Study of the encapsulation of resveratrol with several cyclodextrins using HPLC

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Introduction

Although several beneficial biological effects of *(E)-resveratrol* on human health have been reported in the last decade (Counet et al. 2006, Latruffe et al. 2002), some problems related with its poor solubility in aqueous media, its poor **bioavailability or** its propensity to oxidation have meant that no novel food has been enriched in this important antioxidant compound. For this reason, the encapsulation of this bioactive substance in cyclodextrins (CDs) is proposed in this work to protect it from potential adverse reactions. Moreover, the method described permits a reduction in the *(E)*-resveratrol analysis times in liquid chromatographic methods. To reach these objectives, the effect of several factors in the mobile phase or variations in the temperature, on both the capacity factor and the apparent formation constants (K_F) of *(E)*-resveratrol-CD inclusion complexes will be discussed. To reach these objectives, normal phase HPLC (NP-HPLC) and reversed phase (RP-HPLC) systems were used.

Materials and Methods

Reagents were purchased from Sigma-Aldrich (Madrid, Spain).Twenty microliters of *(E)*-resveratrol (prepared at a concentration of 0.05 mg/ml in methanol) were injected for HPLC analysis using a Merck-Hitachi L-6200 pump and a diode array detector Shimadzu SPD-M6A UV. For the aqueous mobile phase studies a commercially available Agilent Zorbax Bioseries GF-450 (250 x 9.4 mm I.D. 6 µm particle size) was used. For reversed phase a commercially avalaible column LiChrospher RP18 (250 x 4 mm I.D. 5 µm particle size) was used For all experiments the mobile phase flow-rate was 1.00 ± 0.01 mL/min and the UV detector was operated at 306 nm.

Results and Discussion

To evaluate the interaction between *(E)*-resveratrol and the host CD at a molecular level, the effect of different types of CDs on the retention time of *(E)*-resveratrol in aqueous medium was studied using NP-HPLC. As expected, the retention time decreased as the concentration of CD in the mobile phase increased, due to the formation of the *(E)*-resveratrol-β-CD complexes, which enhance the guest solubility in the mobile phase and reduce its residency time in the column (Table 1).

		k			
Type	*no addition*	*0.5 mM*	*1.0 mM*	*1.5 mM*	*2.0 mM*
α-CD	2.54	1.90	1.57	1.31	1.18
β-CD	2.54	1.31	0.88	0.65	0.54
γ-CD	2.54	2.50	2.42	2.38	2.30

Table 1. Effect of the CD concentration on the capacity factors of *(E)*-resveratrol determined using increasing concentrations of α-,β- and γ-CD at 25ºC.

Furthermore, the K_F between *(E)*-resveratrol and different types of CDs were determined. Fitting the values of capacity factors to the equations previously described by López-Nicolás (López-Nicolás 2006; López-Nicolás 2008) provides the corresponding K_F. As regards the different species α-, β- and γ-CD it can be observed that the highest K_F value (K_F =1922 ± 89 M^{-1}) was found for β-CD, followed by α-*CD* (K_F =565 ± 34 M^{-1}) and, finally, γ-CD showed the lowest K_F value (K_F = 55 ± 4 M^{-1}). These results indicated that *(E)*-resveratrol interacts more strongly with β-CD, which was therefore considered the most suitable CD for the present investigation.

Moreover, and in order to evaluate the effect of the presence of an organic modifier in the retention time and capacity factor of *(E)*-resveratrol, RP-HPLC was used. As it was expected, the increase in the capacity factor was always most pronounced for the highest mobile phase H_20 concentration (70 %, v/v) (Figure 1).

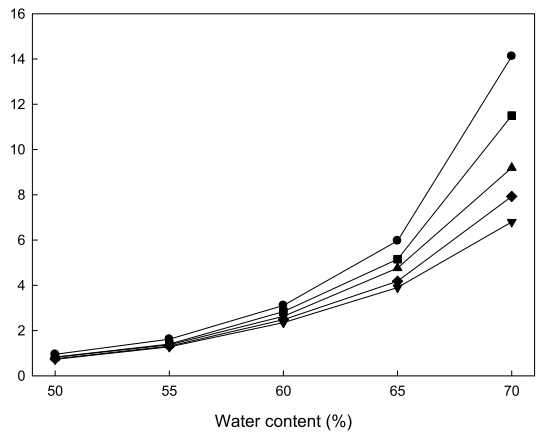

Figure 1. Capacity factors of *(E)*-resveratrol-β-CD complexes as a function of water percentage in methanol-water mobile phases: (●) no β-CD ; (■) β-CD 0.5mM; (▲) β-CD 1mM; (♦) 1.5 mM; (▼) 2mM.

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Finally, the effect of the mobile phase composition on the K_F using both NP-HPLC and RP- HPLC is shown in Table 2.

HPLC System					
	N. Phase	R. Phase			
% H_20	100	70	65	60	50
K_F	1922	1821	1008	663	589

Table 2. Effect of the water content on the K_F between β-CD and *(E)*-resveratrol at 25ºC.

Finally, in this paper the effect of temperature on the K_F was studied (Table 3) at two different mobile phase composition. As it was expected, K_F decreases with decreasing temperature because it is accompanied by a higher degree of complexation

	Tª (ºC)	20	25	30	37
K_F	*100 % H_2O*	2400	1922	1676	1497
	65 % H_2O	1176	1080	744	607

Table 3. Effect of the temperature on the K_F between β-CD and *(E)*-resveratrol

Conclusion

The use of CDs reduces the analysis time for determining *(E)*-resveratrol by HPLC systems. The capacity factors and K_F between this stilbene and CDs are strongly dependent of several factors as: type of CD, temperature or the presence of an organic modifier in the reaction medium.

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