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APPLICATION OF CYCLODEXTRINS TO THE EXTRACTION OF ANTIOXIDANT COMPOUNDS FROM OLIVE OIL

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Olive oil contains powerful antioxidant compounds which impart stability, contribute to various properties of it, and are valuable from the nutritional point of view. Their extraction with as mild conditions as possible led to its investigation using cyclodextrins as a tool. The inclusion ability of α-, β-, and γ-CD was estimated, and it has been demonstrated that the small cavity of α-CD as well as the wide one of γ-CD could enclose less effectively the antioxidant compounds of olive oil than the intermediate in shape cavity of β-CD. The highest yields of antioxidant compounds were achieved when olive oil was mixed with a 2% aqueous solution of β-CD and the resulting precipitate was treated with ethyl alcohol.

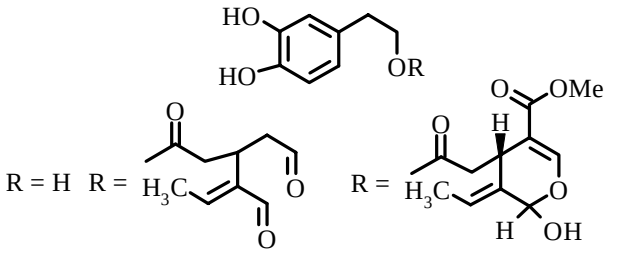
Key words: *α-*, *β-*, *γ-*cyclodextrin, *trans*-cinnamic acid, olive oil antioxidants.

Olive oil is a good source of natural antioxidants which show several functional, nutritional, and sensory properties: inhibition of blood platelet aggregation and phospholipid oxidation [1], protection of human erythrocrytes against oxidative damages [2], correlation with the pungent and bitter taste of oil, reduction of the oxidative process of fruity flavored aromatic compounds, and improvement of the olive oil shelf-life [3].

The present study was undertaken in order to propose a reliable method for the extraction of the phenolic compounds from olive oil with as mild conditions as possible. It is based on the ability of cyclodextrins (CDs) to form stable inclusion complexes with phenolic compounds [4–9]. Cyclodextrins are obtained from the action of *Bacillus macerans* amylase on starch and consist of homogeneous cyclic α-(1→4) linked *D*-glucopyranose units. α-cyclodextrin (α-CD), cyclohexaamylose, which comprises 6 glucopyranose units has, a cavity with an internal diameter of about 6Å, while β-cyclodextrin (β-CD), cycloheptaamylose, comprising 7 glucopyranose units, has a cavity of 8Å. The diameter of γ-cyclodextrin (γ-CD), cyclooctaamylose, comprising 8 such units, is about 10Å [10].

Our interest is focused on the extraction of phenolic compounds from olive oil which, according to the literature [11], contribute most to its antioxidant activity. These are 3,4-dihydroxyphenylethanol, simple and ester form (3,4-DHPEA and 3,4-DHPEA-EDA), and an isomer of oleuropein aglycon (3,4-DHPEA-EA).

trans-Cinnamic acid (CA) has been used as a reference guest compound in order to evaluate the optimum parameters which affect the formation of the inclusion complexes and to verify that the antioxidant compounds can be released from olive oil at satisfactory levels.



3,4-DHPEA 3,4- DHPEA - EDA 3,4-DHPEA -EA

Department of Food Science and Technology, Agricultural University of Athens Iera Odos 75, Votanikos 11855, Athens, Greece fax no:++30210 7254109, e-mail: chem-eng@ath.forthnet.gr. Published in Khimiya Prirodnykh Soedinenii, No. 1, pp. 18-21, January-February, 2005. Original article submitted July 27, 2004.

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TABLE 1. Maximum Amount of *trans*-Cinnamic Acid Determined in the Aqueous and Oil Phase, When 7.40 mg of it Were added in 10 g Olive Oil and Mixed with a 2% Aqueous Solution of α- or β- or γ-CD

	Amount of CA in the phase		CA complexed with CD	
	aqueous, mg*	oil, mg	amount, mg	%**
α-CD	1.097±0.009	0.484±0.003	5.819	79
β-CD	0.126±0.002	0.194±0.001	7.080	96
γ-CD	0.698±0.003	0.199±0.002	6.521	88

*Number of repetitions = 10; **Bound/originally added × 100.

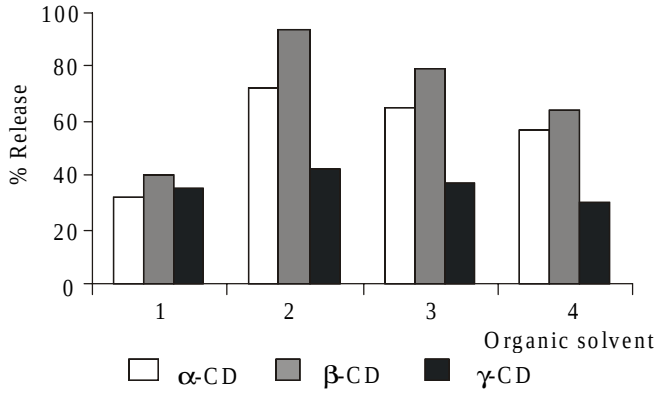

Fig. 1

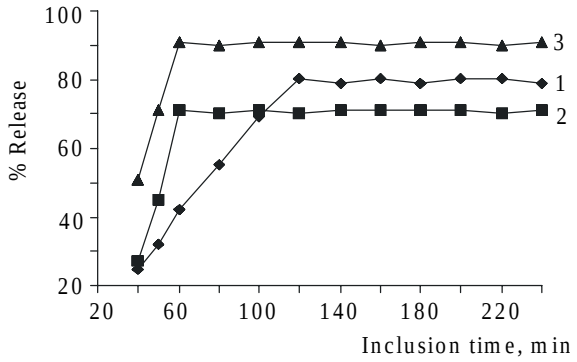

Fig. 2

Fig. 1. % Release of *trans*-cinnamic acid from the inclusion complex with α-, β-, and γ-CD. Organic solvents used: 1 - methanol, 2 - ethanol, 3 - ethyl ether, 4 – chloroform. Reaction time for complex formation: 1 h.
Fig. 2. % Release of *trans*-cinnamic acid from the inclusion complex with α- (2), β- (3), and γ-CD (1) versus time, after treatment of the precipitate with ethyl alcohol. Number of repetitions=10.

Complexation of Cinnamic Acid with α-, β-, and γ-CD. In the first part of the study a known amount of *trans*-cinnamic acid was spiked in olive oil and its percent release, following its complexation with α-, β- and γ-CD, was determined. The influence of the organic solvent used for the extraction of the guest compound, the inclusion ability of each CD, as well as the time of reaction have been evaluated. The aim was to estimate the recovery yields of the antioxidant compounds that can be achieved with the proposed method.

The binding of CA to CDs was assessed initially by evaluating the distribution of CA in the components of the system: oil, water, and cyclodextrin. The concentration of CA in the first two components was determined analytically whereas the complexed amount was estimated by the difference of CA in the oil and water phase and that originally added to the system. Table 1 shows that the amount of CA detected in the aqueous phase was 14.8%, 1.7% and 9.4% of that originally added in the mixtures with α-, β-, and γ-CD respectively. In the case of α-CD, 6.5% of CA remained in the oil phase while in the systems of β- and γ-CD, only 2.6% of CA was measured in the oil phase. Among the three CDs tested, β-CD was estimated to bind approximately 96% of the CA originally added to the system.

These assumptions will be confirmed thereafter by the analytical determination of the amount of CA that can be released from the resulting complex with each CD.

Influence of the Nature of the Organic Solvent on the Release of *trans*-Cinnamic Acid from the Inclusion Complexes with α-, β-, and γ- CD. The complex which resulted from the reaction of CDs with CA was added to either ethanol or methanol or chloroform or ethyl ether, as described in detail in the experimental section, and the percent release of CA was determined by HPLC. Figures 1, 2, and 3 indicate that ethanol is the best solvent for the release of CA from its inclusion complex with the three CDs. It appears that ethanol, due to its shape, can fit better than chloroform and ethyl ether in the CD cavities and due to its lower polarity, in comparison with that of methanol, can penetrate more easily than the latter in them.

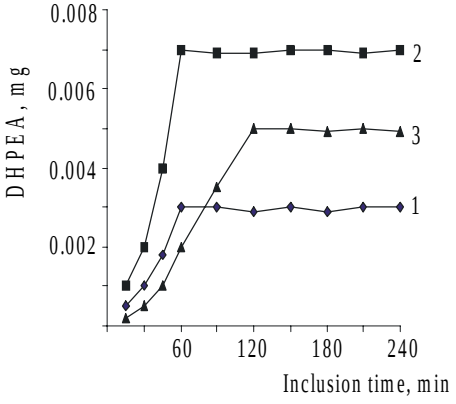

Fig. 3

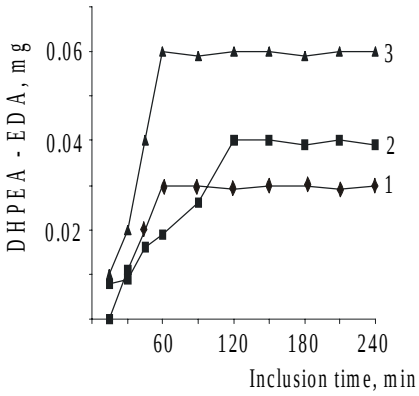

Fig. 4

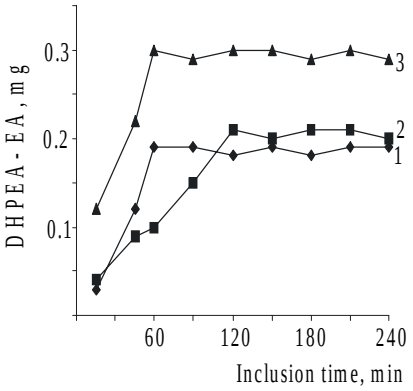

Fig. 5

Fig. 3. Amount of 3,4-DHPEA released from the inclusion complex with α- (1), β- (2) and γ- CD (3) versus time, after treatment of the precipitate with ethyl alcohol. Number of repetitions=10.

Fig. 4. Amount of 3,4-DHPEA-EDA released from the inclusion complex with α- (1), β- (3) and γ- CD (2) versus time, after treatment of the precipitate with ethyl alcohol. Number of repetitions=10.

Fig. 5. Amount of 3,4-DHPEA-EA released from the inclusion complex with α- (1), β- (3) and γ- CD (2) versus time, after treatment of the precipitate with ethyl alcohol. Number of repetitions=10.

Consequently, ethanol molecules can very effectively exclude the complexed compound from the cavity of cyclodextrins resulting in the dissociation of the complex. The experimental results of this work are in accordance with the literature concerning the ethanol being the best solvent for the release of bromobenzene and phenols from a complex with α- and β-CD [12].

Inclusion Ability of α-, β-, and γ-CD. The amount of *trans*-cinnamic acid complexed with CDs was found to have a linear relationship with the time of reaction, reaching a plateau in 1 hour in the case of α- and β- CD and in 2 hours in the case of γ-CD. This plateau corresponded to 91% binding to β- CD, 82% to γ-CD, and 72% to α-CD (Fig. 2). Therefore, it can be assumed that the intermediate in shape cavity of β-CD can more effectively include the CA molecules, an observation which is in accordance with our previous work, where it has been proved that in aqueous model systems the complexation of CA was favored in the case of β-CD. At this point we should consider that the driving force of complexation is the reduction of the free energy of the system, which takes place when the poorly hydrated guest molecule penetrates into the nonpolar CD cavity, exposing there water molecules of high enthalpy. In the case of γ-CD the phenomenon of complexation seems to be slower since its wide cavity can accommodate so many water molecules that their properties resemble water molecules in the bulk of the solvent. On the other hand, the binding force between the components of the complex appears to be greater in the case of β-CD since the CA molecule can penetrate more easily into the β-CD cavity than in the smaller one of α-CD interacting more strongly with its cavity walls.

Complex Formation of Antioxidant Compounds of Olive Oil with α-, β-, and γ-CD. The objective of the present work, which is part of a thorough study on the complexation of CDs with phenolic compounds, is to propose a new method for the extraction of antioxidant compounds from olive oil. Since it has been demonstrated that *trans*-cinnamic acid can be released from olive oil at high recovery levels after its complexation with cyclodextrins, in the second part of the study the amounts of the olive oil antioxidants have been determined following the prescribed extraction procedure.

Having in mind that phenyl alcohols, phenyl acids, flavonoids, and secoiridoids are characterized by different affinities with different organic solvents, the release of the phenolic compounds has been determined with four different solvents (methanol, ethanol, ethyl ether, and chloroform). The results indicated no selective effect of the solvent on the release of the phenolic compounds. In fact the yields of all the antioxidant compounds of interest were higher when the inclusion complexes were treated with ethanol. This observation demonstrates that the predominant factor for the release of phenolic compounds from the inclusion complex is the ability of the solvent to penetrate into the cyclodextrin cavity and not the affinity of each compound for the solvent.

As illustrated in Figs. 3, 4, and 5, the release of the antioxidant compounds was linear versus time and reached its maximum value in one hour for the complexes formed with α- and β-CD and in two hours for those formed with γ-CD. Furthermore, the highest yields of the antioxidants were obtained in the case of β-CD, the complexation with α-CD led to less than half the amounts, whereas γ-CD gave intermediate values for all the compounds under study.

The results indicate that it is the size of the CD cavity and not the phenolic compound that is the parameter determining the extent and time of complexation. This assumption can be easily justified if one takes into consideration the driving force of complexation, which has been discussed above, as well as the fact that the molecular structures of the antioxidant compounds of interest do not differ significantly.

In conclusion, it should be pointed out that the method developed in this study can be considered as a new technique for the extraction of olive oil antioxidants. It has been demonstrated that antioxidant compounds can easily form inclusion complexes with α-, β-, and γ-cyclodextrin and by the use of β-CD they can be released from olive oil at recovery levels of more than 90%. It appears that the proposed procedure has something new to add to the existing ones [11, 13–15]. It may be characterized as a mild and simple extraction technique, as it requires small amounts of only one solvent, simple laboratory equipment, and short preparation time. The high yields of antioxidant compounds, which can be achieved with very good reproducibility, constitute a major asset of the proposed method.

EXPERIMENTAL

Olive oil was purchased from the local market and was purified with a 80/20 v/v mixture MeOH/H_2O to strip off the antioxidants in the experiments involving *trans*-cinnamic acid.

α-, β-CD (purity 99% and 97%, respectively) and cinnamic acid (97%) were purchased from Sigma, and γ-CD (98.5%) from Merck. Methanol, ethanol, ethyl ether, and chloroform were of HPLC grade.

3,4-DHPEA, 3,4-DHPEA-EDA, and 3,4-DHPEA-EA were gifts from Professor G. Montedoro of University of Perugia, Italy.

Methods. The binding and extraction of phenolic compounds was optimized using CA as a reference-guest compound. A. The inclusion quantity of 7.40 mg of cinnamic acid was dissolved in 10 ml olive oil, contained in a 50 ml centrifuge tube. Ten ml of a 2% aqueous solution of α-, β-, or γ-CD was added to the centrifuge tube and the liquid phases were shaken on a SBS orbital agitator at 100 rpm, under nitrogen atmosphere, at 22°C. For the determination of the optimum reaction time, the time of agitation ranged from 40 to 240 min. The water and oil phase were separated from the precipitate by centrifugation at 4000g for 5 min B. Extraction. For the dissociation of the CA-CD complex, the precipitate was mixed with 5 ml ethanol for 10 min. After centrifugation at 3000 g for 4 min, the liquid phase was analyzed by HPLC. Methanol, chloroform, and ethtyl ether substituted for ethanol to assess their potential to dissociate the cinnamic acid – CD complex. Water also substituted for cyclodextrins in the blank.

For the isolation of olive oil antioxidants, the above procedure was used without the addition of *trans*-cinnamic acid.

HPLC Analysis. The qualitative and quantative determination of the phenolic compounds was performed on a Waters HPLC system, consisting of a 600 pump and a 996 PDA detector. The compounds were monitored from 220–400 nm and chromatograms at 278 and 239 nm were extracted. The chromatographic separation was achieved on a Nova Pak C18 column (4.6 × 250 mm) obtained from Waters at room temperature. The mobile phase consisted of 2% acetic acid (A) and methanol (B) at the following gradient elution: 95% A-5% B in 2 min, 75% A- 25% B in 5 min, 60%A- 40%B in 15 min, 50% A -50% B in 25 min, and 40% A- 60% B in 40 min.

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