P-073	**Study of the encapsulation of methyl jasmonate by natural and modified cyclodextrins using RP-HPLC**	

López-Nicolás, J.M. *[#], Escorial Camps, M. and García-Carmona, F.
Dept. Biochemistry ad Molec. Biology. University of Murcia. Murcia, Spain

INTRODUCTION AND OBJECTIVES

Cyclodextrins (CDs) are a group of structurally related natural products formed during the bacterial digestion of starch. These cyclic oligosaccharides consist of α-(1–4) linked α-D-glucopyranose units and contain a somewhat lipophilic central cavity and a hydrophilic outer surface. The most important functional property of CDs is their ability to form inclusion complexes with a wide range of organic guest molecules (López-Nicolás 2008). On the other hand, methyl jasmonate (MJ) is an important compound which is used for regulation of many important processes in plant development, including seed germination and pollen development, flower and fruit development, leaf abscission, and organ senescence. In recent years some works about the use of CDs and MJ to stimulate the production of different metabolites have been published. However, no research has characterized the encapsulation of MJ into the internal cavity of CD. For this reason the main objective of this work is the physicochemical study of the encapsulation of MJ by different CDs.

MATERIAL AND METHODS

Chemicals and reagents

MJ and CDs were purchased to Sigma (Madrid, Spain). The methanol and water used in this study were of HPLC grade purchased from Scharlau S.A. (Barcelona, Spain).

Equipment and experimental procedures

Twenty microliters of MJ (prepared at a concentration of 0.2% in methanol) were injected for HPLC analysis on equipment using a Merck-Hitachi pump L-6200 and a diode array detector Shimadzu SPDM6A UV (Shimadzu, Kyoto, Japan). A commercially available reversed-phase column LiChrospher RP18 (150mm×4mm I.D. 5µm particle size) was used. For all experiments the mobile-phase flow-rate was set and systematically controlled at 1.00±0.01 mL/min and the UV detector was operated at 210 nm. To prepare the correspondent mobile phases, several types of natural and modified CDs, at different concentrations, were added to the binary mixtures methanol:water.

Temperature studiesTo study the effect of the temperature on the encapsulation process of MJ by CD, increasing temperatures from 15 to 40 ◦C were selected. Using the thermodynamic relationship reported by Rodríguez-Bonilla et al., (2010) the thermodynamic parameters (standard enthalpy, entropy and Gibbs free energy change) of transfer of the MJ from the mobile phase to the CD were calculated.

RESULTS AND DISCUSSION

Effect of the cyclodextrin structure on the encapsulation of methyl jasmonate

To characterize the interaction between MJ and the natural CDs at molecular level, the first step was to study the interaction between MJ and several types of CD with differing structure, size and glucose number of glucose units. Three types of natural CD with GRAS status, all approved recently for use as additives in the European Union (α-, β- and γ-CD), were used to this end. The data concerning to the of adding increasing α-,β- and γ-CD concentrations on the retention time of MJ show that the lowest retention time was obtained with β-CD, followed by α-CD and γ-CD. At the molecular level, our data show that the inner diameter of the CD formed by seven units of glucose (β-CD: 6.0–6.4 Å) fitted MJ better than the inner diameter of six units (α-CD: 4.7–5.2 Å) or eight units (γ-CD: 7.5–8.3 Å) of glucose.

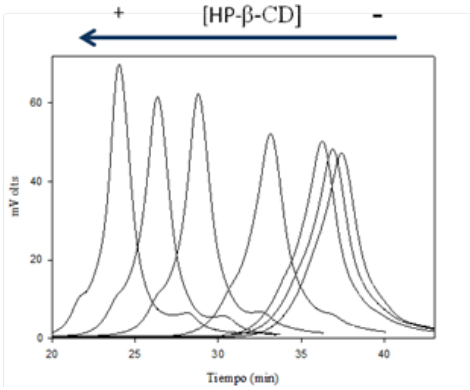

Figure 1. **Effect of increasing concentrations of HP-β-CD (0-12 mM) on retention time of MJ using RP-HPLC.**

Between the modified CDs tested, HP-β-CD showed the better encapsulation behavior. For this reason, and due that this type of modified CD is the most used in culture medium, we selected HP-β-CD for the next steps of our investigation.

Stoichiometry and constant encapsulation values (KF) of the MJ–CD complexes

Because this is the first study where the interaction of MJ with CD is studied, it was necessary to investigate

the stoichiometry for CD complexes of MJ. Using eqs. described by different authors (López-Nicolás, 2008), the reciprocal of k for MJ was plotted as a function of [CD] to determine the stoichiometric ratios for MJ/CD complexes. In our study, a plot of 1/k versus [CD] gave a straight line with a linear correlation higher than 0.99, indicating that the presumed stoichiometry of the MJ/CD complexes formed was 1:1. On the other hand, when 1/k was plotted against $([CD])^2$, a non-linear relationship was obtained (linear correlation of 0.80), which indicates that the stoichiometry of the inclusion complex is not 1:2. The KF values for CD complexes indicate the stability of the complex and the conditions that can modify the inclusion process. Moreover, these KF values have an important role for the development of biotechnological, food, pharmaceutical and other uses for CD. For these reasons and since the KF values between MJ and CD will reveal such as temperature, pH, presence of modifiers, etc. the amount of MJ encapsulated in equilibrium with free MJ, the next step of this investigation was to calculate these constants. The values of apparent KF for mobile phases containing different water percentage were obtained from linear graphs of 1/k versus [CD] and linear regression slopes and intercepts. The data of KF value for a methanol concentration of 40 % employed in the mobile phase was 43.1 M^{-1}. The existence of a strong competition on the part of methanol and solute for the CD cavity reported by different authors explains the dramatic decrease in KF values observed when percentage values between 40 and 60% of methanol content were used.

Effect of temperature on the encapsulation of MJ by HP-β-CD

In order to clarify the role of temperature on the encapsulation of MJ with HP-β-CD, the effect of temperature on the effective KF was studied for the MJ/HP-β-CD interaction between 15 and 30 ºC. For all the temperatures tested the stoichiometry of the MJ/HP-β-CD complexes was 1:1, the reciprocal of k for MJ versus [CD] showing a correlation coefficient higher than 0.99 for a binary mixture of methanol–water as mobile phase. From these data, decreasing KF values were obtained (from 65 M^{-1} to 34 M^{-1}) when the temperature is increased, it being found that an increase in temperature leads to a lower degree of encapsulation of MJ by HP-β-CD.

Thermodynamic parameters for the MJ/HP-β-CD complexes

The next step of our investigation was to study the main thermodynamic parameters of the encapsulation process (ΔH°, ΔS° and ΔG° at 25±0.2 ºC) in order to study mechanistic aspects of the affinity of MJ for HP-β-CD. For this, a van't Hoff plot was used and the Ln KF was plotted versus 1/T. The data showed a lineal representation, with correlation coefficient higher than 0.99. The results obtained to three main conclusions being drawn concerning the nature of the encapsulation of MJ by HP-β-CD: (i) The negative values obtained for enthalpy changes (−19.66±1 $kJmol^{-1}$) indicate the exothermic nature of the interaction processes of MJ with HP-β-CD. This behavior is typical of hydrophobic interactions, van der Waals interactions, the displacement of water molecules from the cavity of HP-β-CD or the formation of hydrogen bonds; (ii)The process presents a negative value for entropy changes (−33.72±2 $Jmol^{-1}$ K^{-1}) due to a decrease in the translational and rotational degrees of freedom of the encapsulated MJ compared with the free ones; (iii)The process is spontaneous, as seen for the negative value obtained for the Gibbs free energy change (−6.90±1$kJmol^{-1}$) for the interactions at 25±0.2 ºC.

Effect of pH on the encapsulation of MJ by HP-β-CD

To study the effect of the pH on the encapsulation process, several pHs fron 4.0 to 11.0 were evaluated. The data show as a strong variation on the pH of the medium not produced significant differences in the KF values due, probably, to the absence of ionizable groups in the structure of MJ.

CONCLUSIONS

Among natural CDs, the interaction of MJ with β-CD was more efficient than with α- and γ-CD. Moreover HP-β-CD was the most efficient modified CD to encapsulate MJ. The decrease in the retention times with increasing concentrations of CD showed that the KF of the MJ/CD complexes were strongly dependent on both the water-methanol proportion and the temperature of the mobile phase employed. Moreover, the data obtained for ΔG°, ΔH° and ΔS° showed that the encapsulation process of MJ by CDs is exothermic and spontaneous.

REFERENCES

- López-Nicolás, J.M. et al. (2008) *Rapid, simple and sensitive determination of the apparent formation constants of trans-resveratrol complexes with natural cyclodextrins in aqueous medium using HPLC*. Food Chem. 109 868-875.
- Rodríguez-Bonilla et al. (2010). *Use of reversed phase high pressure liquid chromatography for the physicochemical and thermodynamic characterization of oxyresveratrol/beta-cyclodextrin complexes.* J. Chromatogr. B 878 1569–1575.

ACKNOWLEDGEMENTS

This work was supported by Programa de ayudas a Grupos de Excelencia de Región de Murcia, de la Fundación Séneca, Agencia de Ciencia y Tecnología de la Región de Murcia.