Time course study of oxyresveratrol inclusion complexes in aqueous solutions

Irene Conesa[1], Silvia Navarro-Orcajada[1], Adrián Matencio[2], Iván Muñoz-Sánchez[1], Lorena Laveda-Cano[1], Desiré Cano-Yelo[1], Francisco García-Carmona[1] & José Manuel López-Nicolás[1]*

[1] Departamento de Bioquímica y Biología molecular-A, Facultad de Veterinaria, Universidad de Murcia-Regional Cmapus of International Excellence "Campus Mare Nostrum" E-30100, Murcia, Spain
[2] Dipartimento Di Chimica, Università di Torino, via P. Giuria 7, 10125, Torino, Italy

*Email: josemln@um.es / tlf: +34 868 884 786

Abstract

The stilbenes are bioactive molecules with a big amount of health benefits and many possibilities in pharmaceutical industry. Several authors have reported a high number of properties for these compounds, including anticancer, antioxidant, anti-inflammatory, antidiabetic, neuroprotective or antimicrobial activities. However, their low aqueous solubility and their ease degradation could lead to low concentration of bioactive compound in the target tissue. For this reason, in the present study, the inclusion complexes of oxyresveratrol with α-, β-, and γ- cyclodextrins are characterized using DSC, TGA, SEM and molecular docking in order to increase the stability of the molecule. All these techniques showed that β-cyclodextrin (β-CD) forms the best complexes.

The stability of oxyresveratrol and oxyresveratrol/ β-CD complexes in different aqueous solutions was evaluated by measuring ºBrix, pH and UV-Vis spectra. The effect of encapsulation on the solubility and antioxidant activity of oxyresveratrol was also analysed. The results indicated that solutions were stable for at least five weeks, especially when stored in darkness, and that cyclodextrin supplementation leads to a higher concentration and antioxidant capacity of the solubilized bioactive compound than when it is not used. These results and the increase in antioxidant activity could be interesting for the pharmaceutical industry and for drugs enriched in oxyresveratrol.

Introduction

Stilbenes are a group of phenolic compounds found in grapes, wine, berries and some nuts. Stilbenes have been demonstrated to have biological activities as antioxidant, anticancer, anti-inflammatory and antimicrobial agents, among others. However, their low solubility in water and their ease degradation could be a problem to achieve the effective concentration of bioactive compound in the target tissue

Cyclodextrins (CDs) are torus-shaped oligosaccharides made up of α-(1,4) linked to glucose. They have an inner cavity whose nature is hydrophobic, unlike the outer surface that is mainly hydrophilic. Because of that, CDs can encapsulate other hydrophobic molecules to form inclusion complexes, which are more soluble than the non-encapsulated molecules. There are three types of natural CDs, α-CD, β-CD and γ-CD, each one with a different size of inner cavity.

In the present study [1], a well-known stilbene, oxyresveratrol (*trans*-3,5,4',6'-tetrahydroxystilbene), is encapsulated in β-CD, and the stability of complexes is evaluated by measuring ºBrix, pH and UV-Vis spectra. Antioxidant activity is also analysed.

Objectives

To characterise the inclusion complexes of oxyresveratrol with natural CDs.

To evaluate the solubility and the antioxidant activity of oxyresveratrol encapsulated in the best CD.

To analyse the stability of oxyresveratrol/CD complexes in different aqueous solutions and conditions (refrigeration and/or darkness) during five weeks.

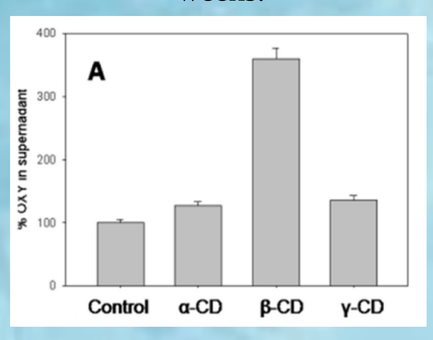

Fig.1: Effect of α-, β- and γ-CD on oxyresveratrol solubility.

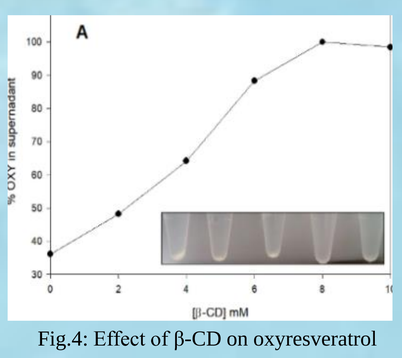

Fig.4: Effect of β-CD on oxyresveratrol solubilization after 24 mixing at 25 °C.

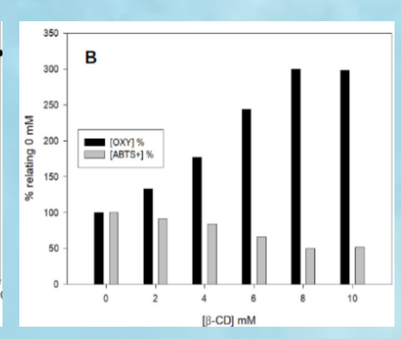

Fig. 5: Effect of higher concentration of oxyresvertrol on a free radical ABTS quantity.

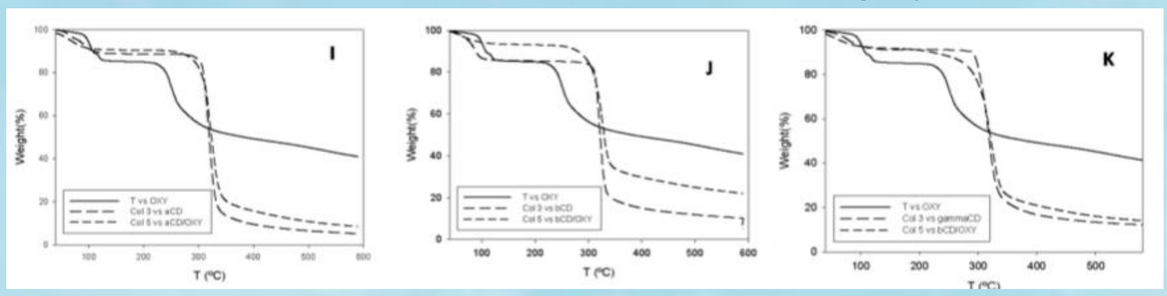

Fig.2: TGA of oxyresveratrol with (I) α-CD, (J) β-CD and (K) γ-CD.

Results and discussion

Selection of the Most Appropriate Cyclodextrin

After incubating oxyresveratrol with natural CDs, it was observed that β-CD was able to encapsulate 9,5 and 7,16 times more oxyresveratrol than α-CD or γ-CD, respectively (Fig. 1). Moreover, TGA profiles (Fig. 2) showed differences on the major degradation peaks of oxyresveratrol with α-CD (at 319 ºC 79.8% weight loss), β-CD (at 326.8 ºC 71% weight loss) and γ-CD (at 322 ºC 77.7% weight loss). DSC can also be used to demonstrate the guest inclusion, the principal degradation peaks disappears when the complex is formed [1].

SEM analysis showed that oxyresveratrol must be included in the CD cavities due to the morphological changes, from irregular structure to regular sphere of the powder. In addition, molecular docking (Fig. 3) also claimed that β-CD was the best CD to form complexes of oxyresveratrol with the formation of hydrogen bonds and the following scores: α-CD = -5.2, β-CD = -9.1, and γ-CD = -5.2.

Effect of β-CD on the Solubility and Antioxidant Activity

Solubility test of oxyresveratrol, in which the concentration of β-CD were increasing from 0 to 10 mM at 25 ºC indicated that the complexation with this agent can completely dissolve 1 mg/mL of oxyresveratrol at least (Fig. 4). At the same time, it was observed that antioxidant activity (measured by $ABTS^{+\cdot}$ method) increased when β-CD solubilised the bioactive compound in the final solution (Fig. 5).

Stability in aqueous solutions

The complexes were solubilized in aqueous solution at pH 3,73 and 6,51. There were no variation of pH during the time course of the experiment. Another parameter analysed was ºBrix, and it was found that the addition of CDs increased the initial value of ºBrix, although this parameter remained stable throughout the experiment. Finally, the measurement of the UV-Vis spectra showed that the relationship between *trans*- and *cis*- oxyresveratrol (absorbance at 301 and 290, respectively) remained stable during five weeks but only with pH 3,73. The determination of the remaining amount of oxyresveratrol pointed to a lower degradation of encapsulated oxyresveratrol than free oxyresveratrol at pH 3,73, while it was similar or higher at Ph 6,51 according to the initial dose of oxyresveratrol. That could be related to the deprotonation of the last citric acid pKa (6,4). Overall, the non-refrigerated darkness condition was the most stable storage.

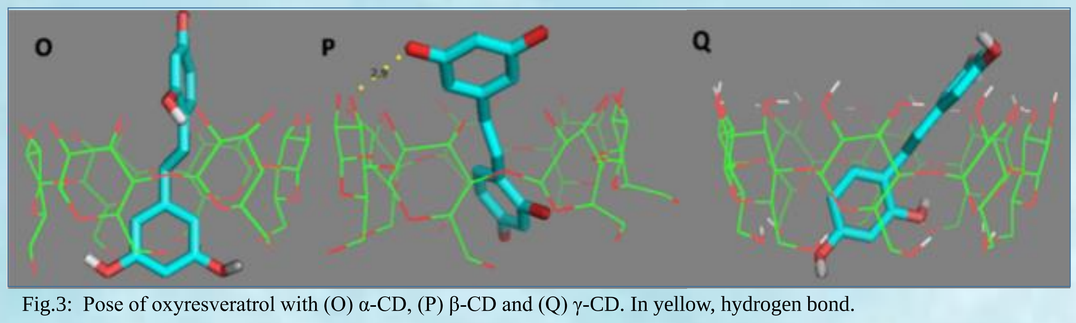

Fig.3: Pose of oxyresveratrol with (O) α-CD, (P) β-CD and (Q) γ-CD. In yellow, hydrogen bond.

Conclusions

The characterization, using DSC, TGA, SEM and molecular docking, of the oxyresveratrol inclusion complexes with natural cyclodextrins, indicated that β-CD was the best cyclodextrin to complex this molecule. Encapsulation was shown to improve the solubility of oxyresveratrol by reaching a final concentration of 4 mM, and therefore, increasing the antioxidant activity of the soluble sample. The time course experiment highlighted that the complexes were stable in aqueous solutions after five weeks (especially in darkness), but with the exception of the oversaturated non-darkness refrigerated stored aqueous solution at pH 6,1. Those results, especially the increase of solubility and antioxidant activity, could be interesting for the pharmaceutical industry in order to reach the active concentration of bioactive compound in the target tissue.

References

[1] Matencio A., Navarro-Orcajada S., Conesa I., Muñoz-Sánchez I., Laveda-Cano L., Cano-Yelo D., García-Carmona F. & López-Nicolás J. M. Food Hydrocolloids, **2020**, 98, 105250

Acknowledgments

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