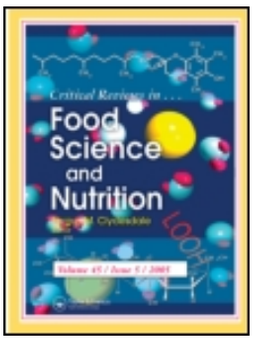

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Cyclodextrins and Antioxidants

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In recent years, the growth of the functional foods industry has increased research into new compounds with high added value for use in the fortification of traditional products. One of the most promising functional food groups is those enriched in antioxidant compounds of a lipophilic nature. In spite of the numerous advantages reported for such antioxidant molecules, they may also have disadvantages that impede their use in functional foods, although these problems may well avoided by the use of encapsulant agents such as cyclodextrins. This explains the recent increase in the number of research papers dealing with the complexation of different guest molecules possesing important antioxidant properties using natural and modified cyclodextrins. This paper presents a review of the most recent studies on the complexes formed between several important types of antioxidant compounds and cyclodextrins, focusing on the contradictory data reported in the literature concerning to the antioxidant activity of the host/guest molecule complexes, the different complexation constants reported for identical complexes, the bioavailability of the antioxidant compound in the presence of cyclodextrins and recommendation concerning the use of natural or modified cyclodextrins. Moreover, the use of cyclodextrins as antibrowning agents to prevent enzymatic browning in different foods is revised. Finally, we look at studies which suggest that cyclodextrins act as "secondary antioxidants," enhancing the ability of traditional antioxidants to prevent enzymatic browning.

Keywords Cyclodextrins, antioxidant, carotenoids, stilbenes, fatty acids, coenzyme Q_{10}

INTRODUCTION

One of the priorities of the food industry is the development of functional foods with high added value such as its the case of antioxidant compounds of lipophilic nature used to fortify hydrophobic solvents. Although the benefits of these compounds have been demonstrated, their use as functional ingredients in aqueous media has several disadvantages. First, the use of these molecules as fortifiers and nutraceuticals is limited by their poor solubility in water. Secondly, the presence of structures in these compounds means that they are easily oxidized by prooxidant agents. Finally, although these compounds are well absorbed by humans when taken orally, their bioavailability is quite low as a result of their rapid metabolism and elimination.

In recent years, several novel foods supplemented with a variety of hydrophilic antioxidant compounds have appeared on the market, although the above mentioned problem has meant that very few of these foods can be enriched with hydrophobic antioxidants molecules. It is for this reason that the complexation of these antioxidant compounds in aqueous medium with molecules such as cyclodextrins (CDs) has been tried in order to increase their stability, bioavailability, and solubility.

CDs are naturally occurring cyclic oligosaccharides derived from starch with six, seven, or eight glucose residues linked by $\alpha(1–4)$ glycosidic bonds (Szente and Szejtli, 2004) (Fig. 1). The steric arrangement of these glucose units in the CD molecule results in a hollow truncated cone with a hydrophilic outside surface, which makes CDs water soluble, and a hydrophobic internal cavity, which enables CDs to form inclusion complexes with various hydrophobic guest molecules (Li et al., 2007). The advantageous changes in guest molecule properties after the formation of inclusions complexes with CDs have led to many applications of CDs in industries related with food, pharmaceuticals, cosmetics, chemicals, agriculture, etc. (Martin del Valle, 2004; Szente and Szejtli, 2004).

CDs are produced from starch or starch derivates by using cyclodextrins glycosyltransferase (CGTase, EC 2.4.1.19). The resulting enzymatic product is usually a mixture of CDs, mainly α-, β-, and γ-CD consisting of six, seven, or eight glucose units, respectively (Fig. 2), and trace amounts of large-ring CDs with more than nine glucose units (Endo et al. 2002). Although a few interesting large-ring CDs (modified CDs) showing novel structural features have been isolated and characterized during the past decade (Zheng et al., 2002; Qi et al., 2007), the most

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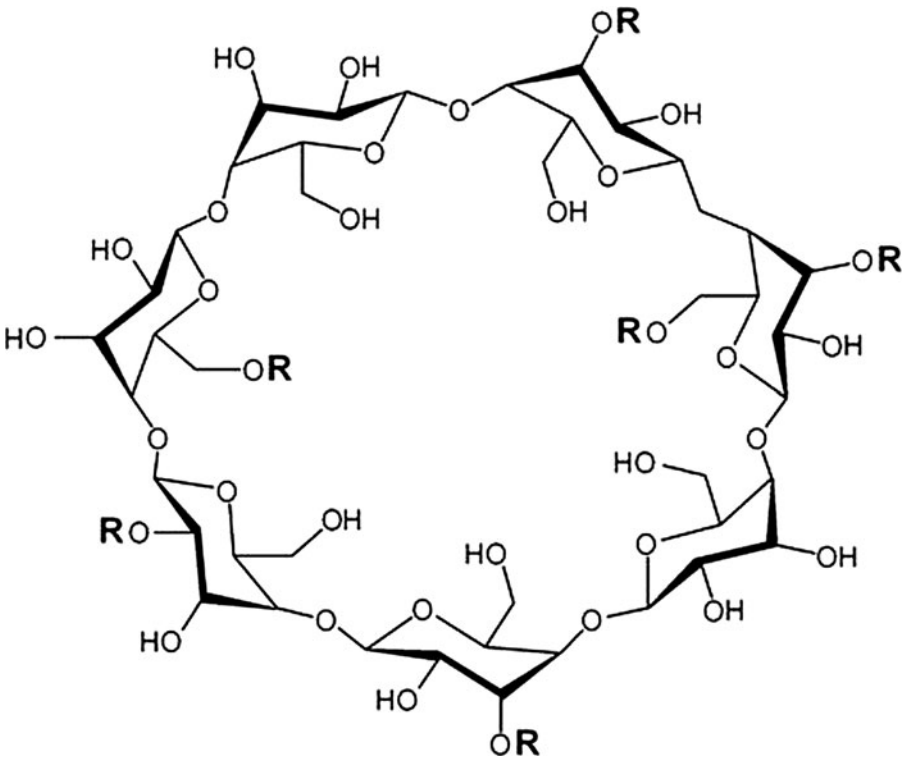

Figure 1 Chemical structure of a cyclodextrin.

extensively studied and utilized products remain α-, β-, and γ-CDs (Szejtli, 1998). Moreover, α-, β-, and γ-CD enjoy GRAS status and have been approved recently as additives in the European Union. Indeed, all three natural CDs have recently been included in the European lists of additives approved for alimentary use with the corresponding E-numbers E-457, E-459, and E-458 assigned to α-, β-, and γ-CD, respectively.

After the first fundamental review on CDs in 1957 (French, 1957), several other excellent reviews and monographs have been published, providing readers with a compilation of CD-related literature (Szejtli, 1990; Singh et al., 2002; Martin del Valle, 2004). As regards the food field, most previous reviews focused on the aspects of CDs related with their impact on the sensory characteristics of foods, food packaging, nutrient

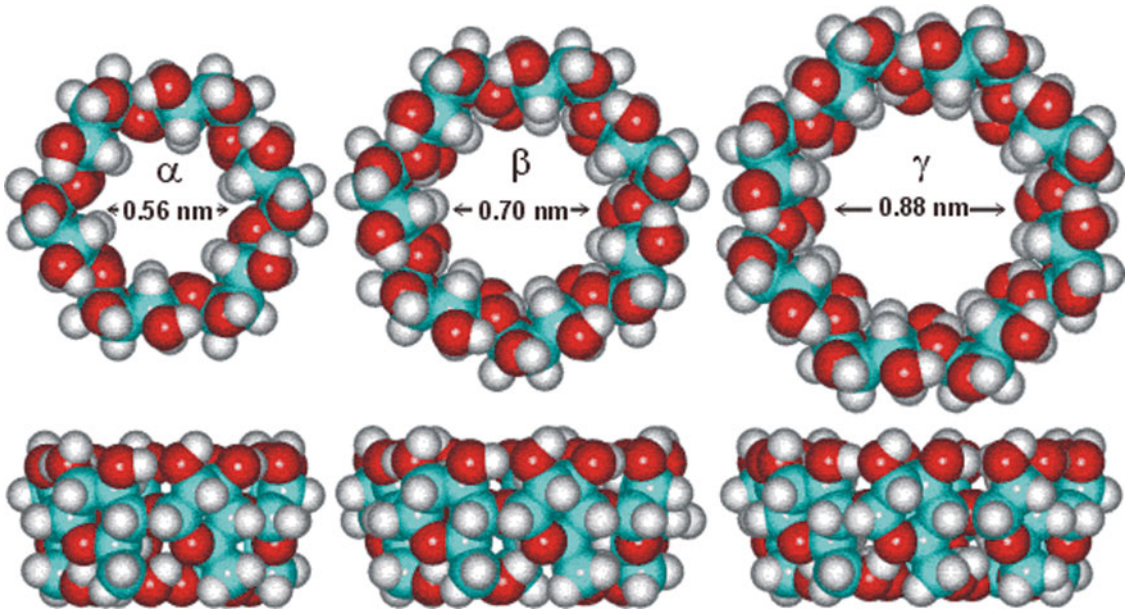

Figure 2 Structures of the natural cyclodextrins α, β, and γ. (Color figure available online.)

sequestering properties, on food preservation, or on the nutritional properties of different food products (Szente and Szejtli, 2004; Carvotto et al., 2006; Astray et al., 2009). However, in this review we focuse on the literature published about the effect of the encapsulation of different lipophilic antioxidants used in the design of functional foods on some aspects such as their solubility, bioavaibility, application, or antioxidant activity.

In recent years, several compounds with antioxidant properties have been complexed with CDs. However, the contradictory data published concerning: (i) the complexation constant (K_F) values, (ii) the nature of the best type of CD for the complexation process, (iii) the effect of the complexation on the antioxidant capacity of the guest molecules, and (iv) the influence of encapsulation on the bioavaibility of the antioxidant compound, means that an exhaustive revision of the complexation process between important groups of antioxidants (carotenoids, stilbenes, fatty acids, vitamins, phenols, and Coenzyme Q_{10}) and both natural and modified CDs is overdue.

Moreover, one of the main factors which must be controlled to prevent the browning of several foods is the enzymatic activity of polyphenoloxidase (PPO) and much research has focused on the use of postharvest chemical treatments to avoid such browning (Sánchez-Ferrer et al., 1995). However, many of these treatments present serious disadvantages for use in the food industry. Although synthetic antioxidants have a higher antibrowning effect in foods than those from natural sources, there is growing consumer interest in natural antioxidants. For this reason CDs have been recently used to prevent the enzymatic browning of different foods by encapsulating the phenols (natural substrates of PPO) present in the same.

Finally, in this review, we revise the capacity of CDs as *"secondary antioxidants"*. Another strategy to avoid the browning of foods would be to look for "*preservers*" of the natural antioxidant capacity of a particular food. In this review, we show how CDs can enhance the ability of other antioxidants to prevent the enzymatic browning due to the protective effect they offer against the oxidation of these antioxidants. In this respect, CDs seem to act as a *"secondary antioxidants"*, reducing food browning and enhancing the naturally occurring antioxidant capacity of the food itself.

CYCLODEXTRINS AND STILBENES

Stilbenes are a small family of plant secondary metabolites derived from the phenylpropanoid pathway, and produced in a number of unrelated plant species (Chong et al., 2009). These compounds are involved in various ways in plant disease resistance and human health. One of the most important properties of stilbenes is their antioxidant activity. In recent years, several works on the encapsulation of these molecules with CDs have been published. However, contradictory results have been reported concerning the effect of the complexation of stilbenes by natural and modified CDs on the physico-chemical and functional properties of these natural molecules. For this reason, we hope to throw light on this problem by revising the data published on the encapsulation of four important stilbenes (resveratrol, pterostilbene, pinosylvin, and oxyresveratrol, Fig. 3) by CDs.

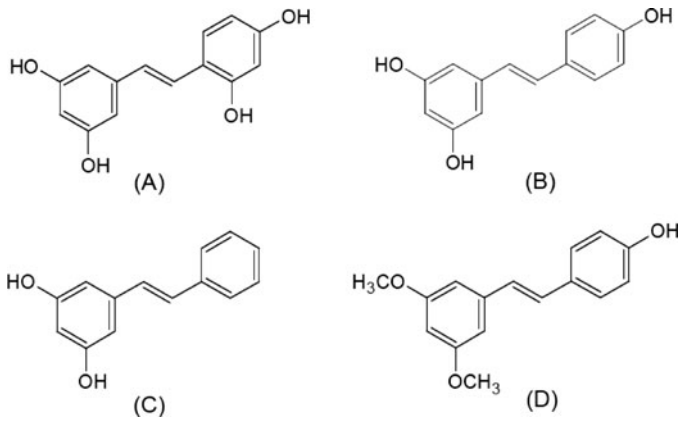

Figure 3 Structure of oxyresveratrol (A); resveratrol (B); pinosylvin (C); and pterostilbene (D).

Resveratrol

Resveratrol (3,5,40-trihydroxy-stilbene) (Fig. 3B) is a phytoalexin found in at least 72 species of plant distributed among 31 genera and 12 families (Jang et al., 1997). In recent years, research into resveratrol has uncovered several beneficial biological effects of this compound on human health (Latruffe et al., 2002). However, the limited number of foods with high levels of resveratrol has led to the search for new strategies to incorporate this in new foods. Recently, transgenic fruits, including lettuce and apple (Ruhmann et al., 2006), with high levels of free resveratrol or its derivates, have been developed. Another alternative is to use resveratrol as an ingredient in the functional food industry as a fortifier and nutraceutical compound.

Although the benefits of this stilbene have been demonstrated, its use has several disadvantages as a functional ingredient in aqueous medium. Although resveratrol is well absorbed by humans when taken orally, its bioavailability is quite low as a result of its rapid metabolism and elimination (Walle et al., 2004). Furthermore, the use of this stilbene as fortifier and nutraceutical is limited by its poor solubility in water (less than 0.05 mg/ml). Finally, the above mentioned presence of conjugated double bounds on the structure of these compounds means that they are easily oxidized by different prooxidant agents (Fan and Mattheis, 2001). However, the above mentioned problems which prevent resveratrol from being used as a fortifier of foods in an aqueous medium have meant that no novel food has been enriched in this important antioxidant compound. Due to these problems, the complexation of resveratrol in aqueous medium using compounds to increase its stability, bioavailability, and solubility is considered a worthwhile goal. The use of CDs is

promising in this respect and its has been reported in several recent papers.

In these works, different methods have been used to determine the K_F values (e.g., liquid chromatography, enzymology, solubility, RMN, Fourier transform infrared spectroscopy, differential scanning calorimetry, or X-ray diffraction). However, most works published have limited their investigation to the determination of K_F values using techniques based on solubility (Bertacche et al., 2006), enzymology (Lucas-Abellán et al., 2007) or liquid chromatography, (López-Nicolás et al., 2006; López-Nicolás and García-Carmona, 2008). However, results concerning the K_F values of resveratrol complexed by CDs under different reaction conditions are contradictory. While some papers have claimed that natural CDs are the most suitable for the inclusion process (Bertacche et al., 2006), others have reported that modified CDs are more appropriate for complexing resveratrol (Lucas-Abellán et al., 2007). Moreover, papers have reported very different K_F values for the same type of complexes. Finally, contradictory data have been published about the effect of the encapsulation with CDs on the antioxidant activity of resveratrol.

Our study may help to resolve this problem by taking a look at several important aspects of the complexation mechanism, such as the aggregation state of resveratrol, the pH values of the reaction medium, the pKa values of the guest molecule and the UV–vis or the fluorospectrometric properties of resveratrol in the presence of CDs.

HPLC is an appropriate technique for determining the stoichiometry and K_F values of resveratrol/CD complexes (López-Nicolás et al., 2006; López-Nicolás et al., 2008). In organic medium, the complexation of resveratrol with β-CD was investigated using reversed-phase liquid chromatography and mobile phases to which β-CD was added. López-Nicolás et al. (2006) reported a decrease in retention times with increasing concentrations of β-CD (0–2.5 mM), and showed that resveratrol forms a 1:1 complex with β-CD, while the apparent K_F values were strongly dependent on the water–methanol proportion of the mobile phase used. Moreover, the K_F values for the resveratrol–β-CD interaction decreased when the temperature was raised from 20 to 37°C In order to gain information about the mechanism of resveratrol affinity for β-CD, the thermodynamic parameters of the complexation were obtained by López-Nicolás et al. (2006). Their results showed that the complex formation of resveratrol with β-CD ($AG^\circ = -17.01$ kJ/mol) is largely driven by enthalpy ($AH^\circ = -30.62$ kJ/mol) and slight entropy ($AS^\circ = -45.68$ J/mol K) changes.

As regards complexation in aqueous medium using HPLC, López-Nicolás and García-Carmona (2008) studied the complexation of resveratrol with natural CDs in aqueous medium under different physicochemical conditions (pH or temperature) which are essential if this antioxidant compound is to be used successfully in the food industry as an ingredient of functional foods due to its poor stability, bioavailability and solubility. In the above paper, a rapid, simple, and sensitive way of determining of the apparent K_F values of resveratrol/CD complexes by HPLC in aqueous medium was investigated for the first time. It was observed that resveratrol forms a 1:1 complex with α-, β-, and γ-CD. The highest value of the apparent K_F values ($K_F = 1922 \pm 89$ M^{-1}) was found for β-CD and a strong dependence of K_F on pH was seen in the region where resveratrol begins the deprotonation of its hydroxyl groups. Moreover, López-Nicolás and García-Carmona (2008) reported that an increase in the system's temperature produced a decrease in the values of K_F.

A complete study of the host–guest interaction of resveratrol with natural and modified CDs was published by Bertacche et al. (2006). The main objective of that paper was to increase the stability and water solubility of resveratrol by complexation with different CDs. Furthermore, the physical–chemical properties of each inclusion compound were investigated by these authors. Complexes of resveratrol with CDs, both native (α-, β-, γ-CD) and modified (HP-CD, dimethyl-β-CD), were obtained by using the suspension method. The authors also prepared an inclusion complex with β-CD by microwave and characterized the solid state of the products using Fourier transform infrared spectroscopy, differential scanning calorimetry and X-ray diffraction. The solution studies were performed by UV–Vis spectrophotometry and H-NMR spectroscopy. In the same paper, phase solubility profiles with all the CDs used indicated the formation of 1:1 stoichiometric inclusion complexes and the K_F values were calculated for each case from the phase solubility diagrams, values in the same order as those shown by other author being reported. Moreover, stability studies in the solid state and in solution were performed, while the photodegradation by UV–Vis spectrophotometry showed that the isomerisation rate *trans* to *cis*, in ethanol solution, decreased with inclusion.

In another paper, Lucas-Abellán et al. (2007) studied the formation of resveratrol/CD inclusion complexes in aqueous solutions using the hydroperoxidase activity of lipoxygenase (LOX) as the enzymatic system. The addition of CDs to the reaction medium had an inhibitory effect on resveratrol oxidation by LOX due to the complexation of phytoalexin into the CD cavity, which is in equilibrium with free CDs and free resveratrol, the only effective substrate for LOX. This inhibitory effect, which has also been described in the case of the oxidation of other substrates, such as fatty acids by LOX (Bru et al., 1995; López-Nicolás et al., 1997), depends on the K_F values between resveratrol and the type of CD used. In the work of Lucas-Abellán et al. (2007), β-CD and maltosyl-β-CD were used and their K_F values were calculated by nonlinear regression of the inhibition curves obtained in the presence of CDs. The values obtained were 4317 and 5130 M^{-1} for β-CD and maltosyl-β-CD, respectively.

In addition, different methods were used by Lucas-Abellán et al. (2008b) to study the complexation of resveratrol with native CDs (α-; β-, and γ-CD) and modified CDs (HP-β-; maltosyl β-; methyl-β-; carboxymethyl- β-; and acetyl-β-CDs) and the K_F were compared. The K_F values between resveratrol and each type of CD were calculated using three different methods: enzymatic, solubility, and fluorimetric. The K_F values obtained by

Lucas-Abellán et al. (2008b) showed that HP-β-CD with their very high K_F of 18,048 $\pm$ 625 M^{-1} were the most effective type of CD for complexing resveratrol. Moreover, comparison of the results obtained by the three methods revealed that the fluorimetric method undervalued the K_F values between resveratrol and all the CDs, while the enzymatic and solubility methods were more precise for calculating the K_F values between resveratrol and CDs. However, these undervaluated K_F values obtained by the fluorimetric method have been discussed recently in a paper published by López-Nicolás and García-Carmona (2010).

In that paper, the authors demonstrated that, at pH values higher than the pK_{a1} of resveratrol, the coexistence of different protonated/deprotonated forms of this antioxidant does not permit the fluorimetric determination of the K_F value of resveratrol with HP-β-CD. However, when the Hildebrand–Benesi equation was used to calculate this constant at physiological pH, the problem was resolved, a K_F value of 14,490 $\pm$ 723 M^{-1} and a 1:1 stoichiometry of the complexation process being found for all the cases tested. The results obtained in this paper resolve the contradictory data published by Lucas-Abellán et al. (2008b) about the complexation process of resveratrol by CDs. Moreover, López-Nicolás and García-Carmona (2010) studied the aggregation state of resveratrol in the presence and absence of HP-β-CD using absorption and steady-state fluorescence at different pH values. The results revealed that this potent antioxidant shows a monomer/aggregate equilibrium which is dependent on the protonation state of resveratrol. This equilibrium can be modified by the presence of HP-β-CD, which reduces the aggregation of the resveratrol molecules, producing individual molecules of the solute and preventing side effects due to aggregation phenomena.

As a second part of this work, our research group recently investigated the influence of the presence of CDs on the hydroperoxidation of resveratrol by LOX, bearing in mind the protonation and aggregation state of this potent antioxidant (López-Nicolás et al., 2009c). When the enzyme uses monomers of resveratrol as substrate, LOX shows a Michaelian behavior but when the resveratrol concentration is increased to values higher than critical it shows strong inhibition. These results can be interpreted as a previously unreported aggregate-induced enzyme inhibition, which can be modified by the use of modulators of the aggregation state of resveratrol such as CDs. Indeed, the addition of increasing concentrations of HP-β-CD produced a change in the LOX enzymatic activity due to the ability of CDs to sequester part of the resveratrol to form soluble inclusion complexes, thereby reducing the concentration of the free resveratrol. Thus, free resveratrol is the only effective substrate and the oxidation of the complexed substrate requires the previous dissociation of the complex. When HP-β-CD is added to the reaction medium, the observed inhibition by substrate aggregation is produced at a higher resveratrol concentration than observed in the absence of any agent. Thus, the concentration of resveratrol at which the LOX activity was inhibited increased in the presence of HP-β-CD, reflecting the formation of inclusion complexes and resulting in the extension of the range of monomeric resveratrol. This result is due to the ability of CDs to increase the critical concentration at which the aggregation of different substrates is produced, thus widening the apparent monomer concentration range (Bru et al., 1995; López-Nicolás et al., 1995). Indeed, as deduced from the fluorescence experiments and our previous studies (López-Nicolás et al., 1995), free and complexed monomers of resveratrol exist in the premicellar region, whereas in the postmicellar region there is the additional presence of resveratrol aggregates. In both regions, the complexed resveratrol simply constitutes a pool of substrate to which the enzyme might or might not have direct access.

In a recent work, the photostability and biological properties of the resveratrol/ HP-β-CD complex were studied by Sapino et al. (2009). When the rate of resveratrol degradation was followed in different systems under UVA irradiation, the results showed the photoprotective effect of the complex.

An important paper on the application of the complexes formed between resveratrol and CDs in cellular biology was published by Bru et al. (2006), who investigated in detail the properties of CDs as elicitors of the defense responses in grapevine cell cultures. This group showed that certain modified CDs are able to elicit some of these responses, as evidenced by the high levels of accumulated resveratrol and stilbene-like compounds, changes in extracellular cell wall-like peroxidase and their isoenzyme expression pattern, as well as the effect on *B. cinerea* growth. In grapevine (*Vitis vinifera* L.), defense responses after microbial infection or treatment with elicitors involve the accumulation of phytoalexins, oxidative burst, and the synthesis of pathogenesis-related proteins. Oligosaccharide fractions from fungal or algal cell walls efficiently induced the defense responses, but a detailed analysis of the elicitor-plant cell surface interaction at the molecular level was precluded due to the lack of chemically pure oligosaccharide elicitors. The above work presented by Bru et al. (2006) was a continuation of the report by the same research group (Morales et al., 1998) in which they investigated the effect of DIMEB, the doubly methylated α-CD in hydroxyls 2 and 6, on resveratrol metabolism in grapevine cell cultures inoculated with the grapevine pathogenic bacteria *Xylophilus ampelinus*. The inoculated cell cultures accumulated significant levels of *trans*-piceid in the cells, whereas no resveratrol was observed in the extracellular medium. When the culture medium was supplemented with 5 mM DIMEB, both infected and noninfected cell cultures accumulated *trans*-piceid in the cells and secreted resveratrol in the spent medium. It was suggested that DIMEB might be acting as an elicitor independent of the presence of the bacterial pathogen.

In order to obtain information about the effect of the complexes between resveratrol and CDs on the bioavailability of the lipophilic antioxidant, Das et al. (2008) studied the impact of aqueous solubility and dose manipulation on the pharmacokinetics of resveratrol, using water-soluble intravenous and oral formulations of resveratrol prepared with HP-β-CD and randomly methylated-β-CD (RM-β-CD), respectively. Sodium salt and a suspension of resveratrol in carboxymethyl cellulose (CMC) were used as the reference intravenous and oral formulations,

respectively. The pharmacokinetics of resveratrol was assessed in Sprague–Dawley rats and the plasma resveratrol concentrations were measured by HPLC. The results showed that both HP-β-CD and RM-β-CD enhanced the aqueous solubility of resveratrol. However, after intravenous administration, rapid elimination of resveratrol was observed at all the tested doses (5, 10, and 25 mg kg^{-1}) regardless of formulation type, with non-linear elimination occurring at 25 mg kg^{-1}. RM-β-CD significantly increased the maximal plasma concentration of orally administered resveratrol, but did not increase the oral bioavailability in comparison with the CMC suspension. Furthermore, in the same paper, the oral bioavailability remained unchanged at all tested doses (15, 25, and 50 mg kg^{-1}). In conclusion, these authors suggested that the aqueous solubility barrier might affect the speed but not the extent of resveratrol absorption. Further dose manipulation (up to 50 mg kg^{-1}) did not have a significant impact on the oral bioavailability of resveratrol. These data rebat the hyphotesis that the use of CDs always increases the bioavailability of the guest molecule.

In another interesting paper (Lu et al., 2006), the molecular modeling of the complexation of resveratrol with two kinds of CD, β-CD and HP-β-CD, was carried out. The molecular modeling showed that part of the A-ring and the B-ring of resveratrol are placed in the cavity of β-CD, and the hydroxyl groups are projected outside. As regards resveratrol in HP-β-CD, the B-ring of resveratrol is included in the cavity of HP-β-CD, and part of the A-ring is points outwards. ^{1}H-NMR spectroscopy showed that H2, H3, H4, and H5 protons of resveratrol are more affected by the complexation, indicating that they are located inside the torus of CDs, which is in agreement with the results of the molecular modeling.

In a recent work, Li et al. (2010) studied the thermal effects of inclusion processes of α, β-, γ-, and Mβ-CD with resveratrol in aqueous solutions by isothermal titration calorimetry with nanowatt sensitivity at the temperature of 298.15 K, and the standard enthalpy changes, stoichiometry and K_F of the inclusion complexes were derived from the direct calorimetric data by nonlinear simulation. Moreover, the thermodynamic parameters were discussed in the light of weak interactions between the host and the guest molecules combining with the UV spectral message. The results indicate that, although all the complexes formed in the aqueous solutions are in 1:1 stoichiometry, the binding processes of α-, β-, and Mβ-CD with the guest are mainly driven by enthalpy, while that of γ-CD with the drug is driven by both enthalpy and entropy.

As indicated previously, resveratrol is a potent lipophilic antioxidant. For this reason, the effect of complexation with CDs on its antioxidant capacity has been described in different papers. For example, Lucas-Abellán et al. (2008a) reported the effect of the complexation of resveratrol with HP-β-CD on the antioxidant capacity of this antioxidant using the oxygen radical absorbance capacity (ORAC) method, with fluorescein (FL) as the fluorescent probe. The method was validated through its linearity, precision, and accuracy for measuring the ORAC of resveratrol in the absence or presence of CDs. Lucas-Abellán et al. (2008a) reported that the complexation of resveratrol in CDs increased the net area under the FL decay curve of resveratrol up to its saturation level, at which the stilbene showed almost double the antioxidant activity it shows in the absence of CDs. For these authors, the antioxidant activity of resveratrol was dependent on the complexed resveratrol because CDs acts as a controlled dosage reservoir that protects resveratrol against rapid oxidation by free radicals. In this way, its antioxidant activity is prolonged and only reaches its maximum when all the resveratrol is complexed.

However, results contrary to those published by Lucas-Abellán et al. (2008a) were reported in another paper concerning the effect on the antioxidant activity published by Lu et al. (2009). These authors compared the scavenging capacity toward the DPPH of free resveratrol and complexed resveratrol at the same concentration. The concentration of resveratrol in resveratrol/6 mM β-CD and resveratrol/6 mM HP-β-CD complexes were 0.68 and 2.72 mM, respectively, as determined by the molar absorption coefficients of 40,800 M^{-1} cm^{-1} at 306 nm and 34,300 M^{-1} cm^{-1} at 307 nm. These authors reported that the differences in scavenging capacity between free and complexed resveratrol are slight, which suggests that the inclusion process had little influence on the antioxidant activity.

Finally, and also concerning the effect of CDs on the antioxidant activity of resveratrol, in the previously mentioned study by Sapino et al. (2009) the radical scavenging activity, the metal-chelating efficiency and the antilipoperoxidative potential of resveratrol were assessed: the data showed that the inclusion phenomenon did not significatively interfere with these biological properties. Finally, in vitro experiments revealed that the skin accumulation of resveratrol was higher when released from the complex then when deposited alone.

Pterostilbene

Pterostilbene (*trans*-3,5-dimethoxy-4′-hydroxystilbene) (Fig. 3D) is a stilbenoid present in different sources such as the berries of some *Vacciunium* species, the leaves of *Vitis vinifera*, the heartwood of sandalwood (*Pterocarpus santalinus*) and *Pterocarpus marsupium* or different medicinal products (Chong et al., 2009). This compound presents a variety of healthy properties, acting, for example, as antihyperglycemic, antioxidative, anticancer, antiinflammatory, anticholesterol, antifungus, hypolipidemic, or analgesic (Remsberg et al., 2008). In spite of these advantages, pterostilbene shows very poor solubility in water, possesses low bioavailability and is easily oxidized by several enzymes (Breuil et al., 1999). For these reasons, the complexation of pterostilbene with molecules which can increase its bioavailability, solubility, and stability in the face of prooxidant agents is strongly desirable, as it when CDs are used.

Recently, the complexation of pterostilbene with CDs was described by López-Nicolás et al. (2009a) using steady state fluorescence. Pterostilbene forms a 1:1 complex with different

natural and modified CDs. Among natural CDs, the interaction of pterostilbene with β-CD was the most efficient. However, all the modified CDs showed higher K_F than β-CD. The highest K_F was found for HP-β-CD (17,520 $\pm$ 981 M^{-1}), in which its value showed a strong dependence on pH in the region where the pterostilbene begins the deprotonation of its hydroxyl group. Moreover, the values of K_F decreased as the system temperature increased. Furthermore, to obtain information on the mechanism of pterostilbene affinity for CD, the thermodynamic parameters of the complexation (ΔH°, ΔS°, and ΔG°) were studied. Finally, a comparison of the K_F values obtained for three types of stilbenes revealed that both the stoichiometry and the K_F values of the complex are dependent on the structure of the guest molecule. While the resveratrol/HP-β-CD and pterostilbene/HP-β-CD complexes showed a 1:1 stoichiometry (with a higher K_F value for the resveratrol-HP-β-CD complexes), *trans*-stilbene showed a 1:2 stoichiometry.

Moreover, Rodríguez-Bonilla et al. (2011b) recently reported on a kinetic mechanism and provided a product characterization of the enzymatic peroxidation of pterostilbene as a model of the detoxification process of stilbene-type phytoalexins. In this work, the addition of increasing concentrations of HP-β-CD produced a change in the peroxidase enzymatic activity that depended on the pterostilbene concentration used. This typical behavior is due to the ability of both natural and modified CDs to sequester part of the pterostilbene to form soluble inclusion complexes, thereby reducing the concentration of the free pterostilbene. Thus, CDs act as substrate reservoir in a dose-dependent manner. Indeed, free pterostilbene is the only effective substrate and the oxidation of the complexed substrate requires the prior dissociation of the complex. Moreover, although CDs have usually been used for complexing the substrates of enzymatic reactions, due to the importance of the pterostilbene oxidation products in the detoxification of phytoalexins, the authors studied the potential effect of the addition of CDs on the three main products of the oxidation of pterostilbene by peroxidase. Indeed, the presence of increasing concentrations of HP-β-CD had different effects on the concentration of the three reaction products determined by HPLC.

Finally, in a recent work, (Rodríguez-Bonilla et al., 2011a), a RP-HPLC method was developed for the determination of pterostilbene in food samples. The novel method is based on the addition of CDs to the mobile phase where the complexation of pterostilbene by CDs is carried out. In order to select the most suitable conditions for the RP-HPLC method, the effect of several physico-chemical parameters on the complexation of pterostilbene by CDs was studied. The results show that the addition of 12 mM HP-β-CD to a 50:50 (v/v) methanol:water mobile phase at 25°C and pH 7.0 significantly improves the main analytical parameters. In addition, it was seen that pterostilbene forms a 1:1 complex with HP-β-CD, showing an apparent complexation constant of 251 $\pm$ 13 M^{-1}. Finally, in order to study the validity of the proposed method, blueberries were analyzed and the concentration of pterostilbene was been determined.

Pinosylvin

Pinosylvin (*trans*-3,5-dihydroxystilbene) $C_{14}H_{12}O_2$, (Fig. 3C) is a stilbenoid with several pharmacological (antimicrobial, antifungal, anticancer, antiinflammatory, antioxidative, and antibacterial) properties (Lee et al., 2005). This compound is present in the wood pulp of pine and eucalyptus trees, and is present in tea oils and herbal remedies (Roupe et al., 2005). As has indicated previously, the disadvantages of stilbenes related with their poor solubility in water, the ease with which they are oxidized by different agents or their tendency to be photodegraded have meant that pinosylvin has not been used as an ingredient in food products. Recently, López-Nicolás et al. (2009b) studied the formation of inclusion complexes between both natural and modified CDs and pinosylvin. Using steady state fluorescence, these authors demonstrated that natural and modified CDs are able to complex pinosylvin following a 1:1 stoichiometry. Their results show that the K_F values were higher for all the modified CDs than for natural CDs, the highest K_F value being that determined for HP-β-CD/pinosylvin complexes (12,112 $\pm$ 761 M^{-1}). The effect of pH and temperature on the K_F values was reported in this work and a thermodynamic study of the inclusion process was carried out to determine the three thermodynamic parameters, ΔH°, ΔS°, and ΔG°. The results show that the complexation of pinosylvin by HP-β-CD is a spontaneous and exothermic process.

Oxyresveratrol

Oxyresveratrol (trans-2,3′,4,5′) (Fig. 3A) is found in different sources such as mulberry (Morus alba L.) fruits and twigs (Lorentz et al., 2003). Its pharmacological properties include a wide range of biological (antioxidant, antiviral, hepatoprotective and cyclooxygenase, and tyrosinase-inhibitory) activities (Lorentz et al., 2003). Recently, Rodriguez-Bonilla et al. (2010) studied the complexation of oxyresveratrol with natural CDs using RP-HPLC and mobile phases to which α-, β-, and γ-CD were added. Among natural CDs, the interaction of oxyresveratrol with β-CD was more efficient than with α- and γ-CD. The decrease in the retention times with increasing concentrations of β-CD (0–4 mM) showed that the K_F values of the oxyresveratrol/β-CD complexes were strongly dependent on both the water–methanol proportion and the temperature of the mobile phase employed. However, oxyresveratrol formed complexes with β-CD with a 1:1 stoichiometry in all the physicochemical conditions tested. Moreover, to obtain information about the mechanism of oxyresveratrol affinity for β-CD, the thermodynamic parameters ΔG°, ΔH°, and ΔS° were obtained. Finally, to better understand on the effect of the structure of different compounds belonging to the stilbenes family on the K_F values, the complexation of other molecules, resveratrol, pterostilbene, and pinosylvin, was studied and compared with the results obtained for the oxyresveratrol/β-CD complexes. The authors show that the stoichiometry for all the

stilbenoid β-CD complexes studied was 1:1, indicating that only one molecule of this type of oxyresveratrol (resveratrol, pterostilbene, or pinosylvin) can be complexed by a molecule of β-CD. However, differences were evident in a comparison of the K_F values of the different complexes. The highest K_F value was obtained for the resveratrol/β-CD complexes, followed by the oxyresveratrol/β-CD complexes, pterostilbene/β-CD complexes, and finally the pinosylvin/β-CD complexes.

CYCLODEXTRINS AND VITAMINS

Since Pitha (1981) reported the use of substituted cycloamyloses for enhancing the water solubility of vitamins A, D, E, and K, the use of CDs as complexant agents of vitamins with high antioxidant capacity has been described in numerous works. Due to the capacity of CDs to form inclusion complex with compounds of a hydrophobic nature, in Pitha's work a revision of the main articles published about the interaction between different CDs and the lipophilics vitamins A, D, E, and K has been resumed. However, as will be seen in forthcoming sections, very few papers have studied the effect of complexation of vitamins by CDs on the antioxidant activity of these fat-soluble compounds. For this reason, further research is needed into the antioxidant capacity of the CD/lipophilic vitamin complexes.

Vitamin A

One of the most important groups of hydrocarbons with important nutritional roles in humans is the so called "Vitamin A" (Loveday and Singh, 2008). The group of compounds which forms "Vitamin A" is made up of retinoids (chemical derivatives of retinol) and provitamin A carotenoids (which are partially converted to retinoids). Supplementation with large, pharmaceutically administered doses of vitamin A can substantially reduce the incidence and severity of some infectious diseases (Villamor and Fawzi, 2005). The fortification of foods is another strategy for combating vitamin A deficiency, although such fortification is not straightforward for several reasons (Loveday and Singh, 2008). Vitamin A is poorly dispersible in aqueous systems such as beverages and high moisture foods, and is highly labile under ambient conditions, a problem that affects food supplementation route (Dary and Mora, 2002). Technologies that enhance the stability of vitamin A in foods are required for ensuring the safety and efficacy of vitamin A fortification of foods. In this review, we discuss the factors affecting vitamin A encapsulation by CDs.

The complexation of provitamin A carotenoids by several CDs will be studied in future sections. For this reason, we shall confine this part of the review to the main papers published on the interaction between CDs and retinoids. Several methods have been reported to prepare retinoid/CD complexes. Semenova et al. (2002) and Guo et al. (1995) prepared them by mixing solutions at room temperature in aqueous alcoholic solutions of ethanol or methanol, respectively. Others authors (McCormack and Gregoriadis, 1998; Muñoz-Botella et al., 2002) reported another method based on the formation of a film of retinoid on the surface of a flask, to which aqueous CD solution is added and stirred for several days. The complexation of all-trans-retinoic acid with β-CD increased the aqueous solubility of all-trans retinoic acid by more than 100 fold (Qi and Shieh, 2002). However, this aqueous solubility increased more than 10 000 times after complexation with HP-β-CD (Lin et al., 2000). This solubility is strongly dependent on several factors such as pH (the β-CD/retinoic acid complex is better at neutral pH than at acidic pH) (Lin et al., 2000; Yap et al., 2005) or the presence in the medium of organic salts Qi and Shieh (2002) reported a 26-fold increase in the β-CD/retinoic acid complex after the addition of 1.5% sodium acetate.

On the other hand, cis-retinoids have also been complexed by several CDs to inhibit their photoisomerization (Munoz-Botella et al., 2002) and photodegradation (Yap et al., 2005). The molar ratio of CD to the retinoid molecule in the inclusion complex is usually 1:1 or 2:1 (Guo et al., 1995; Munoz-Botella et al., 2002), but ratios as high as 4.5:1 have been reported (McCormack and Gregoriadis, 1998). However, different factors such as CD concentration can modify the stoichiometry of the inclusion complexes, for example, HP-β-CD forms complexes with all-trans retinoic acid in a molar ratio of 1:1 at low CD concentration and in a molar ratio of 2:1 at higher CD concentration (Lin et al., 2000).

Recently, Weisser et al. (2009) investigated a topically applied vitamin A loaded amphiphilic CD nanocapsules. In this work, a new cholesteryl-CD derivative, obtained by grafting a single cholesterol on a CD, proved suitable for the manufacture of nanocapsules. These nanocapsules were loaded with a lipophilic drug, for example, vitamin A propionate which is a highly unstable and poorly water soluble molecule of therapeutic interest. The oily nature of vitamin A propionate leads to the formation of nanocapsules with a reproducible size distribution and long term stability. The colloidal suspension can be used to form a gel which permits the encapsulated vitamin A propionate to penetrate the skin.

In another work, Lin et al. (2007) reported the biopharmaceutics of 13-cis-retinoic acid (isotretinoin), a molecule commonly used in the management of severe acne, formulated with modified β-CDs, studying the influence of the presence of CDs on the kinetic profile and bioavailability of isotretinoin administered to rats.

Vitamin K

Vitamin K is a necessary participant in the synthesis of several proteins that mediate both coagulation and anticoagulation (Wallin et al., 2008). Vitamin K deficiency is manifested as a tendency to bleed excessively. Indeed, many commercially-available rodent poisons are compounds that interfere with vitamin K and kill by inducing lethal hemorrhage. Vitamin K serves

as an essential cofactor for a carboxylase that catalyzes the carboxylation of glutamic acid residues in vitamin K-dependent proteins. The key vitamin K-dependent proteins include coagulation proteins (factors II (prothrombin), VII, IX, and X), anticoagulation proteins (proteins C, S, and Z) and others such as bone proteins, osteocalcin and matrix-Gla protein, and certain ribosomal proteins (Yamauchi et al., 2010).

Although the antioxidant activity of vitamin K has been reported in numerous studies (Vervoort et al., 1997), very few papers have reported the complexation between this type of vitamin and CDs. In a study about the solubilization of lipid-soluble vitamins by complexation with CDs, Okada et al. (1990) reported the inclusion complex formation of eight kinds of lipid-soluble vitamins with 6-O-α-D-glucopyranosyl-β-CD (G-β-CD) in aqueous solution and in solid phase, which were assessed by the solubility method and thermal analysis. All lipid-soluble vitamins were highly solubilized in water by complexation with G-β-CD. As regards the complexation of vitamin K with G-β-CD, analysis of the phase solubility diagrams showed, the stoichimetric ratio of the main complex in water to be 1:3 for Vitamin K_1/G-β-CD, 1:3 for Vitamin K_2/G-β-CD, and 1:1 for Vitamin K_3/G-β-CD. Later, Zhenming et al. (2003) studied the interaction of the Vitamin K_3-CD inclusion complex and its analytical application. The inclusion interaction of the complexes between Vitamin K_3 and β-CD, HP-β-CD and sulfobutylether-β-CD (SBE-β-CD) were studied using steady-state fluorescence measurements. The K_F values were calculated and a 1:1 inclusion stoichiometry for Vitamin K_3/CDs was determined. The results showed that the inclusion ability of β-CD and its derivatives was in the order: SBE-β-CD>HP-β-CD>β-CD. Finally, the results obtained were applied to determining Vitamin K_3 in pharmaceutical preparations. In another work, Berzas et al. (2000) published a similar spectrofluorimetric study of the β-CD/Vitamin K_3 complex and reported a different K_F value although the 1:1 stoichiometric ratio was confirmed. This vitamin not naturally fluorescent but yields fluorescence when it is reduced. However, it is possible to yield a fluorescent derivative in aqueous medium when complexed to β-CD and the procedure was applied to pharmaceutical formulations. The procedure was also applied to pharmaceutical formulations. Furthermore, Lengyel and Szejtli (2005) prepared a stable, nonsublimable γ-CD inclusion complex of menadione (Vitamin K_3) in solution, in suspension, and by "kneading". The biological activity of the complex was tested on baby chickens by prothrombin time determination. Reduced prothrombin times showed an enhanced bioavailability of menadione in complexed form. The stability of Vitamin K_3 is greatly improved in pharmaceutical and veterinary products prepared with a menadione- γ-CD -CD inclusion complex.

Vitamin D

Vitamin D is a group of fat-soluble secosteroids, the two major physiologically relevant forms of which are vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol). Vitamin D without a subscript refers to either D_2 or D_3 or both. Vitamin D_3 is produced in the skin of vertebrates after exposure to ultraviolet B light from the sun or artificial sources, and occurs naturally in a small range of foods. In some countries, staple foods such as milk, flour and margarine are artificially fortified with vitamin D, and it is also available as a supplement in pill form. Food sources such as fatty fish, mushrooms, eggs, and meat are rich in vitamin D and are often recommended for consumption by those suffering vitamin D deficiency (Holick, 2005).

The complexation of Vitamin D by different type of CDs has been reported in several studies. Palmieri, et al. (1993) evaluated different complexation methods (phase solubility diagram, HPLC, DSC, X-RAY diffractometry, and NMR) to form inclusion complexex between β-CD and Vitamin D_2 in aqueous solution and solid state. The solid inclusion complexes were prepared by spray-drying, kneading and solid dispersion. The dissolution profiles of the complex either in powder or in tablets were studied in order to select the best inclusion process. Their results show that while kneading provided a good yield, spray drying led to complete complexation and best dissolution.

Studies on the stability and structure of the β-CD/Vitamin D_2 inclusion complex by Peng et al. (1999) showed that the Vitamin D_2 content in the inclusion complex was still 85%–95% after two years, while that in the β-CD and Vitamin D_2 mixture was just 17%–22%. This indicates that Vitamin D_2 in the inclusion complex was much more stable than pure Vitamin D_2. Therefore, adding the inclusion complex β-CD-Vitamin D_2 into "Longmu" Zhuanggu Chongji, a medicine for children to prevent and cure rickets, may not only decrease the expense of Vitamin D_2, but also enhance the stability of the products.

One of the most interesting applications of the complexes between Vitamin D and CDs was reported in the paper by Takeda et al. (1994). These authors studied the application of CDs to the microbial transformation of VD_3 to 25-hydroxy Vitamin D_3 and 1α,25-dihydroxy Vitamin D_3. In this microbial hydroxylation, it was found that CD had the ability to enhance the hydroxylation of Vitamin D_3.

In another paper, Comini et al. (1994) studied the interaction of β-CD with bile acids (cholic, taurocholic, chenodeoxycholic, and lithocholic) and their competition with vitamins A and D_3. These vitamins were seen to compete with cholic and taurocholic acids but not with lithocholic and chenodeoxycholic acids. The affinity of vitamins A and D_3 for β-CD was lower than that of the bile acids. The results of this study suggest that depletion of lipophilic vitamins will not occur it of β-CD, thus providing further support for the safety and suitability of β-CD as an ingredient in foods and orally administered drugs.

In another study, Tian and Holick (1995) studied the catalyzed thermal isomerisation between previtamin D_3 and vitamin D_3 via β-CD complexation, observing a strong increase in the velocity of isomerisation when CDs are present in the reaction medium. This conformation-controlled process may play an important role in the modulation of the previtamin D_3/vitamin

D_3 endocrine system in vivo, as it has been seen to do in the sea urchin.

One of the main applications of the use of CDs in the analytical chemistry field is to reduce of the retention time and the separation of antioxidant compounds analyzed by different analytical techniques. In 1997, Spencer and Purdy studied the separation of fat-soluble vitamins using several types of CDs in high-performance liquid chromatography and micellar electrokinetic chromatography (MECK). The use of MEKC rather than HPLC provided comparable or even improved resolution of these hydrophobic solutes. Both techniques proved successful in the separation of vitamins D_2 and D_3. Dimethyl-13-CD as mobile phase and buffer additive led to the best separation of these two compounds. Moreover, the addition of CD to the running buffer in MEKC provided little improvement in the resolution of these two compounds.

Vitamin E

Tocopherols and tocotrienols share common structures both having a chromanol head and a phytyl tail. They are collectively named vitamin E, which has been shown to be the most effective lipid-soluble antioxidant in nature, interfering with one or more propagation steps of the lipid peroxidation process. Due to their medical, biological, and physiochemical significance, tocopherols have been extensively studied. In contrast with other lipohilic vitamins, the complexation of vitamin E by CDs has been reported in many papers along with the effect of encapsulation on the antioxidant activity.

In a recent report on CD inclusion complex formation and solid-state characterization of the natural antioxidants α-tocopherol and quercetins, Koontz et al. (2009) suggested that, natural antioxidant/CD inclusion complexes may serve as novel additives in controlled-release active packaging to extend the oxidative stability of foods. In this work, variations of the coprecipitation from aqueous solution technique were optimized for the CD complexation of α-tocopherol and quercetin.

The use of CDs to separate different tocopherols has been reported by several authors. For example, Abidi and Mounts (1994) separated α-, β-, γ-, and δ-tocopherols and 5,7-dimethyltocol by normal-phase HPLC on β- or γ-CD-bonded silica (CDS) with fluorescence detection. Generally, the k′ values obtained with hexane mobile phases or with the β-CDS phase were greater than those observed in HPLC using cyclohexane mobile phases or with the γ-CDS phase. With a similar objective, Chang et al. (2006) published a study about CD-modified microemulsion electrokinetic chromatography for the separation of α-, γ-, δ-, and γ-tocopherol acetate. Microemulsion electrokinetic chromatography, claimed to attain high peak efficiency with great solubilization power, has not previously been applied to the separation of tocopherols. The results obtained by these authors were applied to vitamin E preparations with optimum results.

Siró et al. (2006) studied the influence of complexation in β-CD on the release of α-tocopherol from antioxidative low-density polyethylene film into fatty food simulants. The release of α-tocopherol from two formulations (with and without complexation with β-CD) of low-density polyethylene film was examined and it was concluded that complexation with β-CD had a significant effect on the antioxidant release rate. A decrease in the diffusion coefficient of one order of magnitude was calculated in the case of complexed α-tocopherol compared with the free form and total migration of α-tocopherol from both films was observed, meaning that the partition coefficient of tocopherol was not influenced by incorporation with CD. Thus, complexation might be the key to a long-lasting antioxidative effect of this kind of active packaging.

Several works have been published concerning the antioxidant activity of vitamin E complexed with different types of natural and modified CDs. Iaconinoto et al. (2004) studied the influence of complexation with β-CD (β-CD), HP-β-CD or HP-γ-CD on the antioxidant activity during the light-induced decomposition of vitamin E (α-tocopherol). The photodegradation of α-tocopherol was examined in emulsion vehicles and was not significantly influenced by complexation with β-CD, whereas HP-β-CD and HP-γ-CD enhanced the light-induced decomposition of α-tocopherol. On the other hand, accelerated stability studies indicated that the degradation of non-irradiated α-tocopherol was reduced by complexation with HP-β-CD or HP-γ-CD. The radical scavenging activity of α-tocopherol was evaluated in vitro using the xanthine/xanthine oxidase enzymatic system. No significant differences were observed between the free form of the vitamin and its complexes with β-CD, HP-β-CD, or HP-γ-CD. Therefore, complexation of α-tocopherol with these CDs does not interfere with the antioxidant activity of this vitamin.

One of the applications of the encapsulation of vitamin E by CDs is in the development of new assays to measure the antioxidant activity of different compounds. Thus, in an interesting work, Huang et al. (2002) developed an ORAC assay for lipophilic antioxidants including the vitamin E family, using RM-β-CD as the solubility enhancer. RM-β-CD was introduced as the water solubility enhancer for lipophilic antioxidants. For the first time, by using 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid as a standard (1.0), the ORAC values of α-tocopherol, (+)-γ-tocopherol, (+)-δ-tocopherol, α-tocopherol acetate, tocotrienols, 2,6-di-tert-butyl-4-methylphenol, and γ-oryzanol were determined: 0.5 $\pm$ 0.02, 0.74 $\pm$ 0.03, 1.36 $\pm$ 0.14, 0.00, 0.91 $\pm$ 0.04, 0.16 $\pm$ 0.01, and 3.00 $\pm$ 0.26, respectively.

On the other hand, Çelik et al. (2007) reported a simple, low-cost, and widely applicable total antioxidant capacity assay for different molecules named the CUPRAC method. This is an improvement over classical CUPRAC methodology because reports the assay for both lipophilic and hydrophilic antioxidants simultaneously, by making use of their "host—guest" complexes with methyl-β-CD in aqueous medium. The turbidity limit for α-tocopherol was 1:3 (v/v) alcohol–water solutions, but

when these suspensions were mixed with equal volumes of 7% methyl-β-CD aqueous solution, clear solutions were obtained in which the CUPRAC assay could be directly performed.

Moreover, Oyurek et al. (2008) reported another method to determine the antioxidant capacity of several molecules using an assay of lipophilic and hydrophilic antioxidants in the same acetone–water solution containing 2% methyl-β-CD and also using the cupric reducing antioxidant capacity (CUPRAC) method. In this way, the order of antioxidant potency of various compounds irrespective of their lipophilicity could be established in the same solvent medium. Methyl-β-CD was introduced as the water solubility enhancer for lipophilic antioxidants. Two percent methyl-β-CD (w/v) in an acetone–H_2O (9:1, v/v) mixture was found to sufficiently solubilize carotene, lycopene, vitamin E, vitamin C, synthetic antioxidants, and other phenolic antioxidants. This assay was validated through linearity, additivity, precision, and recovery assays, which demonstrated that the CUPRAC assay is reliable and robust.

Finally, one of the most important applications of the encapsulation of vitamin E is in the cosmetic industry. Thus, the formation of an inclusion complex between γ-CD and a broad variety of organic compounds increases the stability and solubility of active cosmetic ingredients and provides a better control over the release of fragrances. Lipophilic vitamin E is essential in skin care because of its nature as a free radical scavenger; however, it is sensitive to light and oxidation-induced degradation. For this reasons, Regiert (2005) studied the light stability of vitamin E by inclusion in γ-CD for its use in the cosmetic industry.

CYCLODEXTRINS AND CAROTENOIDS

The presence of carotenoids in higher plants, algae, fungi, and bacteria has been widely discussed in recent years (Boussiba et al., 1992; Castenmiller and West, 1998; Scotter et al., 2003). Moreover, recently papers have investigated their presence in animals, such as birds and crustaceans. Because carotenoids can only be biosynthesized by plants and microorganisms, their presence in animals is attributed to their ingestion in the food and their accumulation in certain tissues, for example, the feathers of flamingos, egg yolk, and the exoskeleton of invertebrates. Furthermore, over 600 compounds constitute the carotenoid family, among which the best known are α-carotene, β-carotene, lutein, lycopene, astaxanthin, and zeaxanthin. With respect to it structure, carotenoids predominantly occur in their *all-trans* configuration, which is the thermodynamically more stable isomer. However, there is ample proof supporting the presence of *cis* isomers in plants, especially in chlorophyll-containing tissues, but also in a number of fruits (Cortes et al., 2004). Low levels of *cis*-isomers of β-carotene were also detected in hydroponic leafy vegetables, although below the limit of quantification.

Several healthy effects have been attributed to the consumption of a diet rich in carotenoids, such as the decreased risk of certain types of cancer, atherosclerosis and age-related degeneration. Moreover, a key property of carotenoids is their capacity to quench singlet oxygen and free radicals; since this capacity depends on the number of conjugated double bonds, carotenoids inevitably show high antioxidant capacity (Nelson et al., 2003).

In spite of the numerous advantages reported for different carotenoids, these molecules present two main disadvantages that impede their use as added antioxidant agents in functional foods: (i) the ease with which they are oxidized by different agents due to the system of double bonds and (ii) their poor solubility in aqueous solvents. In an attempt to avoid these problems, several papers on the complexation of carotenes with CDs have been published. Below, we review the most recent works on the complexation of the main types of carotenoid with different CDs.

Astaxanthin

Astaxanthin, generally known as all-*trans* astaxanthin, is a red-orange carotenoid pigment, naturally found in many aquatic animals, such as shrimp, crab, and salmon. Because it belongs to the xanthopyll class of carotenoids, astaxanthin is closely related to β-carotene, lutein, and zeaxanthin, sharing with them many of the general metabolic and physiological activities attributed to carotenoids (Higuera-Ciapara et al., 2004).

The use of astaxanthin in the aquaculture industry is increasing since it provides the typical muscle color of salmon a property that is widely accepted by consumers. Different biological functions have been reported for astaxanthin in fish. For example, this type of carotene is associated with protecting cells against oxidative damage and also with reproduction and embryo development (Parajo et al., 1996). Moreover, the use of astaxanthin as nutraceutical agent in human nutrition has increased because of the numerous investigations which justify its antioxidant, anticancer, and antiinflamatory properties (Wei and Yan, 2001). Several astaxanthin products derived from microalgae are available for consumers.

The antioxidant properties of astaxanthin are strongly related with the 11 conjugated carbon–carbon double bonds which are present in its structure. Several authors have shown that the antioxidant property of astaxanthin is 10 times that of β-carotene, and up to 500 times stronger than that of vitamin E (Shimidzu et al., 1996).

However, several difficulties limit the use of astaxanthin as an antioxidant agent to produce novel lipophilic foods is limited by including its sensitivity to heat or light and its poor aqueous solubility.

For this reason, Chen et al. (2007) recently published a paper describing the preparation and stability of an inclusion complex of astaxanthin with β-CD. Using HPLC, IR, and absorbance methods, the complexes investigated by these authors showed a stoichiometry of 1:4 for astaxanthin: β-CD. Moreover, their results showed that the water solubility of the inclusion complex was <0.5 mg/ml, which is better than that of astaxanthin itself. Moreover, the heat stability of the inclusion complex and its

stability in light were greatly enhanced. The aqueous solubility of the inclusion complex was also slightly enhanced (<0.5 mg/ml).

In another paper and in order to distinguish between the complexation of this carotene with natural and modified CDs, Yuan et al. (2008) reported the inclusion complex of astaxanthin with HP-β-CD, using IR spectroscopy. The water solubility of the resulting inclusion complex was >1.0 mg/ml, which is much better than that of astaxanthin and that published by Chen et al. (2007) for the complexes of this type of carotenoid with β-CD. Moreover, when the solid state thermal behavior of the inclusion complex was investigated by thermogravimetric/differential thermal analysis, the temperature at which astaxanthin began to decompose was enhanced to about 290°C. The stability of the inclusion complex in solution was also tested by Chen et al (2007), who found that the complex greatly improved the stability of astaxanthin against light and oxygen. The release of astaxanthin from the inclusion complex was also reported in that paper.

Lycopene

Lycopene is one of the major carotenoids in the diet of North Americans and Europeans, and is therefore a nutraceutical for which is great demand. The most important source of lycopene is tomato and its processed food products, in which lycopene may constitute more than 60% of the carotenoids present. Lycopene is also present in watermelon, apricot, papaya, passion fruit, guava, pink grapefruit, carrots, pepper, persimmon, balsam pear, and a large number or red berries (e.g., cranberries) and the fruits of autumn olive (Charoensiria et al., 2009]).

Several epidemiological studies have suggested that the high consumption of tomatoes and tomato products containing lycopene may protect against CVD and reduce the risk of several types of cancer, most notably those of the prostate, breast, lung, and digestive tract. Serum and tissue levels of lycopene have also been inversely related with chronic disease risk. Such epidemiological leads have stimulated a number of animal models and cell culture studies designed to test this hypothesis and to establish the beneficial effects of lycopene. The evidence gained in from these studies suggests that lycopene has anticarcinogenic and antiatherogenic effects both in vitro and in vivo.

A key property of carotenoids is their capacity for quenching singlet oxygen and free radicals, a capacity that depends on the number of conjugated double bonds, and makes lycopene exceptionally effective compared with other carotenoids. The in vitro quenching constant of lycopene has been found to be more than twice that of β-carotene and 100 times that of α-tocopherol (Di Mascio et al., 1989). Lycopene may also interact with reactive oxygen species, such as hydrogen peroxide and nitrogen dioxide. Despite the many disputes in the literature about lycopene behavior, lycopene presents the same problems for use in functional foods as those mentioned above for astaxanthin. For this reason, its complexation with CDs has been reported in several papers, which we will now look at.

Blanch et al. (2007) reported the stabilization of all-*trans*-lycopene from tomato by encapsulation using α-, β-, and γ-CDs. To that end, two different encapsulation methods were studied: a conventional method and a supercritical fluid extraction process. An optimization procedure taking into consideration the distinct molar ratios of CD/lycopene (1/0.0026, 1/0.005, and 1/0.05) as well as the type of CD to be used was accomplished. Encapsulation was determined by using micro-Raman spectroscopy. As a result, a CD/lycopene molar ratio of 1/0.0026 was selected as it provided the best complexation yields (93.8%), while β-CD seemed to be the most favorable for stabilizing the lycopene. A comparison of the two methods studied showed higher encapsulation yields with the conventional method. The results reported by the above authors (Blanch et al., 2007) showed that the supercritical fluid approach offers numerous advantages, such as the possibility of carrying out the extraction, fractionation, and encapsulation of lycopene from tomato in one step, notably shortening the overall procedure time and minimizing sample handling.

In another paper, Quaglia et al. (2007) produced lycopene-containing powders from tomato products by a solvent-free method making use of β-CD. The powders were prepared by spray-drying a tomato concentrate, one of the most bioavailable forms of lycopene after mechanical treatment with β-CD in different weight ratios. The obtained product was centrifuged to partly eliminate the food matrix and characterized by the amount of lycopene hydrodispersed/hydrosolubilized in the aqueous fraction. The chemical antioxidant activity of sera was also evaluated by these authors and a progressive increase of this antioxidant capacity when CDs were presented in the reaction medium was observed. The powders obtained by spray-drying sera exhibited good flow properties, a lycopene content of between 0.4 and 1.09 mg/g and excellent water dispersability. The process developed, which makes use of β-CD, is of great interest for obtaining nutraceuticals with better bioavailability than that of lycopene alone.

In their study, Larroza Nunes and Zerlotti Mercadante (2007) obtained encapsulated lycopene in a powder form, using either spray-drying or molecular inclusion with β-CD followed by freeze-drying. The encapsulation efficiency using spray-drying ranged from 94 to 96%, with an average yield of 51%, with microcapsules showing superficial indentations and an absence of cracks and breakages. Lycopene/β-CD complexes were only formed at a molar ratio of 1:4, and irregular structures of different sizes that eventually formed aggregates, similar to those of β-CD, were observed after freezedrying. About 50% of the initial lycopene did not form complexes with β-CD. Lycopene purity increased from 96.4 to 98.1% after spray-drying, whereas lycopene purity decreased from 97.7 to 91.3% after complex formation and freeze-drying. Both the drying processes yielded pale-pink, dry, and free-flowing powders.

Recently, Fernández-García et al. (2010) analyzed the assimilation efficiency of carotenoids when they are delivered as

inclusion complexes with β-CD in water and showed that the in vitro intestinal absorption of several carotenoids (lycopene, lutein, and β-carotene) delivered as molecular inclusion complexes with β-CD led to a significant increase in carotenoid assimilation compared with the corresponding carotenoid suspensions in Tween and assimilation was not inhibited by high-density lipoproteins.

In 2006, Vertzoni and coworkers described the solubilization and quantification of lycopene in aqueous media in the form of binary CD systems. The optimized kneading method for preparing the lycopene–CD binary systems improved the solubility of lycopene in water and 5% (w/v) dextrose solution. The lycopene in the binary systems was quantified by a spectrometric method that followed single-step extraction with dichloromethane. The storage stability characteristics of the binary systems were studied at 4°C in solution and at −20°C in the lyophilized products, monitoring the lycopene content at $\lambda_{max} = 482$ nm. The results obtained by these authors with the spectrometric method were confirmed by HPLC. In the presence of CDs, the lycopene concentration in water was 8.0 ± 1.0, 27.1 ± 3.2, and 16.0 ± 2.2 μg/ml for β-CD, HP-β-CD and Me-β-CD, respectively. In 5% (w/v) aqueous dextrose solutions the corresponding values were 16.0 ± 1.8, 48.0 ± 5.1, and 4.0 ± 0.5 μg/ml, respectively. At 4°C, the storage stability of the lycopene–CD binary systems in water or 5% (w/v) aqueous dextrose solutions was limited ($t_{1/2}$ = 1–4 days), although the addition of the antioxidant sodium metabisulfite increased the stability of the lycopene–HP-β-CD binary system in water. At −20°C, the lyophilized lycopene–CD binary systems were stable for at least two weeks.

As regards the effect of the addition of CD on the antioxidant capacity of lycopene, Bangalore et al., (2005) reported the effect that β-CD had on the relation between lycopene concentration and ORAC , which is used as an index of antioxidant activity for many hydrophilic antioxidants but not for lycopene. This study served to validate the ORAC assay for different concentrations of lycopene in the presence of β-CD, a water-solubility enhancer. The lycopene concentration correlated poorly with ORAC in the absence of β-CD. However, these correlations improved with increasing levels of β-CD. The results demonstrated that the inclusion of β-CD in the ORAC assay improves the correlation between ORAC and lycopene concentration, thus expanding the scope of the assay to include fat-soluble antioxidants. These findings open up new avenues for using the ORAC assay as a tool for evaluating lycopene antioxidant activity, enabling us to better understand the relationship between lycopene concentration and antioxidant activity in various food systems.

β-carotene

β-carotene is the major carotenoid present in the human diet as well as in the human organism, where displays pro-vitamin A activity, among its other positive effects on human health. β-carotene is endogenously present in several isomers; all-*trans*-β-β'-carotene is followed, in decreasing order, by lower concentrations of the 15-*cis*, 13-*cis*- and 9-*cis*-isomers (Johnson at. al., 1997). In addition to the β-carotene structure, the form α-carotene also exists, as a result of a shift of one of the conjugated double bonds in the head region of the β-carotene structure. β-carotene is mainly present in the vegetables that form part of the human diet and small amounts in the animal-derived diet. It is mainly green, yellow, orange, and red vegetables like broccoli, Brussels sprouts, tomatoes, spinach, carrots, and paprika as well as colored fruits like apricot, grapefruit, cherry, and papaya, are rich in β-carotene (Biesalski et al., 2001). Additional nutritional sources of β-carotene are foods supplemented with β-carotene as a food colorant, like margarine, butter, and many soft drinks. The bioavailability of β-carotene in the human organism varies greatly and depends on various factors (Van het Hof et al., 2000) such as interaction between individual carotenoids, the amount of fat and individual fatty acids present in the diet and the matrix in which the carotenoids are located. The absorption rate is also modified by diseases of the gastrointestinal tract mainly due to the malabsorption of lipids.

The complexation of β-carotene with several types of CDs has been reported in recent years. For example, Mele et al. (2002) used the formation of complexes between CDs and *trans*-β-carotene in water to evidence the formation of large aggregates using light scattering and NMR spectroscopy. These authors also showed that the NMR spectra of CD/β-carotene in a D_2O solution pointed to a chemical shift of the CD protons upon complexation, indicating that a host-guest interaction had taken place in solution. The pattern of the chemical shift was, however, different from that expected in the case of classical inclusion complexes of defined stoichiometry (e.g., a 1:1 complex), indicating that the formation of a true inclusion compound between CD and β-carotene may be only one of the possible interaction mechanisms. Any possible CDs/β-carotene host-guest associations can be reasonably expected to have a neatly amphiphilic character due to the hydrophilic hosts and the hydrophobic guest. The study of Mele et al. (2002) confirmed that the hydrophobic moiety (β-carotene) of such inclusion complexes in water tends to self-associate, forming larger supramolecular micelles like aggregates, with the hydrophilic CD molecules arranged outside and in fast exchange (on the NMR timescale) with the free CD molecules in solution. Besides, the LS data presented by these authors showed that pure β-CD and γ-CD do not form large self-aggregates in water, at least in the experimental conditions used. This observation is in agreement with recent NMR studies showing that pure β-CD is a monomer in a water solution (Azaroval-Bellanger and Perly, 1994), ruling out previous contrary conclusions obtained from LS experiments.

Another field of application for β-carotene complexation by CDs is in food science and technology. Szente et al. (1998) studied the stabilization and solubilization of natural lipophilic colorants with CDs and provided data on the practical application of the benefits of the molecular encapsulation of natural colorants by CDs. The experimental results confirmed the increased stability of CD-complexed curcumin, curcuma oleoresin, β-carotene, and carotenoid oleoresins in the face of light,

heat, and oxygen. The parent β-CD stabilized carotenoides and, especially, curcumines. Methylated β-CD was found to be the most potent solubilizing agent for both carotenoids and curcuminoids.

The complexation of this potent antioxidant with CDs has also been used in cellular biology, where the use of CDs to complex β-carotene was reported to improve a novel method for the supplementation of cultured cells (Pfitzner et al., 2000). A physiological, water-soluble complex of carotenoids with methyl-β-CD was developed for the purpose of cell supplementation. The bioavailability, cytotoxicity and stability of the formulations were compared to carotenoid solutions in organic solvents and the stability of the different carotenoid solutions (0.5 μM) under cell culture conditions was determined by measuring absorbance 1 and 7 days after treatment. To determine the availability of β-carotene, human skin fibroblasts were incubated for up to 8 days with 5 μM β-carotene in methyl-β-CD or THF/DMSO and the cellular and medium β-carotene contents were determined by HPLC analysis. Depending on the solubilizer used, different orders of stability were found by Pfitzner et al (2000). Methyl-β-CD formulation: β-carotene>zeaxanthin>lutein>lycopene. Organic solvents: zeaxanthin>lutein>lycopene>β-carotene. Two days after supplementation with 5 μM β-carotene in MLCD, cellular β-carotene levels reached a maximum of 140 $\pm$ 11 pmol/μg DNA, leveling off at 100 $\pm$ 15 pmol/ μg DNA until day 8. Incubation with β-CD dissolved in THF/DMSO resulted in a lower β-carotene uptake of 105 $\pm$ 14 pmol/ μg DNA and 64 $\pm$ 20 pmol/ μg DNA, respectively. No cytotoxic effects of these formulations were detected by Pfitzner and coworkers in their paper. The results show that the methyl-β-CD formulation is a better method for investigating carotenoids and other lipophilic compounds in in vitro test systems compared to methods using organic solvents.

In another paper, Lancrajan et al. (2001) used liposomes and β-CD as carriers for the incorporation of three dietary carotenoids (β-carotene and two other carotenoids lutein and canthaxanthin) into plasma and into the mitochondrial, microsomal and nuclear membrane fractions from pig liver cells or the retinal epithelial cell line D407. The uptake dynamics of the carotenoids from the carriers to the organelle membranes and their incorporation was followed by these authors by incubating at pH 7.4 for up to 3h. The results showed the β-CD carrier to be more effective at incorporating lutein, while β-carotene was incorporated into natural membranes from liposomes or from CDs.

As regard the antioxidant capacity of β-carotene, Özyürek et al. (2008) reported the antioxidant capacity of both lipophilic and hydrophilic antioxidants simultaneously, by making use of their "host—guest" complexes with methyl-β-CD in acetonated aqueous medium using the CUPRAC method. In this way, methyl-β-CD was introduced as the water solubility enhancer for lipophilic antioxidants. Two percent methyl-β-CD (w/v) in an acetone–H_2O (9:1, v/v) mixture was found to sufficiently solubilize β-carotene, lycopene, vitamin E, vitamin C, synthetic antioxidants, and other phenolic antioxidants. This assay was validated through linearity, additivity, precision, and recovery assays. The validation results demonstrated that the CUPRAC assay is reliable and robust. In an acetonated aqueous solution of methyl-β-CD, only CUPRAC and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays were capable of measuring carotenoids together with hydrophilic antioxidants. The CUPRAC antioxidant capacities of a wide range of polyphenol and flavonoids were reported in this work as trolox equivalent antioxidant capacity (TEAC) in the CUPRAC assay, and compared to those found by reference methods, ABTS/horseradish peroxidase (HRP)–H_2O_2 and ferric reducing antioxidant power (FRAP) assays. The TEAC coefficients of hydrophilic antioxidants did not differ significantly from those reported in the original CUPRAC method, while TEAC values of β-carotene and lycopene were reported for the first time in this modified CUPRAC assay. The authors demonstrated that synthetic mixtures composed of lipophilic and hydrophilic antioxidants provided the theoretically expected CUPRAC antioxidant capacities, indicating that there were no chemical deviations from Beer's law, and that the observed CUPRAC absorbances were additive.

Moreover, using different techniques, Polyakov et al. (2004) studied the formation of inclusion complexes between β-CD and carotene and carotene derivates, finding that although CD protects the carotenoids from reactive oxygen species, the complexation with CD results in considerable decrease in antioxidant ability of the carotenoids. Their results show that CDs does not prevent the reaction of carotenoids with Fe^{3+} ions, but reduces their scavenging rate toward OOH radicals. This means that different sites are responsible for the interaction of carotenoids with free radicals and Fe^{3+} ions. Because CDs are widely used as carriers and stabilizers of dietary carotenoids, the demonstration that CDs protect the carotenoids from reactive oxygen species and provide their safe delivery to the cell membrane is of importance.

CYCLODEXTRINS AND COENZYME Q_{10}

Coenzyme Q_{10} (CoQ_{10}), also known as ubiquinone, is a lipid-soluble compound that has an important function in the mitochondrial electron transport chain as an electron carrier (Bhagavan and Chopra, 2006). Further, CoQ_{10} is an antioxidant whose activity is particularly important in regenerating vitamin E. Its ability to quench free-radicals also helps to maintain the structural integrity and stability of mitochondrial and cellular membranes, including intracellular membranes. A number of claims that are attributed to CoQ_{10} may be due to its antioxidant properties as well as to its enhancement of the cellular bioenergy capacity. In spite of these advantages, CoQ_{10} presents several inconveniences for use in the food industry as fortifier or nutraceutical. CoQ_{10} is sensitive to light and heat and will decompose when it exposed to light. Moreover, the bioavailability of

CoQ_{10} is low and variable due to its poor solubility in water and high molecular weight (Bhagavan and Chopra, 2006). For these reasons, CoQ_{10} should be treated to protect it from light and heat and to increase its bioavailability. A number of strategies to improve the absorption of CoQ_{10} have been proposed, such as oily solutions, self-emulsified drug delivery systems, esterification, coadministration of pepper extract, binary solid dispersion, and liposome. We shall now present, a review of the principal papers and patents on the complexation of this antioxidant by CDs.

In 2008, Hatanaka et al. reported the physicochemical and pharmacokinetic characterization of water-soluble CoQ_{10} formulations. In this work, a novel liquid (nano-emulsion, NE) and water-soluble powder formulations, including CD–CoQ_{10} complex (CoQ_{10}–CD) and dry-emulsion (DE), were prepared. The physicochemical properties of each formulation were characterized by dynamic light scattering, scanning electron microscopy, powder X-ray diffractometry, and differential scanning calorimetry. In all the powder formulations prepared, CoQ_{10} existed mainly in an amorphous form, as determined by and each powder formulation exhibited high solubility and dispersibility in water, resulting in the formation of a nano-sized emulsions (NE; 60 nm) and micron sized particles (DEs and CoQ_{10}–CD; 0.77–2.4 μm). The pharmacokinetic study of each dosage form, as opposed to a CoQ_{10} crystal suspension, was also carried out in rats after a single oral dose. Although similar kinetic values were seen with Tmax of 1.5 and 1.7 h, respectively, NE exhibited ca 1.7-fold higher AUC and Cmax than the crystalline CoQ_{10}. Considering the significant increase on both Cmax and AUC of CoQ_{10}, NE methodology could be the most effective of the formulations tested for improving of oral absorption of CoQ_{10}.

Recently, Miyamoto et al. (2009) published a structural study of the CoQ_{10} inclusion complex with γ-CD, investigating the molecular composition and three-dimensional structure of the complex using chemical analyses and molecular modeling. Moreover, the molecular ratio of γ-CD and CoQ_{10} in the complex was investigated by NMR as well as by HPLC to determine the γ-CD/ CoQ_{10} ratio, which was found to be 2.5.

Increasing the bioavailability of CoQ_{10} is one of the main goals of the complexation of this potent antioxidant by CDs. An interesting study on the enhancement of the oral bioavailability of CoQ_{10} by complexation with γ-CD in healthy adults was published by Terao et al. (2006). The objective of this study was to compare the effect of the molecular encapsulation of CoQ_{10} by complexation with γ-CD (CoQ_{10}-γ-CD) with that of a mixture of CoQ_{10} and microcrystalline cellulose (CoQ_{10}-MCC) on absorption and bioavailability of CoQ_{10} in supplement form in healthy adults. After 6 and 8h mean CoQ_{10} plasma levels in subjects given a single oral administration of the CoQ_{10}-γ-CD capsule were significantly than those found with the CoQ_{10}-MCC capsule. In addition, the mean plasma levels at 24 and 48h tended to be higher after CoQ_{10}-γ-CD administration. These results indicate that the oral absorption and bioavailability of CoQ_{10} in healthy adult volunteers could be significantly enhanced by complexation with γ-CD, and point to the potential use of γ-CD as formulation aid for orally administered CoQ_{10}.

Cuomo and Rabovsky (2000) also compared the bioavailability of CoQ_{10} in different formulations. The study was undertaken as part of a programme to develop a new CoQ_{10} formula providing high bioavailability of CoQuinone product, but without the synthetic solubilizers found in CoQuinone. Two new formulas were tested. The first, a dry tablet formula, contained CoQ_{10} complexed with CDs, while the second was an all-natural liquid formula based on lecithin, medium chain triglycerides and glycerine monooleate. The dry tablet formula with CDs did not provide high levels of bioavailability, but the new all-natural liquid formula did. For these reasons, further studies are required into the bioavailability of CD/ CoQ_{10} complexes.

Recently, Higashi et al. (2009) prepared four kinds of complexes of CoQ_{10} with γ-CD using the kneading method and the solubility method with or without heating, and compared the resulting pharmaceutical properties. Differential scanning calorimetrical curves and powder X-ray diffraction patterns showed that the complexes formed pseudorotaxane-like supramolecular structures, although those included free γ-CD and CoQ_{10} when prepared without heating. Heating improved the complexation of CoQ_{10} with γ-CD in both methods. The dispersion rate of CoQ_{10} in water increased in the order of CoQ_{10} alone $\sim$physical mixture with γ-CD $<$ solubility/heating product $<$ solubility product $<$ kneading/heating product $<$ kneading product, possibly due to submicron-ordered particle formulation. Of the various ointments containing CoQ_{10} alone, the release of CoQ_{10} from the hydrophilic ointment was fastest, especially when heating was involved. The fast release of CoQ_{10} from hydrophilic ointment could be involved in propylene glycol in the ointment. These results suggest that supramolecular complexes of CoQ_{10} with γ-CD can be prepared by various methods, and among various complexes the pseudorotaxane-like $CoQ_{10}0$/γ-CD complexes prepared by the solubility method with heating show the best potential for preparing of ointments.

In 2008, Nishimura et al. confirmed that CoQ_{10} forms a pseudorotaxane-like supramolecular complex with γ-CD. The X-ray diffraction pattern of the CoQ_{10}/γ-CD complex was different from that of the physical mixture, but almost the same as that of polypropylene glycol/γ-CD polypseudorotaxane. Also, a differential scanning calorimetric study and the FT-IR study confirmed the interaction between CoQ_{10} and γ-CD in the solid state. An 1H-NMR study and the yield study of the supramolecular complex of CoQ_{10} with γ-CD demonstrated that the stoichiometry was 5:1 (γ-CD:CoQ_{10}). The dispersion rate of CoQ_{10} was markedly increased by the formation of the supramolecular complex with γ-CD, possibly due to submicron-ordered particle formulation. In fact, CoQ_{10} was found to form submicron-sized supramolecular particles with γ-CD, when prepared by the solubility method. Consequently, the study showed that CoQ_{10} forms a pseudorotaxane-like supramolecular complex with γ-CD in water.

Milivojevic et al. (2009) recently reported the complexation of CoQ_{10} with β- and γ-CD in aqueous solutions in order to improve the water solubility, thermo- and photo-stability of CoQ_{10}. Complex formation resulted in an increase in water solubility at room temperature and in the pH 6.5 by a factor at least 10^2. The solubility of CoQ_{10} in the presence of CDs linearly increased with temperature and pH. UV light ($\lambda = 254$) and temperature together had a great effect on CoQ_{10} stability. After 120 min of exposure at 80 °C and UV light, about 72.3% of pure CoQ_{10} was degraded. Thermo- and photo-stability was strongly improved by complex formation; more than 64% of CoQ_{10} remaining unchanged.

A significant application of the CoQ_{10}/CD complexes is in the analysis field was described by Yang and Song (2006), who reported a novel polarographic method for the determination of CoQ_{10} in β-CD and iodinate system. These authors improved the stability of CoQ_{10} to light by forming an inclusion complex of CoQ_{10} with β-CD. In addition, the polarographic reductive wave of the inclusion complex was catalyzed by KIO_3 to produce an association/parallel catalytic wave, which was two orders of magnitude greater than the reductive wave of CoQ_{10} as regards analytical sensitivity. The proposed method is highly sensitive and allows CoQ_{10} to be determined under light, making it useful for the rapid analysis of CoQ_{10} in pharmaceutical preparation samples. In addition, it may meet the difficult requirements involved in biological samples. If complex biological samples are treated with the necessary separation and accumulation processes, the method could be applied to the determination of CoQ_{10} in more complex biological samples.

Finally, Uekaji et al. (2011) recently reported the enhancement of the stability and bioavailability of coenzyme CoQ_{10} oxidized form by γ-CD complexation. In a series of the studies, the authors investigated an easy and economical conversion of CoQ_{10} oxidized form to its reduced form in complex powder, using inexpensive vitamin C as the reductant.

CYCLODEXTRINS AND FATTY ACIDS

The complexation of fatty acids (both saturated and unsaturated by natural and modified CDs has been reported in numerous works. For example, the properties and applications of fatty acid/CDs were studied by Szente et al. (1993) in an interesting paper several years ago. Based on that paper, several researchers have focused their research on the encapsulation of these guest molecules. As regards the nature of encapsulation, the CD hydrophobic cavity can include, at least in part, fatty acid chains (Duchêne et al., 2003). The exact nature of the CD and that of the fatty acid (chain length, double bonds) together, have a significant influence on the inclusion characteristics. Depending on the fatty acid chain length (C4–C18), one CD (or more) can interact and include the carboxylic chain. Duchêne et al. (2003), in an excellent paper, that for both short ($\leq$C8) and long ($\geq$C12) chain fatty acids, the highest affinity is obtained with α-CD, which has the narrowest cavity (Gelb and Schwartz, 1989). In the case of intermediate chain fatty acids ($\approx$C10), part of the chain is outside the β-CD cavity so these can better interact with α-CD than with β-CD at the same 1:1 molecular ratio (Szente et al., 1993). These results were confirmed by a study of the water solubility of fatty acids from C6 to C12 in the presence of α- or β-CD (Schlenk and Sand, 1961). As will be discussed below, the presence of double bonds has been investigated by different authors (Jyothirmayi et al., 1991; Szente et al., 1993; López-Nicolás et al. 1995). On the other hand, the influence of fatty acid chain length was demonstrated by Schlenk and Sand (1961), Gelb and Schwartz (1989) and Shimada et al. (1992). The number of CDs capable of interacting with fatty acids increases as in the hydrocarbon chain increases in length. The formation of inclusion compounds occurs through two types of interaction: (1) the creation of hydrogen bonds between the carboxyl of the fatty acid chain and the hydroxyls in position 6 on the CD; and (2) the creation of hydrophobic interactions between the fatty acid hydrocarbon chain and the CD cavity.

In a recent paper, Parker and Stalcup (2008) studied the complexation by β-CD of different fatty acids such as octanoate, 2-octenoate, decanoate, 9-decenoate, and dodecanote, using affinity capillary electrophoresis and isothermal titration calorimetry.

As regards to the application of fatty acid CD complexes, Ajisaka et al. (2002) showed that the addition of medium-chain fatty acid (caprylic capric and lauric acid)/CD complexes to ruminant diets may be effective in reducing methane production.

Moreover, Greenberg-Ofrath et al. (1993) used CDs as carriers of cholesterol and fatty acids in the cultivation of mycoplasmas. The design of fully or partly defined media for mycoplasma cultivation needs the essential lipids, cholesterol and long-chain fatty acids to be provided in an assimilable and nontoxic form. Greenberg-Ofrath et al. (1993) introduced CDs as carriers of these lipids, thus providing alternatives to serum or bovine serum albumin. β-CD was found to inhibit the growth of the sterol-requiring *M. capricolum* in both serum and BSA media, but it stimulated the growth of the sterol-independent *A. laidlawii*. In sharp contrast to β-CD and DIMEB, HP-β-CD added at 5 and 10 mM to a basal medium supplemented with lipids permitted the growth of *M. capricolum*.

Linoleic acid (LA) and conjugated linoleic acid (CLA) are the two polyunsaturated fatty acids most studied in connection with encapsulation with CDs.

Linoleic Acid

Using nuclear magnetic resonance techniques, Jyothirmayi, et al. (1991) studied the complexation of linoleic and arachidonic acid by α- and β-CD. Moreover, the complexation of polyunsaturated fatty acids by several CDS was reported many years ago by our group. For example, López-Nicolás et al. (1995) and Bru et al. (1995) studied the equilibria of LA/CD complexes to investigate the behavior of "soluble lipids" in solution as a function of factors that typically affect biochemical processes, such as pH, temperature, and CD structure. The above complexes

were formed with a stoichiometry of 1:2 in solution. The first CD molecule interacts with linoléico acid and through hydrogen bonds when the pH is below the fatty acid pK_a; hydrophobic interactions may also play an important role at high pH. The second CD molecule makes only hydrophobic contact with the linoleic and hydrocarbon chain. The formation of hydrogen bonds was dependent on the inner diameter of the CD, whereas the strength of the hydrophobic interactions between CD and LA were related with the presence of hydrophobic groups in the CD. The first CD molecule interacts more strongly with linoleic and at increased temperatures. The quantitative description of the linoleic and-CD interaction allows absolute control of the effects of the lipid on biochemical processes.

Later, López-Nicolás et al. (1997) reported that the structural resemblance between CDs and starch in its helical conformation makes the former a suitable model system for studying the oxidation of polyunsaturated fatty acids such as LA, which naturally occurs and associated with amylose as inclusion complexes in several plant storage tissues. For the oxidation of linoleic and by LOX in the presence of β-CD, the authors proposed a model in which free linoleic is the only effective substrate; thus the oxidation of the complexed substrate required the previous dissociation of the complex. Consistently, β-CD was shown to slow down the reaction rate of LOX oxidation, which was mainly due to the increases in K_m and V_{max} remaining unchanged. The apparent inhibition produced by β-CD (increased K_m) is due to the removal of effective substrate in the form of inclusion complexes. This "sequestered" substrate can, however, be converted since it is in equilibrium with the free form.

Moreover, the advantages of the presence of CDs in a reaction catalyzed by immobilized LOX were reported for the first time by Pérez-Gilabert and García-Carmona (2005). In this study, the authors showed that the steady-state rate in the presence of β-CD was seven times higher than in control experiments using the same concentration of LA; furthermore, the percentage of substrate conversion (and product accumulation) in the presence of β-CD was higher than in the control assays. The operational stability of the immobilized enzyme increased in the presence of β-CD, while an increase in the percentage of the main reaction products was also observed.

Finally, Hadaruga et al. (2006) made a thermal stability study of LA/α- and β-CD complexes using bionanoparticles that were obtained by a solution method and were characterized by differential scanning calorimetry and transmission electron microscopy. The authors analyzed pure LA, the corresponding thermally (50–150°C) degraded raw LA samples and those recovered from the complexes by gas chromatography–mass spectrometry, after conversion to the methyl esters. The nanoparticles obtained in that work showed good yields of 88% and 74% for α- and β-CD complexes, respectively. The main degradation products (for the thermally degraded raw samples) were aldehydes, epoxy, dihydroxy derivatives, homologues, and isomers of LA. The same authors observed the good thermal stability of nanoparticles, especially for the LA/α-CD complex, which contained a relative concentration of above 98% fatty acid in the case of temperature degradations of 50 and 100°C. However, a lower concentration of 92% was observed in the case of the LA/βCD complex while, for the temperature degradation of 150°C, the LA was partially converted to more stable geometrical isomers.

As regards the practical applications of the fatty acid/CD complexes, Regier (2007) showed that when in the form of a molecular inclusion compound with α-CD, LA is effectively protected against oxidation. Finally, investigations by this author into the storage and light stability, using olfactory tests and headspace analysis of the formulations, pointed to the stability of the suitable inclusion compound. Moreover, the reversible complexation makes it possible for the first time to use LA in various cosmetic formulations and personal-care products.

Conjugated Linoleic Acid

CLA is a collective term describing a mixture of positional and geometrical isomers of LA including a conjugated double bond in various positions (Uehara et al., 2008). It is industrially manufactured by alkali-induced conjugation of LA-rich oils such as sunflower oil, in the presence of propylene glycol. After conjugation, a mixture consisting of almost equivalent amounts of the isomers, 9-*cis*, 11-*trans*-CLA (c9t11), and 10-*trans*, 12-*cis*-CLA (t10c12), which have two double bonds (*cis* and *trans*) at different positions of the aliphatic chains, is obtained. The two CLAs exhibit various biochemical properties, including a reduction of cancer incidence, beneficial effects in atherosclerosis, decreased body fat content and improved immune functions. It has been reported that the biochemical properties of c9t11 and t10c12 differ in that the c9t11 isomer exhibits anti-tumor activity, whereas the t10c12 isomer decreases body fat, increases energy expenditure, and suppresses the development of hypertension (Uehara et al., 2008).

As indicated previously, free fatty acids and their derivatives are compounds which can be complexed in the hydrophobic inner cavity of CDs (Jyothirmayi et al., 1991). Thus, polyunsaturated fatty acids encapsulated in several types of CDs have been shown to be completely protected against oxidation even in pure oxygen (Reichenbach and Min, 1997). For this reason, Kim et al. (2000) expected that CLA could be protected against oxidation by microencapsulation in CDs and investigated for first time the oxidative stability of CLA microencapsulated in (α-, β-, γ)-CDs at various mole ratios, when reacted at 35°C. These researchers studied the process of complexation by measuring headspace-oxygen depletion in air-tight serum bottles and by measuring the peroxide values. The rate of oxygen depletion determined by Kim et al. (2000) was reduced from 41.0 (control) to 21.5, 2.1, 1.2, and 1.1 μmol/L h^{-1} by CLA/ α-CD microencapsules at 1:1, 1:2, 1:4, and 1:6 mole ratios, respectively, indicating that CLA oxidation was completely protected by a 1:4 mole ratio of CLA/ α-CD. Such a protective effect by CLA/β-CD or CLA/γ-CD microencapsules was achieved at a 1:6 mole ratio, but the effect by CLA/β-CD was slightly greater

than that of CLA/γ-CD. The protective effect of α-, β-, γ-CDs for CLA oxidation was confirmed by their POV-reducing abilities in CLA/CDs. The results suggested that α-CD was the most effective for the protection of CLA oxidation by microencapsulation, followed by β-CD and γ-CD.

However, In a later and similar work, Park et al. (2002) characterized the inclusion complex of CLA with natural CDs with opposite results. These authors prepared the complexes to determine the mole ratio of CLA complexed with CDs and the oxidative stability of CLA in the CLA/CDs inclusion complexes. When measured by GC, NMR, and T_1 value analyses, 1 mol of CLA was complexed with 5 mol of α-CD, 4 mol of β-CD, and 2 mol of γ-CD. The results presented by these authors showed that the oxidation of CLA at 35°C for 80h was completely prevented by the formation of CLA/CDs inclusion complexes.

To date, the most important application of the complexes between CLA and CDs is that reported by Liu et al. (2005) in a study on the separation of CLA isomers by CD-modified micellar electrokinetic chromatography. Analytical methods for the determination of the composition of CLA isomers are important for both research and routine inspection purposes. Gas chromatography is one of the methods used to analyze CLA isomers, but it produces overlapping peaks of some CLA isomers, such as 9*cis*, 11*trans* and 8*trans*, 10*cis* and their geometric isomers (*cis,cis* and *trans,trans*) and a retention time of more than 50 min is needed with this method. Furthermore, fatty acids needed to be methylated with the GC method. Another method, Ag^+-HPLC, allowed well-resolved separation of three groups of geometric isomers (*trans,trans, cis*/*trans*, and *cis,cis*) of a commercial CLA mixture, and each group could be further separated into positional isomers, such as 11,13-CLA; 10,12-CLA; 9,11-CLA; and 8,10-CLA. However, the four peaks of each group partially overlapped and were separated using three columns in series, while the retention time was prolonged up to 60 min. To resolve these problems, Liu et al. (2005) used the complexes between CLA and CDs. In this paper, a CD-modified micellar electrokinetic chromatography (CD-MEKC) method was developed to separate the CLA isomers. All seven CLA isomers (9*cis*,11*cis*-CLA, 9*cis*,11*trans*-CLA, 9*trans*,11*trans*-CLA, 10*trans*,12*cis*-CLA, 11*cis*,13*cis*-CLA, 11*cis*,13*trans*-CLA, and 11*trans*,13*trans*-CLA) were completely separated in the optimized conditions [4% (w/v) β-CD, 54 mM sodium dodecyl sulphate (SDS), 80 mMborate (pH 9.0), 8 M urea, 4% (v/v) ethanol, 30 kV and 15°C]. The CD-MEKC method reported was better than to the gas chromatographic and silver-ion high-performance liquid chromatographic methods generally used in analyzing CLA isomers.

Recently, Yang et al. (2009 and 2010) compared the formation of inclusion complexes between β-CD/CLA and amylose/CLA in different papers. In the first work (2009) a delivery system for bioactive CLA through a self-assembled amylase/CLA complex was investigated are compared with a β-CD/CLA complex. The results show the amylose-CLA complex offers better antioxidative protection against on CLA than β-CD/CLA complex, supporting the strong complexing interaction between CLA and amylose demonstrated by thermogravimetric analysis. Moreover, in a second paper (Yang et al., 2010), these authors confirmed the formation of an amylase/CLA/βCD three-component complex. Therefore, β-CD can be used to manipulate the crystallization process of amylose to modulate food product quality, and the amylose-β-CD complex could also be applied to improve the delivery efficiency of CLA and other bioactive compounds.

CYCLODEXTRINS AS ANTIOXIDANT MOLECULES

CDs as Inhibitors of Food Browning

Color is a sensory property with a strong influence on food acceptance as it contributes decisively to the initial perception of a food's condition, ripeness, degree of processing, and other characteristics. One of the main factors that can alter the color of food and so limit its commercial shelf life is browning since the organoleptic and nutritional properties of foods may be strongly altered if this undesirable reaction is not controlled. Therefore, the control of the browning during the processing stages of food has always been a challenge for food researchers (Walker, 1977; Sapers, 1993; Sapers et al., 2001). The degree of browning depends on the presence of oxygen, reducing substances, metallic ions, pH, temperature, and the activity of different oxidizing enzymes. One of the main factors that must be controlled is the enzymatic activity of PPO (monophenol dihydroxyphenylalanine: oxygen oxidoreductases, EC 1.14.18.1) (Sánchez-Ferrer et al., 1995). The presence of this enzyme in different fruits has been reported by several authors in the past decade, and much research has focused on the use of postharvest chemical treatments to avoid enzymatic browning (Sapers and Miller, 1988). However, many of these treatments present serious disadvantages for use in the food industry because they can have negative effects on the sensorial properties of the products (Abreu et al., 2003). Moreover, some chemical treatments have been associated with severe allergy-like reactions in certain populations, for which reason the Food and Drug Administration has restricted their use to only a few applications to inhibit the browning of foods (Sapers, 1993). Therefore, alternative methodologies are being investigated to extend the shelf life of foods, among them fresh juice fruit.

Recently, the use of CDs has been proposed by several authors for the control of enzymatic browning in different fruits, acting as "*primary antioxidants*". To this end, the effectiveness of CDs as browning inhibitors was determined as the difference between the colors observed in the CD-treated sample and the controls, using the color space CIE-L^*, a^*, and b^* system. Different types of CDs (natural and modified) have been used to study the evolution of the color parameters of different fruit juices such as pear (López-Nicolás and García-Carmona, 2007), peach (López-Nicolás et al., 2007a), apple (López-Nicolás et al.,

2007b), and grape (Nuñez-Delicado et al., 2005). In all cases, both the scalar (L^*, a^*, and b^*) and the angular coordinates (H^* and C^*) were evaluated to define the color of fruits juice completely in the absence and presence of each type of CDs. To evaluate the behavior of pear, peach, apple, and grape juice enzymatic browning after the addition of CDs, increasing concentrations of different CDs were used. The evolution of the space CIE-L^*, a^*, and b^* parameters, reflecting inhibition of the darkening of the fruit juices with time shows that CDs are able of complexing PPO substrates, thereby preventing their oxidation to quinones and subsequent polymerization to brown pigments. Moreover, kinetic models to evaluate fruit juice enzymatic browning in the absence and presence of CDs have been proposed. The different K_F values between the mixtures of diphenols present in fruits juice and different CDs were calculated.

As mentioned above, the use of CDs as antibrowning agents in fruit juices has received growing attention. However, there has been no detailed study of the behavior of these molecules as substances, which can also lead to the darkening of foods. For this reason, the role of CDs as activators and inhibitors of latent banana pulp PPO was studied by Sojo et al. (1999). In this work, the effect of CDs on *o*-diphenol oxidation catalyzed by banana PPO was studied. The oxidation of dopamine, the natural substrate of banana, in the presence of CDs was unaffected, because this hydrophilic phenol does not form inclusion complexes with CDs. However, when a hydrophobic phenol such as *tert*-butylcatechol (TBC) was used, a marked inhibition was observed with β-, HP-β-CD, and maltosyl-β-CDs. This inhibition was due to the complexation of TBC in the CD core, demonstrating that banana pulp PPO worked only toward free substrate and not toward the complex TBC/CDs. In addition, the effect of some inhibitors in the presence of CDs and dopamine as substrate was studied. Increasing concentrations of CDs, in the presence of two inhibitors (4-iodophenol and cinnamic acid) were able to activate the inhibited enzyme to reach the noninhibited level by complexing the inhibitors in the hydrophobic core of the CDs. This dual effect of CDs as activator and inhibitor was tested in crude banana pulp extracts, with surprising activation effects never before described being observed. To confirm these data, a kinetic study of the activation of banana juice enzymatic browning by the addition of maltosyl-β-CD was reported by López-Nicolás et al. (2007c). In this paper, when the color of fresh banana juice was evaluated in the presence of different CDs, the evolution of several color parameters was the opposite of that observed in other fruit juices. Moreover, a kinetic model based on the complexation by CDs of the natural browning inhibitors present in banana was developed for the first time to clarify the enzymatic browning activation of banana juice. Finally, the apparent K_F values between the natural PPO inhibitors present in banana juice and maltosyl-β-CD were calculated. The results presented in this paper show that any antioxidant agent used to avoid enzymatic browning must be tested individually for each food because an opposite effect to that desired may be produced.

Knowledge of the kinetic models of the enzymatic processes occurring in foods is essential to understand their macroscopic behavior, as in the case of organoleptic properties, such as color. The surprising observation that CDs chosen as natural antibrowning agents of fruit juice may behave as pro-browning agents, depending on the fruit source, is explained in this investigation through a biochemical model reflecting the interaction between natural compounds present in banana juice and CDs. Therefore, the presence of hydrophobic or hydrophilic phenols in the fruit structure and the inability of CDs to complex dopamine mean that a recognized antibrowning agent, such as CDs, can be converted into a browning agent, leading to changes in color parameters not previously suspected. In Fig. 4, we can observe that the negative values of the enzymatic browning percent variation of pear, peach, grape, and apple juices indicate that the addition of 90 mM maltosyl-β-CD as a browning inhibitor is effective to the extent calculated. However, the positive percent variation value obtained for banana juice indicates the activation of the enzymatic browning by the addition of maltosyl-β-CD.

To resume, the main phenolic compounds present in these fruit juices that can be oxidized by PPO are shown in Table 1. As can be seen in this table, in the case of apple, grape, pear, and peach juices the phenolic compounds are of a hydrophobic nature and have been reported as guest molecules for different CDs. However, the main natural phenol present in banana is dopamine, a hydrophilic compound that is not complex by CDs.

CDs as "secondary antioxidants"

In addition to their role as antibrowning agents in complexing the substrates of PPO and preventing the oxidation of the phenols present in fruit juices, other authors (López-Nicolás et al., 2007a; López-Nicolás and García-Carmona 2007) have reported the use of CDs as secondary antioxidants to improve the color of fresh juices. As is known, the synthetic antioxidants, metabisulfite and L-cysteine, have a higher antibrowning effect on fruits juice than those from natural sources, such as ascorbic acid (AA) or CDs. However, there is a growing interest in natural antioxidants for use in food, although another strategy would be to look for "preservers" of the natural antioxidant capacity of a particular food. In this approach, the development and use of new natural "*secondary antioxidants*" is a fresh and challenging task, as demonstrated by the oxidation of phenols by LOX (Nuñez-Delicado et al., 1997), in which CDs act as secondary antioxidants in synergism with AA, and seem to act as a "*secondary antioxidants*", reducing fruits juice browning and enhancing the naturally occurring antioxidant capacity of the food. Thus, CDs can enhance the ability of AA to prevent enzymatic browning became of the protective effect provided by the CDs against AA oxidation.

This capacity of CDs to function as a secondary antioxidant in juices was evaluated and a kinetic model was proposed (López-Nicolás et al., 2007a; López-Nicolás and García-Carmona 2007). AA is the best-known chemical agent for

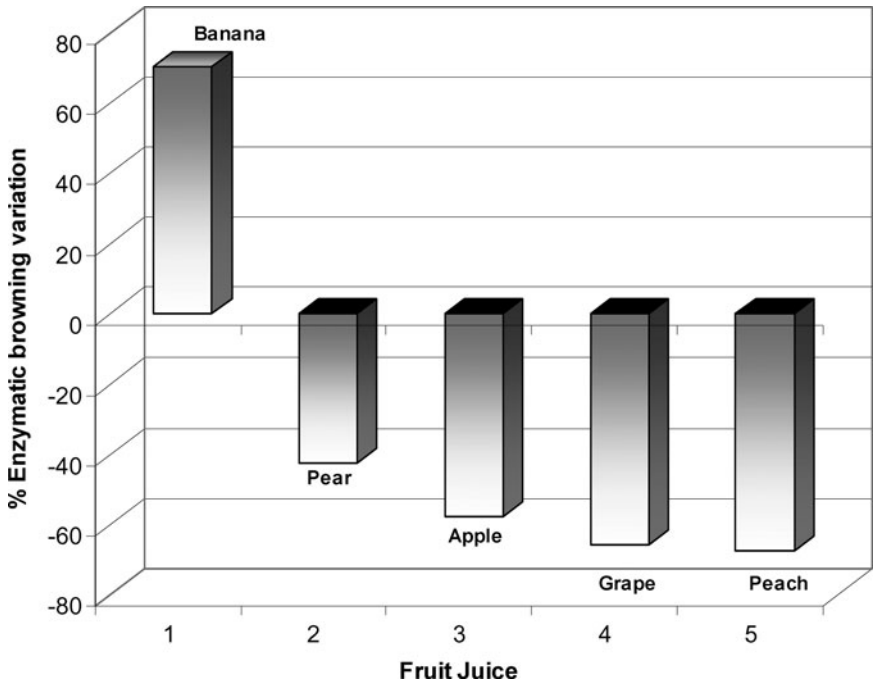

Figure 4 Percent variation of enzymatic browning in several juices in the presence of 90 mM maltosyl-β-CD. Each data point is the mean of 3 replicates. (Adapted from López-Nicolás et al., 2007c).

reducing the browning reaction. However, once the added AA has been completely oxidized to DHAA, *orto*-Quinones (*o*-Qs) accumulate and suffer browning. More stable forms of AA derivatives, such as erythorbic acid, 2- and 3-phosphate derivatives, phosphinate esters and ascorbyl-6-fatty acid esters, have been developed to overcome these problems, but the results have not been very satisfactory. In the model proposed by us, CDs can enhance the ability of AA to prevent enzymatic browning due to the protection that AA is offered against oxidation by *o*-Qs (Fig. 5). In the absence of CDs, the total concentration of PPO substrate is available to be oxidized to *o*-Qs by PPO in the presence of O_2. However, in the presence of CDs, AA is protected, due to the complexation of PPO substrates in the hydrophobic cavity of CDs. CDs slow down the production of *o*-Qs and, hence, the oxidation of AA, because only free substrates, in equilibrium with CD-bound phenol (CD-S), are oxidized by PPO in the presence of O_2 to *o*-Qs. In this way, the reaction is slowed down and the shelf life of the food is prolonged.

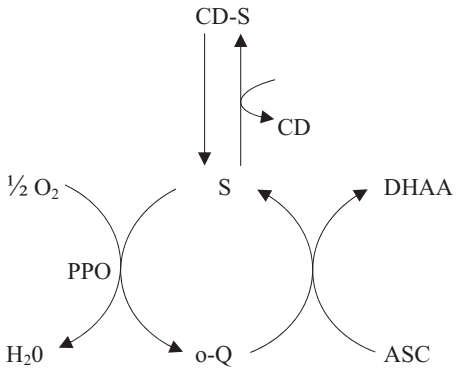

Figure 5 Use of maltosyl-β-CD as secondary antioxidant on the browning of pear juice. PPO: polyphenol oxidase, CD: cyclodextrin, S: free PPO substrate, CD-S: complex between PPO substrates and CD, o-Q: orthoquinone, AA: ascorbic acid, and DHAA: dehydroascorbic acid. (Adapted from López-Nicolás and García-Carmona, 2007).

Table 1 Effect of adding CDs on the browning of several fruit juices and the complexation of their main polyphenolic compounds (Adapted from López-Nicolás et al., 2007c)

Fruit juice	Principal polyphenolic compounds	PPO substrates	Complexation by CDs	Effect of CD on browning
Pear, Apple, Peach, Grape	Chlorogenic acid	+	+	Inhibitor
	Caffeic acid	+	+	
	Catechin	+	+	
	Epicatechin	+	+	
	Quercetin	+	+	
Banana	Dopamine	+	–	Activator

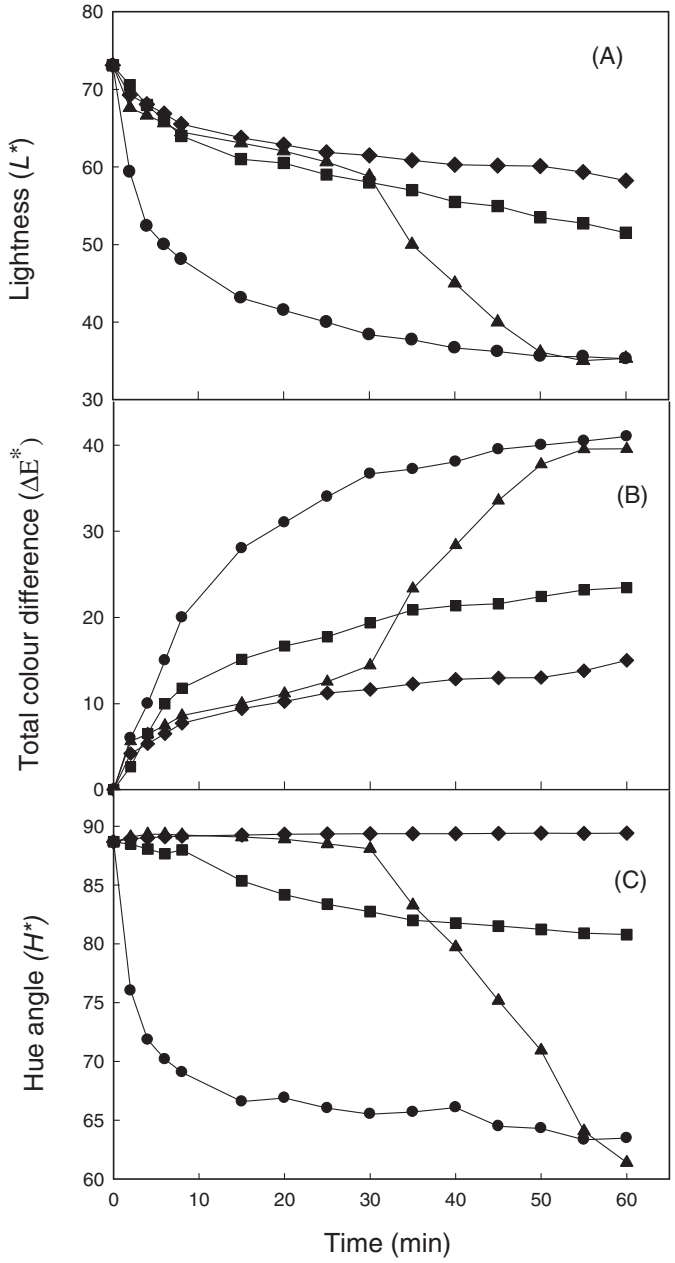

Figure 6 Evolution of lightness (L*) (A), total color difference (ΔE*) (B) and Hue angle (H*) (C) in the absence of any agent (●) and in the presence of 2.28 mM AA (●), 90 mM maltosyl-β-CD (■), and 90 mM maltosyl-β-CD plus 2.28 mM AA (♦). (Adapted from López-Nicolás and García-Carmona, 2007).

To confirm this hypothesis, we show an example of the evolution of pear juice color in the absence and presence of 2.28 mM AA and 90 mM maltosyl-β-CD, which was published in an interesting work by López-Nicolás and García-Carmona 2007 (Fig. 6A). Pear juice lightness (L^*) fell significantly when 90 mM maltosyl-β-CD was added to the medium. Moreover, when 2.28 mM AA was added, the rapid decay in L^* observed in the absence of any reagent was drastically reduced in the first 30 min. After which, L^* quickly decayed to reach the same values as observed in the absence of any agent. Furthermore, in Fig. 6A, we can see that when both 2.28 mM AA and 90 mM maltosyl-β-CD were added simultaneously to pear juice, the decrease in lightness (L^*) was less than in the absence of either of them individually. Moreover, in the presence of both agents, the pronounced decay observed in L^* values after the first 30 min was eliminated and the initial lightness of pear juice was almost totally maintained. This behavior is probably due to the preservation of the antioxidant capacity of AA by maltosyl-β-CD. To corroborate these results, the evolution of total color difference (AE^*) in the presence and absence of these enzymatic browning inhibitors was tested. As shown in Fig. 6B, the presence of AA in the medium reduced the change in this parameter more effectively than maltosyl-β-CD but only during the first 30 min of the reaction time. After this time, the total color difference increased sharply to reach the maximum variation observed in the absence of any regent. However, in the presence of 2.28 mM AA plus 90 mM maltosyl-β-CD, the changes in AE^* were lower than when these reagents were added independently. Moreover, the inhibition rate of AE^* was maintained after the first 30 min. These results for L^* and AE^* confirm the hypothesis that the hydrophilic nature of AA precludes its inclusion in maltosyl-β-CD, and so the apparent CD-mediated protection of AA in pear juice is probably due to the complexation of the mixture of phenols present in pear juice into the hydrophobic cavity of the maltosyl- β -CD.

Although CDs are widely used as browning inhibitors in different fruit juices, the effect of the addition of CDs on others organoleptic properties, for example, odor and aroma, has been reported in few works. Recently, some studies concerning the addition of CDs on the flavor profile of pear fruit juices have been published (Andreu-Sevilla et al. 2011, López-Nicolás et al., 2009d). Moreover, correlation of the results show in these papers, concerning the color and aroma of pear juice in the presence of CDs, with the consumer preferences has been also reported. Different descriptive sensory analysis of pear juices in both the presence and absence of CDs were carried out and odor/aroma attributes (fresh, fruity, pear-like, unnatural, etc.), plus global color, odor, aroma, and quality, were quantified using a trained panel of judges. The addition of α-CD at 90 mM resulted in pear juices with the best color but with low aromatic intensity and low sensory quality. On the other hand, the addition of α-CD at 15 mM led to a pear juice also with an acceptable color but at the same time with a high intensity of fruity and pear-like odors/aromas, making it the best appreciated juice by the panel.

CONCLUSIONS

In spite of the sharp increase in recent years of research into the encapsulation of lipophilic antioxidants by cyclodextrins, several problems still remain about the host/guest interaction and contradictory reports have been published. Although the positive effect of cyclodextrins on the solubility, stability, and protection against prooxidant agents such as light, heat

or oxidative enzymes have been demonstrated, the influence of encapsulation on such important factors as K_F values, the bioavailability of the guest molecule and the antioxidant capacity of the complexed compound is still not clearly defined and conflicting data have been reported by different authors for the same CD/antioxidant complex. This review shows that two principal factors are related with these problems. Firstly, several authors compare the K_F values determined under different physico-chemical conditions leading to erroneous conclusions. Indeed, factors such as pH of the medium or antioxidant pK_a play a fundamental role in encapsulation and are often not taken account. Secondly, although the analytical techniques to evaluate the complexes formed are well defined, the different assays used to measure the antioxidant capacity of the lipohilic antioxidant compounds may produce contradictory results. Thus, the antioxidant capacity of a host/guest complex cannot be determined indistinctly by techniques such as FRAP, ABTS, DPPH, or ORAC because each of them measures different properties of the complex. This fact may explain why, for the same type of CD and lipohilic antioxidant, some researchers have reported an increase in the antioxidant capacity of the complex, while others have observed a decrease in the same property. Yet other authors have found that encapsulation of the antioxidant compound has no effect on its antioxidant activity. Finally, it should be clearly stated that while it is important to publish new studies that demonstrate the interaction between others lipophilic antioxidant compounds and cyclodextrins, it is even more necessary to look for practical applications of the complexes that have already been reported.

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ABREVIATURES

AA = ascorbic acid
ABTS = 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
CD = cyclodextrin
CGTase = glycosyltransferase
CLA = conjugated linoleic acid
CMC = carboxymethyl cellulose
CoQ_{10} = coenzyme Q10
CUPRAC = cupric reducing antioxidant capacity
CVD = cardiovascular disease
DHAA = dehydroascorbic acid
FL = fluorescein
FRAP = ferric reducing antioxidant power
G-β-CD = 6-O-α-D-glucopyranosyl-β-CD
HP-β-CD = hidroxy-propil- β- cyclodextrin
HP-γ-CD = hidroxy-propil- γ - cyclodextrin
K_F = complexation constant
LA = linoleic acid
LOX = lipoxygenase
MECK = micellar electrokinetic chromatography
Mβ-CD = methyl- β-CD
o-Q = orthoquinone
ORAC = oxygen radical absorbance capacity
PPO = polyphenoloxidase
RM-β-CD = randomly methylated-β-CD
SBE-β-CD = sulfobutylether-β-CD
TEAC = trolox equivalent antioxidant capacity
TBC = *tert*-butylcatechol

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