di-hydrogen
120 phosphate (reagent grade) were purchased from Panreac (Barcelona, Spain).

121

122 *2.2. Complexation and phase solubility diagrams*

123 The complexation process of OA and MA were evaluated by developing phase
124 solubility diagrams, according to a modified method of that described by Higuchi and
125 Connors (Higuchi & Connors, 1965). Excess amounts of OA and MA were added to 10
126 mL of aqueous solutions of increasing concentrations from 0 to 13 mM for β-CDs and 0

127 to 50 mM for α-, γ-, HP-α-, HP-β- and HP-γ-CDs. The different phase solubility diagrams
128 were prepared in glass test tubes and maintained in an ultrasonic bath (Ultrasons H.P.
129 Selecta, Spain) for 60 min to reach equilibrium.

130 The effect of temperature on the complexation process was studied by developing
131 solubility experiments in distilled water at different temperatures (4 ºC, 25 ºC and 65 ºC).
132 The effect of pH on the complexation process was studied by developing solubility
133 diagrams in buffered solutions of CDs at pH 3.0 (sodium acetate buffer), 6.5 (sodium
134 phosphate buffer) and pH 9 (sodium borate buffer).

135 After 60 min of ultrasound treatment, solutions were centrifuged in a
136 microcentrifuge (Eppendorf Centrifuge S415D, Germany) at 10000 *xg* for 10 minutes and
137 filtered using 0.45 μm nylon membrane filters (Chromafil. Macherey-Nagel, Germany).
138 Phase solubility diagrams were made in triplicate.

139 The complexation constant Kc between the triterpenic acids and CDs was
140 calculated from the slope of the phase solubility profile and the solubility of the triterpenic
141 acid in aqueous solution (S_0) by using the equation (1):

142 $$K_C = \frac{slope}{S_o \cdot (1 - slope)} \quad (1)$$

143 The complexation efficiency (CE) is the ratio between dissolved complex and free
144 CDs concentration. It is independent of S_0, and was calculated from the slope of the phase
145 solubility profiles by using the equation (2).

146 $$CE = \frac{[disolved - complex]}{[CD]_f} = S_0 * K_C = \frac{slope}{(1 - slope)} x100\% \quad (2)$$

147 The molar ratio OA:CD and MA:CD was calculated using the CE value with the
148 equation (3).

149 $$\text{Triterpenic Acid:CD} = 1:\left(1+\frac{1}{CE}\right) \quad (3)$$

150

151 *2.3. Thermodynamic parameters of the complexation process*

152 The thermodynamic parameters calculated were the Gibbs free energy transfer
153 (ΔG^0_{tr}), standard free energy change (ΔG^0), standard enthalpy change (ΔH^0), and standard
154 entropy change (ΔS^0).

155 The ΔG^0_{tr} represents the free energy of transferring a compound from water to the
156 CD hydrophobic cavity, and was calculated by equation (4).

157 $$\Delta G^0_{tr} = -RT\cdot\ln\frac{S}{S_0} \quad (4)$$

158 in which S is the molar solubility of OA or MA in a CDs aqueous solution, R is the gas
159 constant and T is the absolute temperature in K.

160 The ΔG^0 values of the complexation reaction were calculated from the Kc using
161 the equation (5).

162 $$\Delta G^0 = -2.303RT\cdot\log K_C \quad (5)$$

163 The ΔH^0 values were also calculated from Kc by using the van't Hoff equation
164 (6):

165 $$LogK_C = -\frac{\Delta H^0}{2.303R}\cdot\frac{1}{T} + C \quad (6)$$

166 The ΔS^0 could be also calculated by using the equation (7):

167 $$\Delta G^0 = \Delta H^0 - T\cdot\Delta S^0 \quad (7)$$

168 *2.4. OA and MA determination by HPLC*

169 OA and MA were quantified by HPLC analysis using an HPLC Agilent
170 Technologies model 1200 equipped with a DAD detector set at 203 nm, injecting 20 μL
171 of centrifuged and nylon filtered complexes. Separations were carried out on an
172 endcapped (5 μm) HPLC Cartridge 250-4 LiChospher 100 RP-18. The column
173 temperature was set to 30 ºC, and the flow rate was 1 mL/min. The mobile phase used
174 was water (A) *versus* acetonitrile (B) for a total running time of 26 min, during which the
175 gradient changed as follows: solvent B was maintained at 70 % from 0 to 10 min, then
176 increased to 80 % and maintained for 13 min, before returning to initial conditions in 3
177 min. Time retentions were 9.1 min for MA and 19.9 min for OA. The data were processed
178 by Agilent ChemStation software, and the OA and MA concentrations were expressed in
179 mM.

180

181 **3. Results and discussion**

182 *3.1. Complexation of OA and MA*

183 In order to study the ability of CDs to increase the aqueous solubility of OA and
184 MA, phase solubility studies were carried out in distilled water at 25 ºC with different
185 types of native and modified CDs. The results obtained by using these types of CDs are
186 shown in Fig. 1 and 2 (■) for OA and MA, respectively.

187 The presence of α- or HP-α-CDs did not increase the aqueous solubility of OA or
188 MA, indicating that inclusion complexes were not formed, probably due to the small size
189 of the hydrophobic cavity of these two types of CDs (data not shown).

190 In the case of β- and HP-β-CDs, the phase solubility diagrams showed an A_L type
191 profile, for both OA (Fig. 1A and 1B) and MA (Fig. 2A and 2B), indicating that water

192 soluble complexes were formed. The slope value was lower than 1 in all cases, indicating
193 the 1:1 stoichiometry of the complexes (Higuchi & Connors, 1965), whereby each
194 molecule of OA or MA enters one molecule of the CD. Li et al. (2009) observed an A_L-
195 diagram for OA and HP-β-CDs and suggested that this kind of diagram could be normal
196 for compounds with low water solubility.

197 Assuming the formation of 1:1 complexes, the complexation constant (Kc) was
198 calculated by using linear regression analysis from the phase solubility diagrams
199 according to equation (1). Kc values in water at 25 ºC are shown in table 1 (T 25 ºC).

200 The Kc value obtained was higher for native than for modified CDs in both OA
201 and MA, indicating a greater affinity of the native β-CDs for the triterpenic acids
202 compared to their modified HP-β-CDs. The Kc value obtained for the β-CDs-OA
203 complex was 825 M^{-1} whereas in the case of HP-β-CDs the Kc value was 201 M^{-1}. In the
204 case of MA, the Kc values were quite similar: 2653 and 2537 M^{-1} for β- and HP-β-CDs,
205 respectively. Chemical modification by adding hydroxypropyl groups to native β-CDs
206 does not favour its capacity to entrap OA or MA in the hydrophobic cavity. These results
207 agree with those obtained for oxaliplatin, when β-CDs showed higher Kc (1438 M^{-1}) than
208 HP-β-CDs (664 M^{-1}) (Zhang et al., 2016). However, the results contrast with those
209 obtained for many other compounds, such as resveratrol (Lucas-Abellán et al., 2008) or
210 diazepam and nitrazepam (Hadžiabdić, Elezović, Rahić & Mujezin, 2012). It is important
211 to note that the stability of the complexes formed between β- or HP-β-CDs and MA were
212 higher than those formed with OA as their Kc values indicated. This fact might well be
213 due to the presence of an additional OH group in the chemical structure of MA, which
214 would interact with the surrounding OH groups of the CD cavity.

215 In the case of native γ-CDs, phase solubility diagrams obtained for both OA and
216 MA were of the Bs type (Fig. 1C and 2C). This type of phase solubility diagrams indicated

217 that the solubility of triterpenic acids increased with CDs concentration until reaching a
218 maximum, before remaining constant or decreasing for higher CDs concentrations. The
219 complexes formed with γ-CDs presented limited solubility (approx. 0.15 mM),
220 decreasing in the presence of γ-CDs concentrations above 2.5 mM for OA (Fig. 1C), and
221 5 mM for MA (Fig. 2C). This effect could be explained by the formation of
222 supramolecular complexes of high molecular weight that precipitate (more than one γ-
223 CDs is added to formed complexes). This B_S type phase solubility diagram contrast with
224 those of the A_L type obtained for the complexation of picoplatin (Zhang et al., 2014),
225 fisetin (Zhang et al., 2015) or oxaliplatin (Zhang et al., 2016) with γ-CDs.

226 The value of Kc for γ-CDs was calculated from the initial linear portion of the
227 phase solubility diagrams, from 0 to 1 mM (Fig. 1Ci and 2Ci), for which the slope value
228 was lower than 1, indicating that the stoichiometry of the complexes formed was 1:1. The
229 Kc values obtained were 4895 M^{-1} for OA and 18723 M^{-1} for MA (Table 1, T 25 ºC),
230 which indicated that the interaction between the triterpenic acids and γ-CDs was much
231 stronger than the corresponding interactions observed in the case of β-CDs. It is also
232 important to point that the complexes formed between MA and γ-CDs were more stable
233 than those formed with OA, as indicated by the higher Kc value obtained. As in the case
234 of β-CDs, this could also be due to the presence of an additional OH group in the chemical
235 structure of MA, which would interact with the surrounding OH groups of the CD cavity.
236 The Kc values obtained between γ-CDs and OA or MA were higher than those obtained
237 for oxaliplatin (1322 M^{-1}, Zhang et al., 2016) or fisetin (196 M^{-1}, Zhang et al., 2015),
238 while in the case of picoplatin (10318 M^{-1}, Zhang et al., 2014), OA showed a lower Kc
239 value, and MA a higher value.

240 As regard the behaviour of HP-γ-CDs, A_L type diagrams were obtained for both
241 OA and MA (Fig. 1D and 2D), indicating the high water solubility of the complexes

242 formed in contrast with those obtained for native γ-CDs. The slope of the diagram was
243 lower than 1 for both triterpenic acids, thus indicating a 1:1 stoichiometry of the
244 complexes formed (Higuchi & Connors, 1965), as usually occurs in the case of modified
245 CDs (Zhang et al., 2016). The Kc values for HP-γ-CDs complexes formed in pure water
246 were 645 M^{-1} and 5474 M^{-1} for OA and MA, respectively (Table 1, T 25 ºC). The value
247 obtained for OA was similar and that obtained for MA was higher than that obtained for
248 oxaliplatin (664 M^{-1}) (Zhang et al., 2016). These values are much lower than those
249 obtained with the native γ-CD.

250 The Kc value describes the strength of the interaction between any compound and
251 CDs, and can be used to compare the affinity of any compound for different CDs types.
252 But a more accurate parameter for the determination of the solubilizing effect of CDs is
253 their complexation efficiency (CE) because it is independent on S_0 (Li et al., 2009). CE
254 represents the molar ratio between complex and free CDs concentration (Loftsson &
255 Hreinsdóttir, 2007). For 1:1 complexes, the CE can be calculated from the slope of phase
256 solubility diagram with equation (2) (Loftsson & Brewster, 2010; Loftsson & Brewster,
257 2012).

258 The comparison of CE parameters is more convenient than comparing Kc values
259 when the study involves different types of CDs or different complexation conditions for
260 the same compound. In this study, CE was also used to calculate the OA:CD and MA:CD
261 molar ratio in solution with equation (3), which can be correlated to the expected increase
262 in compound solubility with different CDs (Loftsson & Hreinsdóttir, 2007). The values
263 obtained for CE and molar ratio are shown in table 1 (T 25 ºC).

264 For the complexation of OA in water at 25 ºC, the CE values obtained ranged from
265 0.52 % for HP-β-CDs to 12.7 % for γ-CDs. The most effective CDs in the complexation
266 of OA were γ-CDs, indicating that about one of every 9 γ-CDs molecules in solution is

267 forming water soluble complexes, as the molar ratio value indicated for this CD type
268 (Molar ratio of 1:9, Table 1, T 25 ºC). However, only one of every 193 HP-β-CDs
269 molecules in solution forms water soluble complexes with OA (Molar ratio 1:193, Table
270 1, T 25 ºC).

271 The same behaviour was observed for MA, in which the lowest value of CE was
272 obtained for HP-β-CDs (0.89 %) and the highest for γ-CDs (6.25 %), indicating that γ-
273 CDs are also the most effective for the complexation of MA. About one in every 16 γ-
274 CDs molecules in solution forms water soluble complexes with MA (Molar ratio 1:16,
275 Table 1, T 25 ºC), while one in every 113 HP-β-CDs molecules in solution forms water
276 soluble complexes (Molar ratio 1:113, Table 1, T 25 ºC).

277 Comparing the OA and MA results obtained for γ-CDs, OA showed a higher CE
278 (12.7 % *vs* 6.25 %) and a lower molar ratio (1:9 *vs* 1:16) than MA, indicating that
279 complexation with OA was more effective, although MA-γ-CDs complexes were more
280 stable as indicated its higher Kc value (4895 M^{-1} for OA *vs* 18723 M^{-1} for MA).

281

282 *3.2. Effect of temperature on complexation of OA and MA. Thermodynamic parameters*

283 The influence of temperature on the complexation process of OA and MA with
284 native or modified CDs was also studied. As can be seen in table 1, the Kc value for the
285 triterpenic acids with each type of CDs used increased with temperature, indicating that
286 higher temperatures favour entrapment of the triterpenic acids in the hydrophobic cavity
287 of CDs.

288 The thermodynamic parameters were calculated from phase solubility diagrams
289 in the presence of increasing concentrations of CDs at several temperatures (Fig. 1 and
290 2). The Gibbs free energy of transfer (ΔG^0_{tr}) represents the free energy of transfer of a

compound from water to the CDs cavity and provides information about whether the reaction condition is favourable or unfavourable for the solubilisation in the aqueous carrier solution. The ΔG^0_{tr} values with increasing concentrations of CDs were calculated using equation (4) (Hadžiabdić et al., 2012).

The negative values of ΔG^0_{tr} obtained in all cases indicated the spontaneous inclusion of OA and MA in each type of CD (Table 2). Moreover, in all the CD types studied ΔG^0_{tr} values were more negative as CDs concentration increased, indicating that the reaction was more favourable as the CD concentration increased. Similar results were described for diazepam and nitrazepan (Hadžiabdić et al., 2012).

All the thermodynamic parameters obtained are shown in table 2, where it can be seen that OA and MA solutions in the presence of β-, γ-, HP-β- or HP-γ-CDs were characterized by negative ΔG^0 values, indicating the spontaneous complexation of the triterpenic acids in the hydrophobic cavity of each CD (Karathanos, Mourtzinos, Yannakopoulou & Andrikopoulos, 2007). Similar results have been described for diazepan, nitrazepan or carvacrol (Hadžiabdić et al., 2012; Santos, Kamimura, Hill & Gomes, 2015). The ΔG^0 values increased in the following order for OA: γ-CDs< β-CDs< HP-γ-CDs< HP-β-CDs and for MA: γ-CDs< HP-γ-CDs< β-CDs< HP-β-CDs. In both cases, the order agrees with the order in the decrease of Kc values. In other words, the lower the ΔG^0, the higher the Kc value.

Moreover, the positive values of ΔH^0 obtained for OA or MA with all the CD types used also indicated the endothermic reaction (Connors, 2002). The values of ΔH^0 increased in the same order as ΔG^0 and so in the reverse order of the Kc values, indicating that complexation with higher Kc values were less endothermic and had more negative values for ΔG^0 (more spontaneous reaction).

315 The positive values obtained for ΔS^0 could be due to the entry of OA or MA in
316 the hydrophobic cavity of CDs governed by hydrophobic interactions, which involve a
317 breakdown of the water structure around the OA or MA, creating a large positive ΔS^0 and
318 a small positive ΔH^0. These results agree with those described for diazepam and
319 nitrazepan (Hadžiabdić et al., 2012).

320

321 *3.3. Effect of pH on complexation of OA and MA*

322 Different pH values determine the presence of neutral or protonated OA or MA
323 forms in the solution. Ionization may significantly influence their solubility and the
324 complexation process. The solubility of these triterpenic acids was tested in a pH range
325 from 3.0 to 9.0 and was seen to slightly increased with pH in both cases (Table 1), as
326 previously observed by Jäger et al. (2007) for OA. To study the influence of OA and MA
327 ionization on their complexation process with CDs, phase solubility diagrams were
328 carried out at different pH values (3.0, 6.5 and 9.0) (Fig. 3 and 4).

329 With each type of CDs used, the phase solubility diagram profile obtained was
330 similar to that obtained when using pure water. The experimental data showed that the
331 Kc increased with the pH of the media (Table 1), indicating the influence of the OA or
332 MA ionization in the complexation process. These results were similar to those obtained
333 in the case of risperidone (Jug, Kos & Bećirević-Laćan, 2009). Despite the fact that
334 ionized forms of triterpenic acids (pH 9.0) were slightly more soluble than the non-
335 deprotonated forms, the higher Kc values obtained at pH 9.0, were probably due to that
336 the protonated form establishing stronger interactions with the hydrophobic cavity of each
337 CDs. OA and γ-CDs at pH 9.0 showed the highest Kc value, 38482 M^{-1} (20-fold higher
338 than at pH 3.0) (Table 1).

As solubility and Kc values varied with the pH value of the medium, it was thought that the CE would also be affected by this parameter. Indeed, for all the CD types studied, CE clearly increased with pH value for both OA and MA (Table 1). The highest CE value obtained was 134 % for OA and γ-CDs at pH 9.0, indicating that at this basic pH one of every 2 γ-CDs molecules in solution forms water soluble complexes (molar ratio 1:2, Table 1). This CE value was 47-fold higher than that obtained for OA and γ-CDs at pH 3.0. In contrast, the lowest CE value obtained was 0.15 % for MA and β-CDs at pH 3.0, indicating that at this acid pH one in every 668 β-CDs molecules in solution is forming water soluble complexes with MA (molar ratio 1:668, Table 1). HP-β-CD and MA showed similar values to those observed for β-CD.

In conclusion, this paper shows that, due to their low aqueous solubility and chemical structure, OA and MA are complexed by β-, γ-, HP-β- or HP-γ-CDs, but not by α- or HP-α-CDs. Contrary to expectations, native β- and γ-CDs exhibited higher Kc values than their corresponding HP modified CDs, indicating the higher strength of the interaction between OA or MA with native than with modified CDs. In both OA an MA, γ-CDs showed a B_S type phase solubility diagram, and presented the highest Kc values (6-fold greater than that presented by β-CDs for OA and 7-fold in the case of MA). As CE values represent the solubilizing potential of CDs, the values obtained indicate that a high Kc value does not always correspond with a high CE value because of the influence of S_0 on the Kc value. For this reason, it is more accurate to use the CE to study the effect of physical or chemical parameters which affect S_0 in the encapsulation process. The pH and temperature affected to the complexation process of OA and MA. The deprotonate form of OA or MA at pH 9.0 forming the most stable union with γ-CDs. Moreover, an increase in the temperature of the medium resulted in an increase in complex formation with both OA and MA. Therefore, these results pointed to the fact that in order to extract

364 both OA and MA from food by-products like those derived from olive oil production,
365 basic pH, temperatures from 50 to 60 ºC and γ-CDs should provide properly results for
366 industry scale extractions.

367

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373

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Table 1. Aqueous solubility (S_0), complexation constant (Kc), correlation coefficient of the phase solubility diagram (r^2), complexation efficiency (CE) and Molar Ratio (D:C), of oleanolic and maslinic acid for different CDs, Temperatures (T) and pH. ±SD. Standard deviation of triplicate diagrams.

			Oleanolic Acid					Maslinic Acid				
			S_0 (μM)	Kc (M^{-1})	r^2	CE (%)	Molar Ratio (D:C)	S_0 (μM)	Kc (M^{-1})	r^2	CE (%)	Molar Ratio (D:C)
β-CD	T	4ºC	14±0.9	729±15	0.966	1.02	1:99	2.8±0.2	2119±102	0.999	0.59	1:169
		25ºC	26±1.2	825±17	0.990	2.15	1:47	3.5±0.4	2653±82	0.989	0.93	1:108
		65ºC	33±0.9	953±7	0.998	3.15	1:33	4.0±0.3	3062±465	0.997	1.22	1:83
	pH	3.0	14±1.0	605±21	0.975	0.85	1:119	2.1±0.2	7152.1±68	0.968	0.15	1:668
		6.5	24±1.3	613±20	0.970	1.53	1:66	3.0±0.4	4493±194	0.964	1.35	1:75
		9.0	35±2.5	1882±14	0.992	6.59	1:16	5.0±0.7	11909±507	0.990	5.95	1:18
HP-β-CD	T	4ºC	14±0.9	71.5±10	0.974	0.10	1:1001	2.8±0.2	1758±77	0.990	0.49	1:204
		25ºC	26±1.2	201±11	0.995	0.52	1:193	3.5±0.4	2537±62	0.994	0.89	1:113
		65ºC	33±0.9	322±43	0.991	1.06	1:95	4.0±0.3	3293±181	0.997	1.32	1:77
	pH	3.0	14±1.0	518±10	0.981	0.73	1:139	2.1±0.2	763±68	0.975	0.16	1:625
		6.5	24±1.3	340±9	0.999	0.82	1:123	3.0±0.4	1911±72	0.998	0.57	1:176
		9.0	35±2.5	796±41	0.936	2.79	1:37	5.0±0.7	1288±57	0.987	0.64	1:157
γ-CD	T	4ºC	14±0.9	4721±578	0.999	6.61	1:16	2.8±0.2	17496±278	0.998	4.90	1:21
		25ºC	26±1.2	4895±207	0.999	12.7	1:9	3.5±0.4	18723±1559	0.999	6.25	1:16
		65ºC	33±0.9	5147±293	0.997	17.0	1:7	4.0±0.3	21355±441	0.995	8.54	1:12
	pH	3.0	14±1.0	2020±133	0.991	2.83	1:36	2.1±0.2	9173±805	0.994	1.93	1:53
		6.5	24±1.3	8468±486	0.981	20.3	1:6	3.0±0.4	17914±968	0.997	5.37	1:19
		9.0	35±2.5	38482±3141	0.954	134	1:2	5.0±0.7	14247±195	0.997	7.12	1:15
HP-γ-CD	T	4ºC	14±0.9	591±67	0.963	0.83	1:122	2.8±0.2	5365±130	0.991	1.50	1:68
		25ºC	26±1.2	645±20	0.994	1.68	1:61	3.5±0.4	5474±314	0.990	1.92	1:53
		65ºC	33±0.9	1220±186	0.977	4.03	1:26	4.0±0.3	7493±187	0.993	3.00	1:34
	pH	3.0	14±1.0	1548±52	0.992	2.17	1:47	2.1±0.2	5686±84	0.998	1.19	1:84
		6.5	24±1.3	2050±88	0.973	4.92	1:21	3.0±0.4	5248±49	0.991	1.57	1:64
		9.0	35±2.5	8077±362	0.943	28.2	1:5	5.0±0.7	4499±44	0.989	2.25	1:45

Table 2. Thermodynamic parameters: Standard Free Energy Chance (ΔG^0), Standard Enthalpy Change (ΔH^0) and Standard Entropy Change (ΔS^0). Gibbs free energy of transfer (ΔG^0_{tr}) for the solubilisation process of Oleanolic and Maslinic Acids at 4, 25 and 65 ºC and at different cyclodextrin (CD) concentration (mM).

Cyclodextrin		Oleanolic Acid			Maslinic Acid		
		ΔG^0 (kJ/mol)	ΔH^0 (kJ/mol)	ΔS^0 (J/mol)	ΔG^0 (kJ/mol)	ΔH^0 (kJ/mol)	ΔS^0 (J/mol)
β-CD		-16.64	3.403	67.26	-19.54	4.546	80.81
HP-β-CD		-13.14	18.369	105.74	-19.42	7.783	91.30
γ-CD		-21.05	1.098	74.33	-24.38	2.563	90.40
HP-γ-CD		-16.03	9.617	86.07	-21.33	4.466	86.56
CD	mM	ΔG^0_{tr} (kJ/mol)			ΔG^0_{tr} (kJ/mol)		
		4ºC	25ºC	65ºC	4ºC	25ºC	65ºC
β-CD	2.5	-1.51	-2.37	-3.23	-5.09	-4.87	-5.29
	5.0	-3.07	-3.74	-4.63	-6.46	-6.37	-7.19
	10.0	-4.92	-5.40	-6.56	-7.91	-8.39	-9.05
	13.0	-5.05	-6.15	-7.23	-8.44	-8.82	-9.94
HP-β-CD	5	-0.70	-1.96	-2.68	-6.02	-6.59	-7.49
	15	-2.40	-3.43	-4.77	-8.39	-9.39	-10.47
	30	-3.54	-4.97	-6.70	-9.68	-10.58	-12.17
	50	-4.09	-5.87	-8.35	-11.15	-12.03	-13.86
γ-CD	0.10	-0.96	-1.01	-1.19	-2.97	-2.65	-3.20
	0.25	-1.81	-1.72	-2.27	-4.56	-4.18	-4.48
	0.50	-2.65	-2.88	-3.19	-6.04	-5.55	-6.35
	1.00	-3.90	-4.15	-4.74	-7.36	-7.26	-7.94
HP-γ-CD	5	-4.49	-3.92	-6.00	-8.63	-9.01	-10.11
	15	-6.27	-6.13	-8.64	-11.23	-11.38	-12.81
	30	-7.39	-7.51	-9.95	-12.59	-12.58	-14.88
	50	-8.30	-8.57	--	-13.53	-13.82	-15.96

Figures

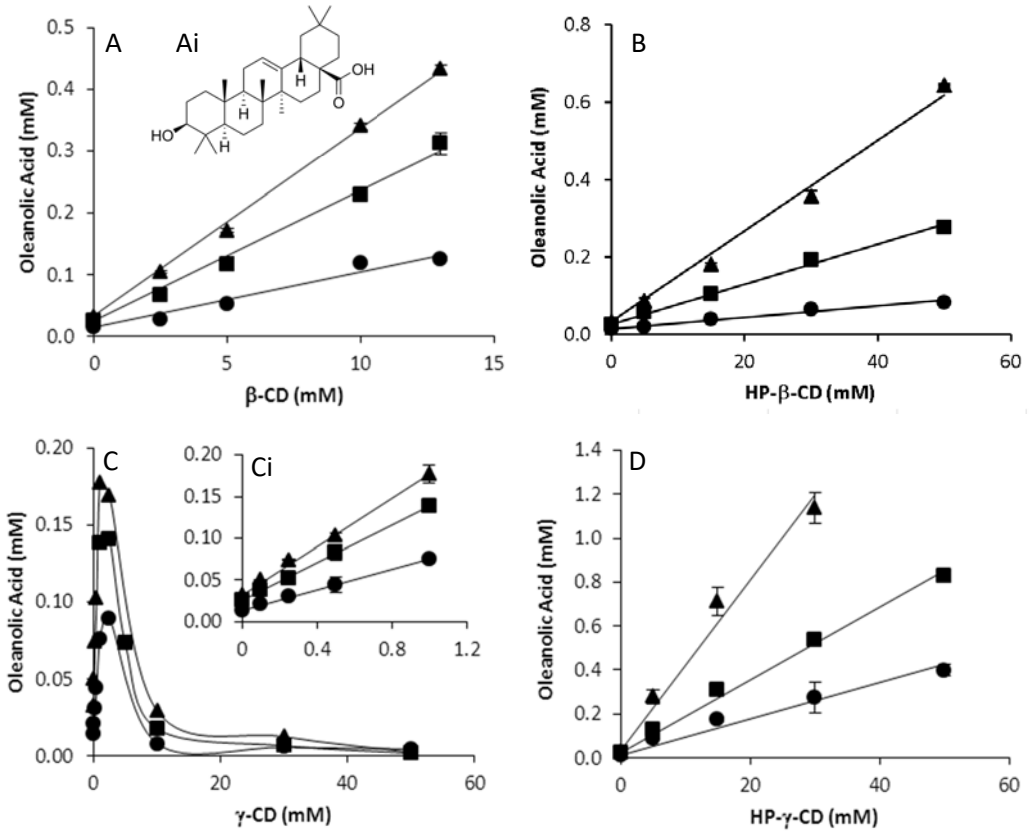

Figure 1. Phase solubility diagrams of oleanolic acid with β-CD (A), HP-β-CD (B), γ-CD (C and Ci) and HP-γ-CD (D) in aqueous solution at 4 ºC (●), 25 ºC (■) and 65 ºC (▲). Oleanolic acid chemical structure (Ai).

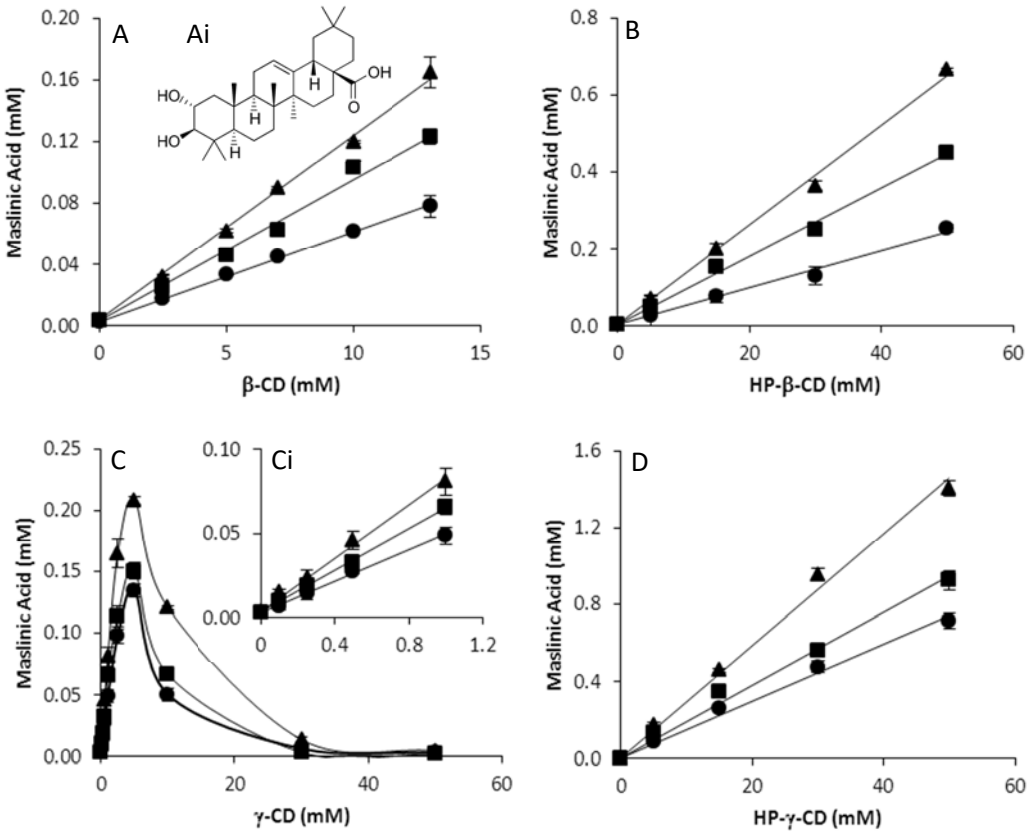

Figure 2. Phase solubility diagrams of maslinic acid and β-CD (A), HP-β-CD (B), γ-CD (C and Ci) and HP-γ-CD (D) in aqueous solution at 4 ºC (●), 25 ºC (■) and 65 ºC (▲). Maslinic acid chemical structure (Ai).

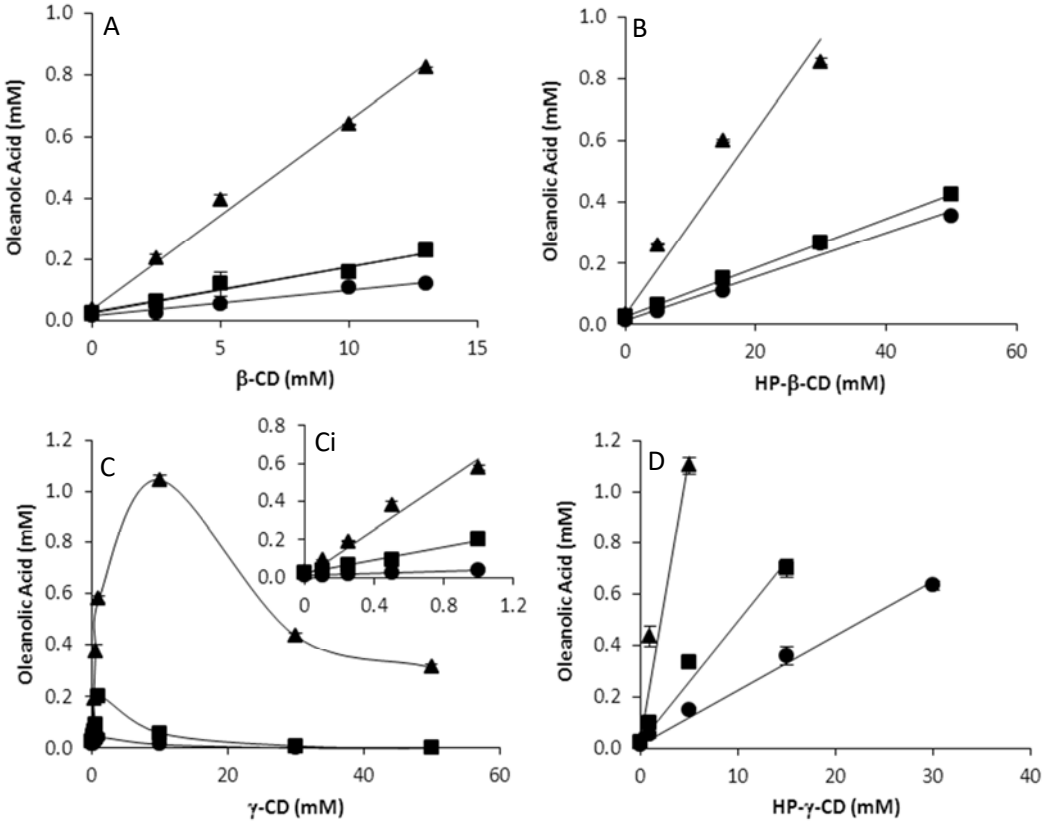

Figure 3. Phase solubility diagrams of oleanolic acid and β-CD (A), HP-β-CD (B), γ-CD (C and Ci) and HP-γ-CD (D) at pH 3 (●), pH 6.5 (■) and pH 9 (▲) at 25 ºC.

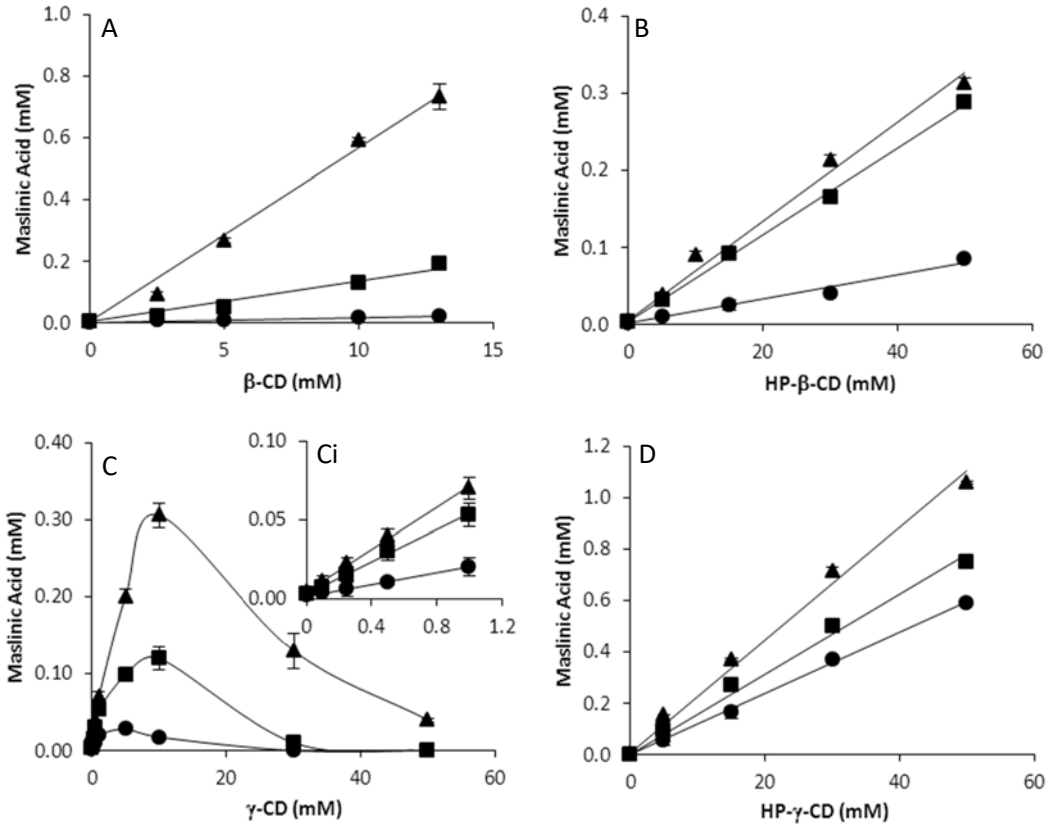

Figure 4. Phase solubility diagrams of maslinic acid and β-CD (A), HP-β-CD (B), γ-CD (C and Ci) and HP-γ-CD (D) at pH 3 (●), pH 6.5 (■) and pH 9 (▲) at 25 ºC.