Study of the antioxidant activity, solubility, stability and cyclodextrin complexation of gnetol

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Gnetol, a natural stilbene particularly found in genus *Gnetum*, has anti-inflammatory, anti-thrombotic, cardio-protective, and anti-cancer activity. However, the applications of this stilbene as a bioactive ingredient in foods, cosmetic or pharmaceutical industries, may be limited due to its low water solubility and its easy degradation. The encapsulation in cyclodextrins could solve these problems.

This study compares the antioxidant activity of gnetol with other stilbenes analogues, encapsulates it in different cyclodextrins and evaluates the effect that this process has in water solubility. In addition, the effect of pH and temperature on the encapsulation constant is analysed.

Our results reveal that the antioxidant activity of gnetol was higher than that of resveratrol, and similar or higher to that of oxyresveratrol. Moreover, the solubility in water of gnetol was 0.31 mg/mL, but the highest concentration of HPβCD used could increase almost threefold the basal solubility, as well as its stability after storage for a week. Gnetol-HPβCD encapsulation constants were influenced by changes in pH and temperature, decreasing when either of these parameters increased. All these results could increase the interest in gnetol, as well as aid in the development of more stable inclusion complexes that improve its aqueous solubility and stability for industrial use.

Introduction

Stilbenes are a group of phenolic compounds found in grapes, wine, berries and some nuts. They have biological activities as anti-inflammatory, anti-thrombotic, cardio-protective and anti-cancer, among others. However, their low solubility in water and their easy degradation could be a problem to use them in different industries [1].

Cyclodextrins (CDs) are torus-shaped oligosaccharides made up of α-(1,4) linked 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[2].

This study [3] compares the antioxidant activity of gnetol with other stilbenes, encapsulates it in different cyclodextrins and evaluates the effect that this process has in water solubility. In addition, the effect of pH and temperature on the encapsulation constant is analysed and evaluates the effect that this process has in gnetol water solubility and stability.

Antioxidant activity of gnetol and other stilbenes

The comparison of the antioxidant capacity measured by the two techniques (the capacity to scavenge $ABTS^{*+}$ free radicals and the capacity to reduce the ferric complex) revealed a good agreement in the stilbene order (piceatannol >gnetol≥ oxyresveratol> resveratrol) [4] . The antioxidant activity is highly dependent on the number and position of the hydroxyl substituent. (Figure 1)

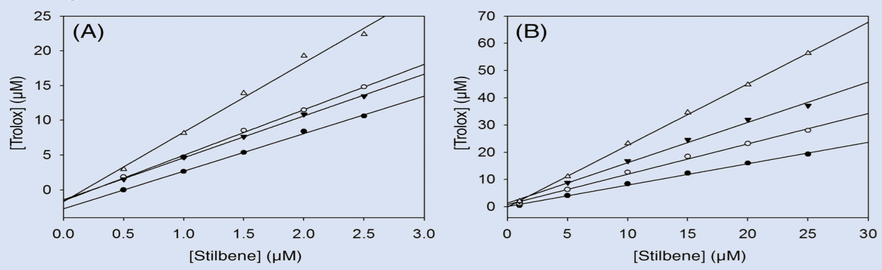

Figure 1. Antioxidant activity of (●) resveratrol, (o) oxyresveratrol, (▼) gnetol, and (△) piceatannol measured by the (A) $ABTS^{*+}$ radical cation and (B) ferric reducing antioxidant power methods.

Stoichiometry and encapsulation constant for gnetol in CDs

Naturals and modified cyclodextrins were tested for the encapsulation of gnetol (αCD; βCD; γCD; MβCD and HPβCD), for all of them the stoichiometry of the inclusion complexes was 1:1, which means that one molecule of CD interacts only with one molecule of gnetol. Encapsulation constants were determined for each complex, and it was revealed that HPβCD formed the best inclusion complexes with gnetol (figure 2), with a K_F of 4542.90 ± 227.15 mol L^{-1}.

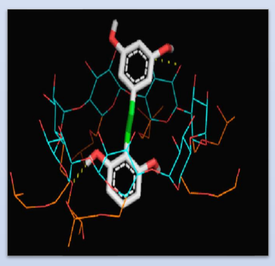

Figure 2. Gnetol-HPβCD complexes.

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Effect of pH and temperature on the encapsulation constant of gnetol

The pH of the medium can alter the protonation state of gnetol and, hence, influence encapsulation constants with CDs. As it can be observed in figure 3A, all constant decreased when pH was increased, indicating that the inclusion complexes were more stable at lower pH values.

In addition, temperature is a relevant factor in the stabilization of inclusion complexes, since hydrogen bonds are usually weakened by heating. Overall, encapsulation constants showed a decreasing trend with rising temperature (figure 3B), which is in good agreement with previous results reported for other stilbenes. HPβCD´s encapsulation constant decreased 23% when the temperature passed from 15ºC to 25ºC, and 61% when it reached 35ºC [3] .

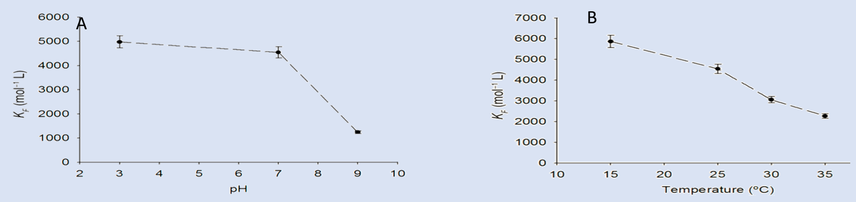

Figure 3. Influence of pH (A) and temperature (B) on the encapsulation constants of gnetol with HPβCD.

Aqueous solubility and stability of gnetol after the encapsulation process

In the absence of CDs, the solubility in water of gnetol was 0.31 mg mL^{-1}.The addition of CDs successfully increased its solubility; HPβCD could solubilize 0.82 mg of gnetol in 1 mL of water; that is, almost threefold the basal solubility (Figure 4). Furthermore, lower concentrations of CDs also make significant differences, just 1 mmol L^{-1} of HPβCD can enhance the aqueous solubility of this stilbene by 62%.

The shelf life of gnetol after 7 days' storage was improved when CDs were present in the medium (figure 5). Whereas free gnetol revealed a loss of 25% and 44% at 4°C and 25°C storage respectively, inclusion complexes with more than 1 mmol L^{-1} HPβCD retained the initial amount of stilbene. Fully complexed gnetol was very stable at both temperatures, which may be desirable for storage, delivery, and marketing of functional foods fortified with this stilbene.

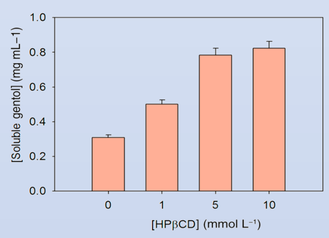

Figure 4: Water solubility of gnetol in the absence and presence of increasing concentrations of HPβCD.

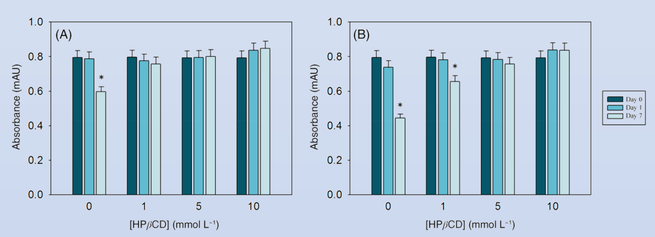

Figure 5: Stability of gnetol in free form and complexed in cyclodextrins when stored (A) refrigerated (4 °C) and (B) at room temperature (25 °C).

Conclusions

The antioxidant activity of gnetol was higher than that of resveratrol, and similar or higher to that of oxyresveratrol. In the encapsulation process, gnetol showed a greater affinity for HPβCD and the encapsulation constants were influenced by changes in pH and temperature, decreasing when either of these parameters increased. This encapsulation process successfully enhanced the aqueous solubility of gnetol, achieving almost a threefold increase in basal solubility with HPβCD supplementation. In addition, the shelf life of gnetol improved after 1 week of storage, regardless of temperature when a medium–high concentration of HPβCD was added. All results add interest to this stilbene and its applications for industrial use.