Research Article

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Molecular encapsulation and bioactivity of gnetol, a resveratrol analogue, for use in foods

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Abstract

BACKGROUND: Gnetol is a stilbene whose characterization and bioactivity have been poorly studied. It shares some bioactivities with its analogue resveratrol, such as anti-inflammatory, anti-thrombotic, cardioprotective and anti-cancer activities. However, the low solubility of stilbenes may limit their potential applications in functional foods. Encapsulation in cyclodextrins could be a solution.

RESULTS: The antioxidant activity of gnetol was evaluated by 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation and ferric reducing antioxidant power methods (Trolox equivalents 13.48 μmol L^{-1} and 37.08 μmol L^{-1} respectively at the highest concentration) and it was higher than that of resveratrol, and depending on the method, similar or higher to that of oxyresveratrol. Spectrophotometric and spectrofluorimetric characterization of gnetol is published for the first time. Moreover, its water solubility was determined and improved almost threefold after its molecular encapsulation in cyclodextrins, as well as its stability after storage for a week. A physicochemical and computational study revealed that cyclodextrins complex gnetol in a 1:1 stoichiometry, with better affinity for like 2-hydroxypropyl-β-cyclodextrin ($K_F = 4542.90 \pm 227.15$ mol^{-1} L). Temperature and pH affected the encapsulation constants.

CONCLUSION: These results could increase interest of gnetol as an alternative to the most studied stilbene, resveratrol, as well as aid in the development of more stable inclusion complexes that improve its aqueous solubility and stability so that it can be incorporated into functional foods.

Supporting information may be found in the online version of this article.

Keywords: gnetol; stilbenes; antioxidant; cyclodextrin; encapsulation; fluorescence

INTRODUCTION

Stilbenes are a group of phenolic compounds isolated from plants, in which they act as phytoalexins to protect from biotic and abiotic stress. They share a common backbone structure of 1,2-diphenylethylene but differ in the type and position of substituents on the aromatic rings[1] (Fig. 1).

The study of these bioactive compounds has increased in recent years owing to their potential as antioxidant, antimicrobial, anti-inflammatory, anti-cancer, anti-obesity, and cardioprotection and neuroprotection agents.[2] Resveratrol (*trans*-3,4′,5-trihydroxystilbene) is the most known stilbene, although there are less-studied analogues with enhanced biological activity or better physicochemical properties. For example, some hydroxylated analogues, like piceatannol (*trans*-3,3′,4′,5-tetrahydroxystilbene) and oxyresveratrol (*trans*-2′,3,4′,5-tetrahydroxystilbene), have demonstrated better *in vitro* antioxidant activity than resveratrol.[3,4] Despite this, the disparity in the principle of the methods used to measure antioxidant activity makes the comparison between studies difficult.

Stilbenes have been suggested to have hormetic activity; therefore, the dosage is key to their beneficial effect. Resveratrol has been reported to be well tolerated in humans at doses of <1 g or in treatments of short duration (less than a month). At higher doses, some studies reported minor gastrointestinal disorders or nephrotoxicity, although in some cases this also occurred in the placebo group.[5,6] Moreover, there are not enough human studies on stilbenes other than resveratrol to determine their toxicity (e.g. piceatannol, oxyresveratrol, or gnetol).[2]

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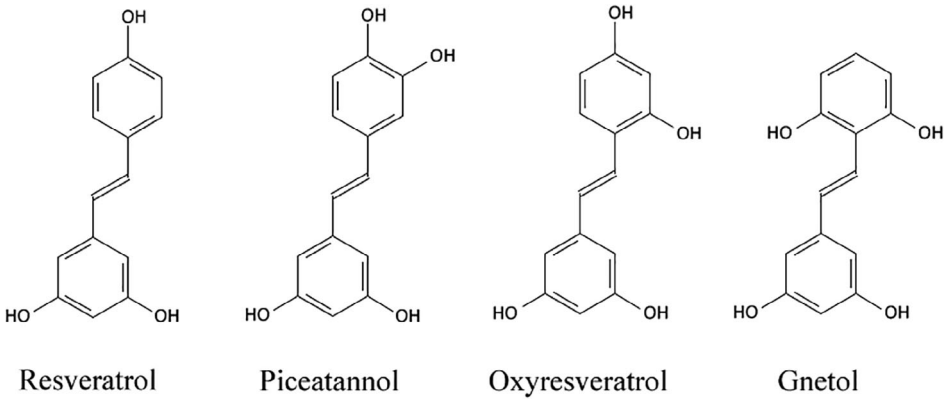

Figure 1. Chemical structure of common *trans*-stilbenes.

Among hydroxylated stilbenes, information about the activity and characterization of gnetol (*trans*-2′,3,5,6′-tetrahydroxystilbene) is scarce and is mainly related to *Gnetum* plant extracts instead of the isolated compound.[7-9] Even so, it has been described that gnetol inhibits cyclooxygenase-1, tyrosinase, acetylcholinesterase, histone deacetylase, and cytochrome P450s 2C9 and 3A4, which could indicate that it has anti-inflammatory, anti-thrombotic, cardioprotective and anti-cancer activity.[10] Pharmacokinetic studies have shown that, despite being less bioavailable than resveratrol, gnetol has a longer half-life after oral administration in rats.[2]

However, the applications of gnetol as a bioactive ingredient in functional foods may be limited due to the low water solubility of this family of compounds. Molecular encapsulation in cyclodextrins (CDs) has proved to be efficient in solving these kinds of issues with a wide range of bioactive compounds,[11-13] including stilbenes such as resveratrol,[14] piceatannol,[15] oxyresveratrol,[16] pterostilbene,[17] and pinosylvin.[18] However, the complexation of gnetol in these agents has not been evaluated.

CDs are cyclic oligosaccharides consisting of α-(1, 4)-linked glucose units capable of forming inclusion complexes with hydrophobic compounds. Since their outer surface is mainly hydrophilic, they can increase the solubility of their guest molecules, as well as protect them from isomerization, oxidation, volatilization, and adverse reactions with other components in the sample. A recent review[13] also affirms that they can be used to increase the production of bioactive compounds, to remove undesired components from foods, to carry active substances, to design nanosensors, and even to package food.

According to the method of production, CDs can be classified as natural or modified. The most common are natural CDs with six (αCD), seven (βCD), and eight (γCD) glucose units, which are also approved as food additives (E-457, E-459, and E-458). In addition, modified CDs are also relevant for their pharmaceutical applications, like 2-hydroxypropyl-β-cyclodextrin (HPβCD) in the treatment of rare diseases, such as Niemann Pick disease type C.[19]

Bearing the foregoing in mind, this research aims to increase the knowledge of gnetol by comparing its antioxidant activity with other stilbene analogues, encapsulating it in natural (αCD, βCD and γCD) and modified (HPβCD and methyl-β-cyclodextrin (MβCD)) CDs, and evaluating the effect that this process has on water solubility. The influence of pH and temperature on the encapsulation constants is analysed, as well as the molecular docking between gnetol and CDs. Additionally, essential physicochemical information about this stilbene is published for the first time, such as absorption and fluorescence emission spectrums and molar attenuation coefficient, which could help future research.

MATERIAL AND METHODS

Materials

Natural CDs (αCD, βCD and γCD), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), iron(III) chloride, 4,5,6-tripyridin-2-yltriazine (TPTZ), and Trolox were purchased from SigmaAldrich (Madrid, Spain). MβCD (DS = 5.4) and HPβCD (DS = 5) were from Carbosynth (Compton, UK). Stilbenes were purchased from TCI (Zwijndrecht, Belgium).

Antioxidant activity measurement

The antioxidant activities of gnetol, oxyresveratrol, piceatannol, and resveratrol were measured by two different approaches of single electron transfer reactions: radical scavenging capacity on a stable free radical (the ABTS method) and ferric reduction ability (the ferric reducing antioxidant power (FRAP) method).

The ABTS assay is based on the reduction in absorbance of $ABTS^{\bullet+}$ radical cation in the presence of antioxidant molecules. This radical cation was prepared according to the enzymatic method described in Rodríguez-Bonilla *et al.*[4] $ABTS^{\bullet+}$ radical cation, stilbene samples, and 0.2 mol L^{-1} sodium phosphate buffer (pH 7) were mixed at a ratio of 10:1:4 to achieve a final volume of 300 µL. Absorbance at 414 nm was measured after a 6 min incubation in a Synergy HT plate reader (BioTek Instruments, Winooski, VT, USA).

The FRAP assay is based on the reduction of the colourless ferric TPTZ complex to the blue ferrous TPTZ complex after the interaction with antioxidant compounds. The ferric TPTZ complex was made according to Rodríguez-Bonilla *et al.*[4] and mixed with stilbene samples at a ratio of 8.3:1. After a 6 min incubation, absorbance at 593 nm was measured in the same plate reader as before.

In both methods, the antioxidant capacity of Trolox, a vitamin E analogue, was measured to determine the Trolox equivalent activity of stilbenes. The concentrations displayed refer to final concentrations.

Fluorescence studies

The fluorescence emission spectrum and maximum excitation and emission wavelengths were obtained using a Shimadzu (Kyoto, Japan) RF-6000 spectrofluorometer equipped with

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thermostatically controlled cells, with both excitation and emission bandwidths set at 5 nm. Relative fluorescence intensity was measured in a Kontron (Zurich, Switzerland) SFM-25 spectrofluorometer equipped with thermostatically controlled cells and with a xenon lamp source and 2 mm quartz cells, with both excitation and emission bandwidths set at 2 nm.

For the determination of the encapsulation constants, the gnetol concentration was fixed at 25 μmol L^{-1} and CDs were varied between 0 and 10 mmol L^{-1}. The influence of pH was evaluated using 0.1 mol L^{-1} sodium acetate buffer (pH 3), 0.1 mol L^{-1} sodium phosphate buffer (pH 7), and 0.1 mol L^{-1} sodium borate buffer (pH 9). Before reading, the inclusion complexes were incubated 30 min at 15, 25, or 35 °C.

Determination of the encapsulation constants in CDs

Gnetol complexes with CDs were analysed by the Benesi–Hildebrand method,[20] in which two mathematical models are proposed. In the first model with 1:1 stoichiometry, one CD molecule encapsulates one guest molecule (Eqn (1)); in the second model (Eqn (2)), with 1:2 stoichiometry, two CDs can complex one guest molecule.

$$\text{gnetol} + \text{CD} \rightleftarrows \text{gnetol}-\text{CD} \quad (1)$$

$$\text{gnetol} + 2\text{CD} \rightleftarrows \text{CD}-\text{gnetol}-\text{CD} \quad (2)$$

Assuming that, the encapsulation constant K_F can be obtained as follows:

$$K_F = \frac{[\text{Inclusion complex}]}{[\text{gnetol}][\text{CD}]^x} \quad (3)$$

where [Inclusion complex], [gnetol], and [CD] are equilibrium concentrations, and x is equal to 1 in a 1:1 model and 2 in a 1:2 model.

The encapsulation constant is a relevant parameter to characterize the interaction between ligands and receptors. The higher the value, the stronger the interaction between molecules, and hence the more stable the inclusion complexes.

This method allows calculation of the encapsulation constant by measuring the fluorescence intensity by means of the following formula:

$$\frac{1}{F-F_0} = \frac{1}{(F_\infty - F_0)K_F[\text{CD}]^x} + \frac{1}{F_\infty - F_0} \quad (4)$$

where F_0 is the fluorescence intensity of gnetol in the absence of CDs, F_∞ is the fluorescence intensity when all gnetol is complexed with CDs, and F is the observed fluorescence intensity at each CD concentration. The linear plot of $1/(F-F_0)$ *versus* $1/[\text{CD}]^x$ gives $1/[(F_\infty - F_0)K_F]$ and $1/(F_\infty - F_0)$ as the slope and intercept respectively.

Determination of the thermodynamic parameters

The thermodynamic parameters of enthalpy $\Delta H°$, entropy $\Delta S°$, and Gibbs free energy $\Delta G°$ of the most stable inclusion complex with gnetol were calculated using the following equation:

$$\ln K_F = \frac{-\Delta H°}{RT} + \frac{\Delta S°}{R} \quad (5)$$

where T is the temperature, R is the gas constant, and $\Delta H°$ and $\Delta S°$ are respectively the standard enthalpy and entropy changes of complex formation. The linear plot of $\ln K_F$ *versus* $1/T$ gives $-\Delta H°/R$ and $\Delta S°/R$ as the slope and intercept respectively, and Gibbs free energy change can be obtained from

$$\Delta G° = \Delta H° - T\Delta S° \quad (6)$$

Molecular docking

The molecular structure of gnetol was obtained from the PubChem database (National Center for Biotechnology Information, Bethesda, MD, USA). The structures of αCD and βCD were extracted from a crystal from the Protein Data Bank (PDB ID: 2XFY and 1Z0N), and γCD was obtained from the London South Bank University website. The modified CDs HPβCD and MβCD were built by adding hydroxypropyl or methyl groups to the βCD. The topology of modified CDs was obtained using PRODRG with default parameters. Default topology was used for the remaining molecules. Input files for docking were generated using AutoDock tools (version 1.5.6) with default parameters and charges. Molecular docking was carried out with AutoDock Vina[21] using default parameters with a seed of 5000 and considering the flexible atoms of CDs. Graphical representations of the docking results were prepared using PyMOL (version 1.3) molecular graphics system (Schrödinger LLC, New York, NY, USA) with default parameters to display hydrogen bonds.

Determination of the molar attenuation coefficient and aqueous solubility of gnetol

The molar attenuation coefficient ε of gnetol was established for the first time by measuring its absorbance between 200 and 600 nm at increasing concentrations in ethanolic solution in a Jasco (Madrid, Spain) V-630 spectrophotometer with Thorlabs CV10Q1400 cuvettes.

Aqueous solubility was determined with test tubes containing a saturated concentration of gnetol (1 mg mL^{-1}) in water. They were incubated at room temperature and in darkness, in the absence and presence of increasing concentrations of HPβCD. Then, the samples were centrifuged at 15 600 xg for a 1 min, and the supernatants containing soluble gnetol diluted 1:100 in ethanol to measure the absorbance at 310 nm and quantified with the calculated ε.

Stability test of free and encapsulated gnetol

Samples containing 25 μmol L^{-1} gnetol in 0,1 mol L^{-1} sodium phosphate buffer (pH 7) in the absence and presence of different concentrations of HPβCD (1, 5, and 10 mmol L^{-1}) were stored at room temperature (25 °C) and under refrigeration (4 °C) in the dark for a week. The absorbance at 310 nm was read after 30 min incubation (day 0), 24 h (day 1) and 1 week (day 7) in a Jasco V-630 spectrophotometer with Thorlabs CV10Q1400 cuvettes.

Data analysis

All experiments were carried out in triplicate. Regressions were made using Sigma-Plot (version 10.0.0.54). A *t*-test was carried out using Rstudio (version 0.99.878) with a significance of $P < 0.05$. Other mathematical operations were carried out using wxMaxima (version 12.04.0).

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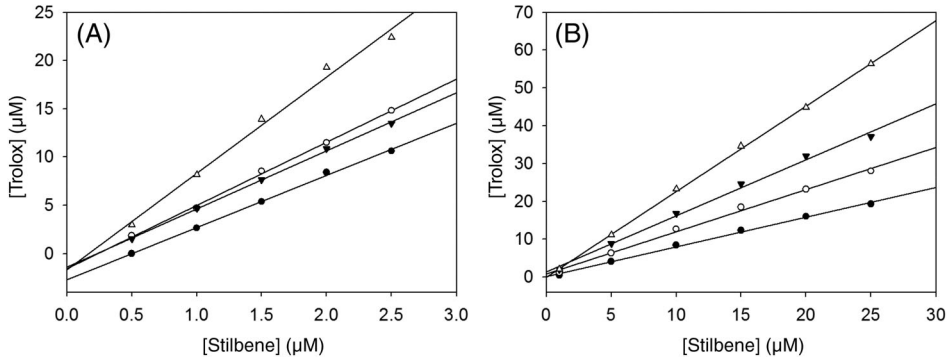

Figure 2. Antioxidant activity of (●) resveratrol, (○) oxyresveratrol, (▼) gnetol, and (△) piceatannol measured by the (A) 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation and (B) ferric reducing antioxidant power methods.

RESULTS AND DISCUSSION

Antioxidant activity of gnetol in comparison with other stilbene analogues

The capacity to scavenge $ABTS^{\bullet+}$ free radicals differed among the stilbenes reviewed (Fig. 2(A)). Resveratrol, with one less hydroxyl group, was the worst antioxidant tested; at its highest concentration it had an activity equivalent to 10.61 μmol L^{-1} of Trolox. Gnetol and oxyresveratrol with four hydroxyl groups showed better results than resveratrol, reaching 13.48 μmol L^{-1} and 14.82 μmol L^{-1} of Trolox equivalents respectively at 2.5 μmol L^{-1}. However, there were no significant differences between them. By contrast, piceatannol, that just changes the position in one functional group compared to oxyresveratrol, was significantly more effective in scavenging $ABTS^{\bullet+}$ free radicals than other analogues (22.38 μmol L^{-1} Trolox equivalents at 2.5 μmol L^{-1}; i.e. double resveratrol activity).

The capacity of stilbenes to reduce the ferric complex was highly dependent on the number and position of the hydroxyl substituents (Fig. 2(B)). Resveratrol remains as the worst antioxidant, with even less activity than Trolox (19.32 μmol L^{-1} Trolox equivalents at 25 μmol L^{-1}). The addition of an extra hydroxyl group at C2′ of the 4′-hydroxyl ring increased this value by 45% (oxyresveratrol), and its addition at C3′ tripled it (piceatannol). Gnetol, with hydroxyl groups at C2′ and C6′ instead of C4′, exhibited an intermediate ferric reduction potential between piceatannol and oxyresveratrol, with a Trolox equivalent activity of 37.08 μmol L^{-1} at the highest concentration analysed. In this method, all differences among the highest concentrations of stilbenes were significant.

The comparison of the antioxidant capacity measured by the two techniques analysed revealed a good agreement in stilbene order (piceatannol > gnetol ≥ oxyresveratrol > resveratrol), with the ABTS method getting higher Trolox equivalents than the FRAP method at the same concentrations of bioactive compound. The greater activity of piceatannol could be explained by its ability to form a stable semiquinone radical with a higher capacity to capture unpaired electrons by sharing its hydrogen atom at C3′ with the adjacent C4′.[3] It seems that this mechanism of action is affected when the hydroxyl groups move from an *ortho* to a *meta* position on the ring and when both functional groups move closer to the methylene bridge.

Previous studies[22] determined the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity of these stilbenes expressed as half-maximal inhibitory concentration: picetannol 5.88 μmol L^{-1}, gnetol 7.25 μmol L^{-1}, oxyresveratrol 8.43 μmol L^{-1}, and resveratrol 14.45 μmol L^{-1}. Our outcomes are similar regarding antioxidant order, although half-maximal inhibitory concentration values are lower if data are extrapolated – piceatannol 2.25 μmol L^{-1}, oxyresveratrol 3.40 μmol L^{-1}, gnetol 3.65 μmol L^{-1}, and resveratrol 4.48 μmol L^{-1} – probably due to the influence of the different reaction medium (water in the ABTS method and methanol in the 2,2-diphenyl-1-picrylhydrazyl method).

Absorbance and fluorescence characterization of gnetol

Gnetol shows a common stilbene spectrum similar to resveratrol, although its two peaks of maximum absorbance are at 219 and 310 nm; the second is the main maximum wavelength, with an upper shoulder at 320 nm and a lower shoulder at 337 nm (Fig. S1A). Concentrations between 1 and 50 μmol L^{-1} gave a perfect correlation (R^2 = 1) with absorbance at 310 nm (Fig. S1A inset), obtaining a molar attenuation coefficient of 40 003 mol^{-1} L cm^{-1}.

As was expected, the polyphenolic structure of gnetol makes it fluorescent, with a main maximum emission wavelength at 503 nm and a secondary peak at 390 nm, after excitation at 280 nm (Fig. S1B). Concentrations between 5 and 100 μmol L^{-1} gave a perfect correlation with fluorescence intensity read at both wavelengths (Fig. S1B inset). For the encapsulation in CDs, excitation at 280 nm and emission at 390 nm were selected.

Determination of the stoichiometry and encapsulation constants for gnetol in CDs

Relative fluorescence of gnetol was plotted at increasing concentration of natural and modified CDs. Complexation curves with βCD and their derivatives presented a rapid increased of fluorescence intensity that reached a plateau at 4 mmol L^{-1} of βCD and 2 mmol L^{-1} of HPβCD or MβCD (Fig. 3(A)). However, αCD and γCD were not as efficient at raising the fluorescence signal of gnetol, which was reflected in their lower encapsulation constants (Table 1).

All CDs gave a lower linear correlation when a 1:2 model was presumed with Eqn (4), indicating that the stoichiometry of the inclusion complexes was 1:1 (Table 1 and Fig. 3(b)), which means that one molecule of CD interacts only with one molecule of gnetol.

Encapsulation constants were determined for each complex (Table 1), and it was revealed that modified CDs formed the best inclusion complexes with gnetol, with a K_F of 4542.90 ± 227.15 mol^{-1} L and 2756.92 ± 137.85 mol^{-1} L for HPβCD and MβCD respectively. Among natural CDs, βCD gave the most stable complexes, with an encapsulation constant of 597.93 ± 29.90 mol^{-1} L, whereas both αCD and γCD were the worst CDs to complex gnetol, with K_F < 200 mol^{-1} L.

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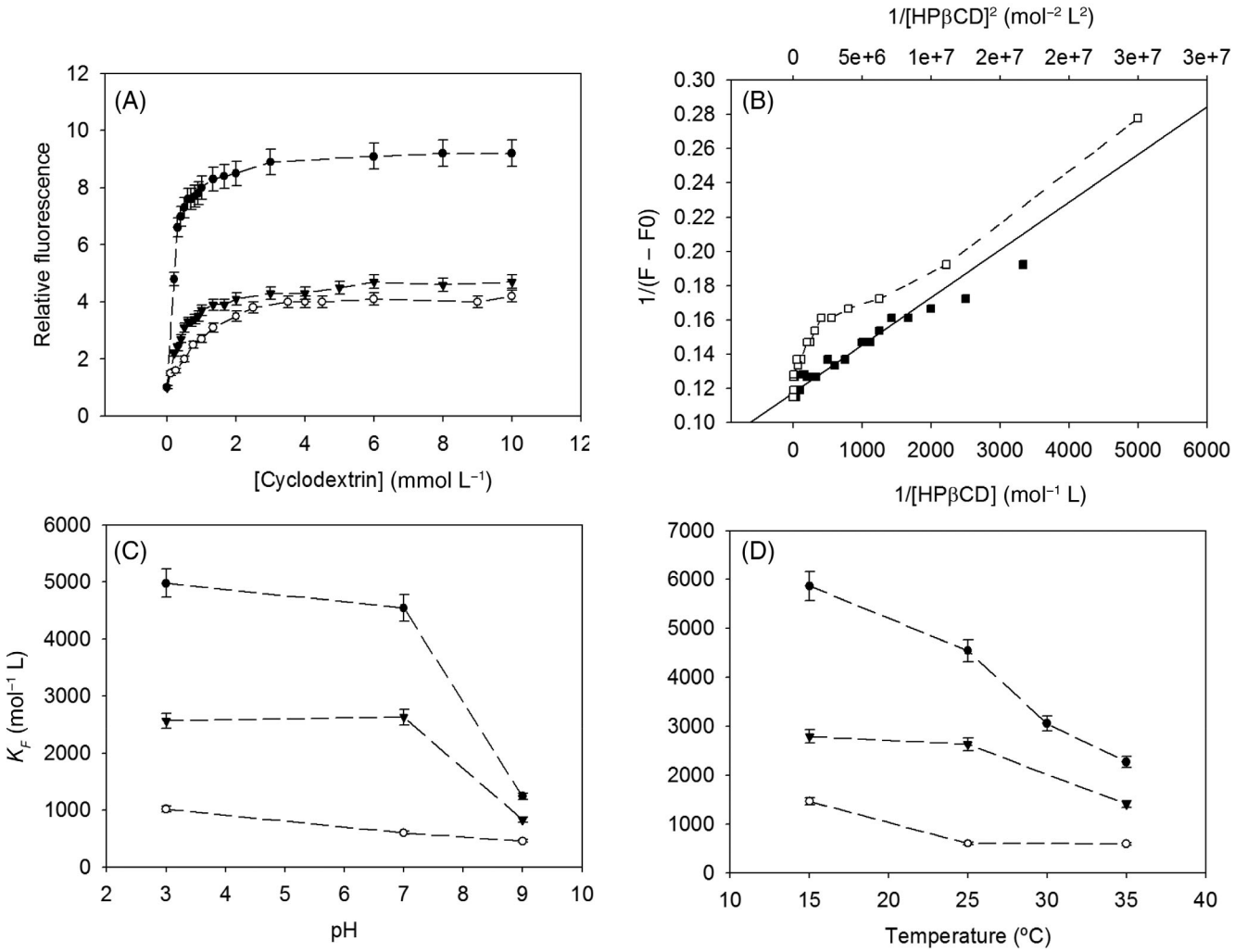

Figure 3. (A) Complexation curves of gnetol with *β*-cyclodextrin (*β*CD, ◦), 2-hydroxypropyl-*β*-cyclodextrin (HP*β*CD, ●) and methyl-*β*-cyclodextrin (M*β*CD, ▼). (B) Benesi–Hildebrand fitting of gnetol–HP*β*CD complexes to 1:1 model (■) and 1:2 model (□) according to Eqn (4) (25 °C pH 7). Influence of (C) pH and (D) temperature on the encapsulation constants of gnetol with natural and modified *β*CDs.

Table 1. Encapsulation constants K_F according to Eqn (4) and docking scores of gnetol–cyclodextrin (CD) complexes (25 °C pH 7)

	R^2			
CD	1:1	1:2	K_F (mol^{-1} L)	Score
*α*CD	0.988	0.874	161.35 ± 8.07	−7.30
*β*CD	0.996	0.959	597.93 ± 29.90	−9.60
*γ*CD	0.983	0.956	60.29 ± 3.01	−6.80
HP*β*CD	0.951	0.938	4542.90 ± 227.15	−11.10
M*β*CD	0.976	0.790	2756.92 ± 137.85	−9.70

HP*β*CD: 2-hydroxypropyl *β*-cyclodextrin; M*β*CD: methyl-*β*-cyclodextrin.

Effect of pH on the encapsulation constants of gnetol

The pH of the medium can alter the protonation state of guest molecules and, hence, influence encapsulation constants with CDs. This fact should be considered if gnetol is aimed to be incorporated as a bioactive compound in functional foods with different pH, such as juices (acidic pH) and milk (neutral pH).

As can be observed in Fig. 3(C), all constant decreased when pH was increased, indicating that the inclusion complexes were more

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stable at lower pH values. Whereas HPβCD and MβCD constants showed a slightly descent from acidic to neutral pH, βCD constant was constantly decreasing until it reached alkaline pH. The general change of encapsulation constants from acid to basic pH was greater for HPβCD (75%), followed by MβCD (68%) and βCD (55%).

This behaviour was similar to that of other stilbenes, such as resveratrol[14] and piceatannol,[15] in which the notorious decrease in the constants from pH 9 onwards was associated with the dissociation constant pK_a of stilbene, from which its hydroxyl groups are deprotonated. This could point to the fact that the dissociation constants of gnetol are close to those of other analogues, and that CDs have a stronger attraction to the protonated form of gnetol than the deprotonated form.

Temperature influence on the encapsulation constants of gnetol and thermodynamic parameters of the most stable inclusion complex

Temperature is a relevant factor in the stabilization of inclusion complexes, since hydrogen bonds are usually weakened by heating. Since functional foods can suffer from variations in temperature during storage and distribution, the determination of the encapsulation constants at different temperatures is needed.

Overall, encapsulation constants showed a decreasing trend with rising temperature (Fig. 3(D)), which is in good agreement with previous results reported for other stilbenes, such as resveratrol,[14] oxyresveratrol,[23] and piceatannol.[15]

There were small differences among CDs. HPβCD's encapsulation constant decreased 23% when the temperature passed from 15 °C to 25 °C, and 61% when it reached 35 °C. Meanwhile, MβCD's encapsulation constant almost did not vary at lower temperatures and decreased 47% from 25 °C to 35 °C. By contrast, βCD's encapsulation constant decreased 59% from 15 °C to 25 °C and remained stable till 35 °C (Fig. 3(D)).

Thermodynamic parameters were established for the best inclusion complex (gnetol–HPβCD) with the encapsulation constants at 15, 25, 30, and 35 °C (K_F (15 °C) = 5866.45 ± 293.32 mol^{-1} L^{-1}, K_F (25 °C) = 4542.90 ± 277.15 mol^{-1} L^{-1}, K_F (30 °C) = 3053.73 ± 152.69 mol^{-1} L^{-1}, and K_F (35 °C) = 2270.03 ± 113.50 mol^{-1} L^{-1}). The negative value obtained for enthalpy changes (−34.93 ± 1.75 kJ mol^{-1}) revealed that the encapsulation process was exothermic, and was greater than the encapsulation of oxyresveratrol[23] (−32.61 ± 1 kJ mol^{-1}), piceatannol[15] (−24.6 ± 1.2 kJ mol^{-1}), and resveratrol[14] (−22.56 kJ mol^{-1}). These results are related to hydrophobic interactions due to the displacement of water molecules from the inner cavity of CD, increased van der Waals interactions between the molecules, the formation of hydrogen bonds, and other interactions.

The changes in entropy were also negative (−48.52 ± 2.43 J mol^{-1}), indicating that the complexation leads to a more ordered system, probably due to the decrease in the translational and rotational degrees of freedom of the complexed gnetol compared with the free gnetol. By contrast, previous studies showed that the complexation of oxyresveratrol gives lower entropy (−56.13 ± 2 J mol^{-1}[23]), whereas the complexation of resveratrol and piceatannol provided higher values (−12.30 L mol^{-1} and −3.7 ± 0.2 J mol^{-1} respectively[14,15]).

The process was spontaneous, because the Gibbs free energy change was negative (−33.72 ± 1.69 kJ mol^{-1} 25 °C). Compared with other stilbenes, the encapsulation of gnetol was more spontaneous than the encapsulation of piceatannol (−23.5 ± 1.2 kJ mol^{-1} 25 °C[15]), resveratrol (−18.89 kJ mol^{-1} 25 °C[14]), and oxyresveratrol (−15.88 ± 1 kJ mol^{-1} 25 °C[23]).

Molecular docking of gnetol–CD complexes

The scores obtained from the molecular docking of gnetol with each CD were properly correlated with the experimental results previously reported (Table 1). Scores are used to predict the binding force between two docked molecules involving non-covalent interactions. A stronger bond is reflected by a lower score.

As expected, HPβCD gave the lowest score (−11.10), followed by MβCD and βCD, which gave similar results (−9.70 and −9.60 respectively), and finally αCD (−7.30) and γCD (−6.80). It seems that the seven sugar-rings structure is more suitable than the other sizes of CD tested.

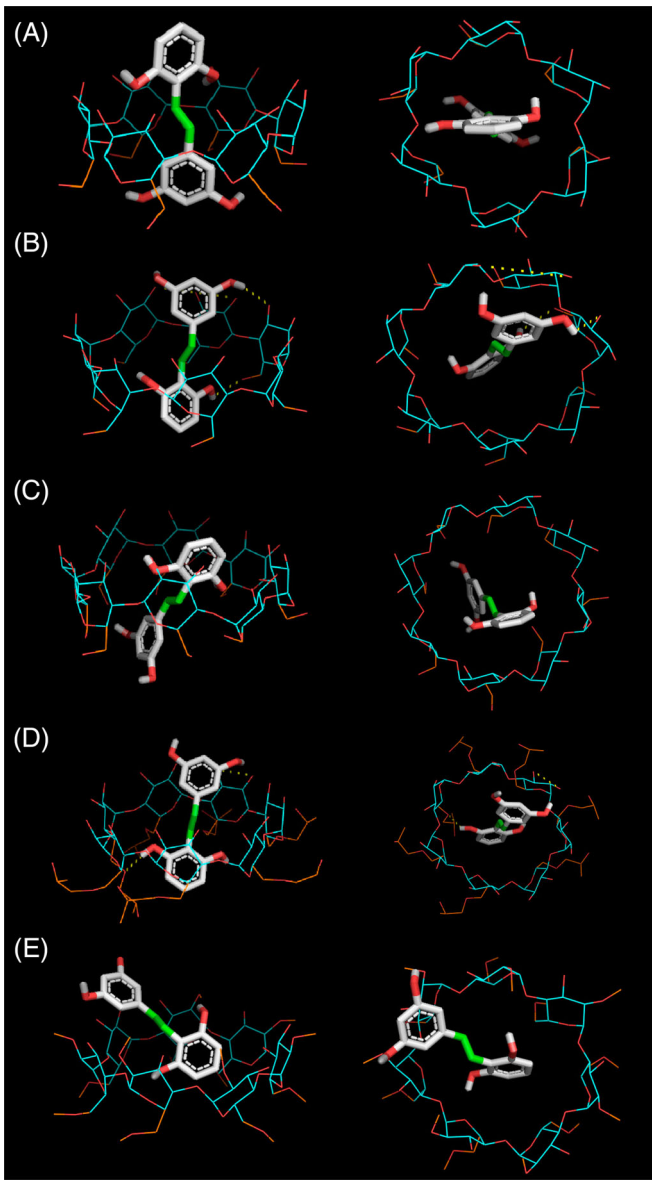

Figure 4. Molecular docking of gnetol with (A) α-cyclodextrin, (B) β-cyclodextrin, (C) γ-cyclodextrin, (D) 2-hydroxypropyl-β-cyclodextrin, and (E) methyl-β-cyclodextrin. Flexible atoms of cyclodextrins are coloured orange, and non-flexible atoms are blue. Hydrogen bonds are yellow dotted lines.

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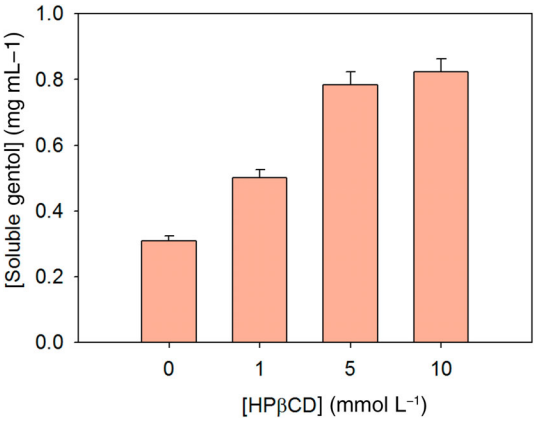

Figure 5. Water solubility of gnetol in the absence and presence of increasing concentrations of cyclodextrins. HPβCD: 2-hydroxypropyl-β-cyclodextrin.

The molecular modelling of the complexes revealed that βCD (Fig. 4(B)), γCD (Fig. 4(C)), HPβCD (Fig. 4(D)), and MβCD (Fig. 4(E)) mainly capture gnetol by the 2′,6′-hydroxyl ring and part of the methylene bridge (except MβCD), whereas αCD (Fig. 4(A)) mainly interacts with the 3,5-hydroxyl ring and the methylene bridge. It stands out that the orientation of the guest molecule in the cavity of natural and modified βCDs differs from αCD and γCD. Gnetol enters into βCD, HPβCD, and MβCD with the 2′,6′-hydroxyl ring south facing from the wider secondary face to the narrower primary face of the CD; that is, from the part of the CD where the secondary hydroxyl groups are positioned to the part of the CD where the primary hydroxyl groups are positioned (Fig. 4(B)–(E)). Meanwhile, in the complexes with αCD and γCD, the 3,5-hydroxyl ring of gnetol is south facing from the secondary to the primary face of the CD (Fig. 4(A) and (C)). These variations in the interaction between host and guest molecules could explain the different affinity and K_F values obtained by fluorescence spectroscopy.

The formation of hydrogen bonds is highlighted in the gnetol dockings with HPβCD and βCD. These weak interactions help to stabilize inclusion complexes and their presence is corroborated the greater encapsulation constants obtained for the natural and modified βCD (Table 1).

Aqueous solubility of gnetol after the encapsulation process

In the absence of CDs, the solubility in water of gnetol was 0.31 mg mL^{-1}. Despite being low, it was ten times higher than the solubility of its analogue resveratrol (0.03 mg mL^{-1}, PubChem).

The addition of CDs successfully increased gnetol solubility. It was observed that the highest concentration of HPβCD could solubilize 0.82 mg of gnetol in 1 mL of water; that is, almost threefold the basal solubility (Fig. 5). Furthermore, lower concentrations of CDs also make significant differences, with the supplementation of just 1 mmol L^{-1} of HPβCD able to enhance the aqueous solubility of this stilbene by 62%.

This improvement in water solubility could be useful for the development of functional foods enriched in gnetol.

Stability of gnetol inclusion complexes during storage

The shelf life of gnetol after 7 days' storage was improved when CDs were present in the medium (Fig. 6). 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. Storage for 1 day gave no significant differences among the samples.

Temperature proved to be a relevant factor in the preservation of this bioactive compound when it was in free or partially complexed form (1 mmol L^{-1} HPβCD), with a higher loss when stored at room temperature. By contrast, 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.

Thus, encapsulation in CDs is an effective technique for preserving gnetol, even more than simple refrigerated storage.

CONCLUSIONS

Gnetol was proved to be a bioactive compound able to scavenge ABTS$^{\cdot+}$ free radicals and to reduce ferric complexes. Its antioxidant capacity was between that of piceatannol and resveratrol, and depending on the method of measurement it was higher or similar to oxyresveratrol activity. This stilbene has an absorption spectrum similar to resveratrol, although the maximum was at 310 nm. The molar attenuation coefficient at this wavelength was 40 003 mol^{-1} L cm^{-1}.

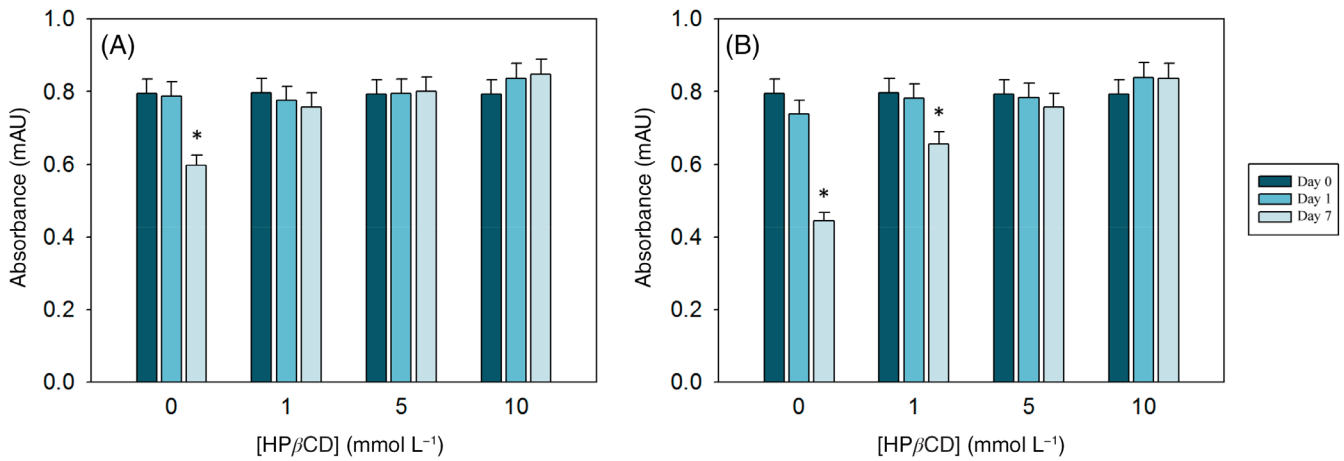

Figure 6. Stability of gnetol in free form and complexed in cyclodextrins when stored (A) refrigerated (4 °C) and (B) at room temperature (25 °C). *Significance $P < 0.05$. HPβCD: 2-hydroxypropyl-β-cyclodextrin.

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The complexation of gnetol was fitted to a 1:1 stoichiometry, as in the case of other stilbene analogues, and showed a greater affinity for CDs with seven glucose units; especially the modified HPβCD. Encapsulation constants were influenced by changes in pH and temperature, decreasing when either of these parameters increased.

Scores from molecular docking were in good agreement with the encapsulation constants obtained from the fluorometric assay. Molecular modelling revealed the formation of hydrogen bonds in gnetol complexes with HPβCD and βCD, and the way gnetol enters the internal cavity of different CDs was correlated with experimental results.

The encapsulation process successfully enhanced the aqueous solubility of gnetol, achieving almost a threefold increase in basal solubility with 10 mmol L^{-1} HPβCD supplementation, although lower concentrations were also significantly effective. 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.

These results add interest to this stilbene and its applications as a bioactive compound in functional foods.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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