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Abstract

Naringenin and hesperetin are two of the most abundant flavanones found in citrus with beneficial effects on human health. However, their poor water solubility imposes considerable limitations to their use in functional foods or nutraceuticals development. In order to increase their aqueous solubility and find a new stable system, the effect of different factors as temperature, pH or complexation agents as cyclodextrins has been studied. The solubility of both flavanones increased exponentially with temperature (25-90 ºC): 10-fold in the case of naringenin and 20-fold for hesperetin. The solubility of both flavanones also increased with the media pH (3.5-8.5): 314-fold in the case of naringenin and 3.5-fold for hesperetin. Flavanones solubility also increased with β- or HP-β-CDs concentration. By the addition of β-CDs 13 mM naringenin solubility increased 9.3-fold and hesperetin solubility 30-fold. Using HP-β-CDs 100 mM naringenin solubility increased 143-fold and hesperetin 467-fold. In summary, the higher increase in naringenin solubility was reached by increasing pH up 8.5. However, in the case of hesperetin, the higher increase was obtained by complexation with HP-β-CDs 100 mM. Moreover, the presence of CDs, not only increased the aqueous solubility of flavanones, but also improved the stability of hesperetin at pHs 3.5 and 6.5.

Keywords

Naringenin, hesperetin, complexation, cyclodextrins, temperature

1. Introduction

Flavanones are a group of flavonoids that are mainly found in citrus, honey, mint and tomato (Tomas-Barberan & Clifford, 2000). Among the most abundant flavanones in these foods are naringenin (4', 5, 7-trihydroxyflavanone) and hesperetin (3', 5, 7 trihydroxy-4-methoxyflavanone) (Bravo, 1998). In recent years there has been a growth in interest in these flavanones due to the beneficial effects attributed to them, including their protective role against lipid peroxidation of the membranes involved in several physiological and pathological disorders such as aging, inflammation, atherosclerosis and ischemia (Harbone & Willian, 2000; Kühnau, 1976; Benavente-García & Castillo, 2008; Hollman, Katan, Rice-Evans & Packer, 1998; Erlejman, Verstraeten, Fraga & Oteiza, 2004).

In addition, naringenin has been reported to have beneficial biological effects on human health as an antioxidant, free radical scavenger, anticancer, anti-inflammatory or immunomodulatory agent, and memory enhancer (Erlun, 2004; Julian, Moyano, Yañez, olea-Azar, 2007; Burdock, 1998). In recent years, naringenin has been found to have a neuroprotective effect in the ICV-STZ induced model of Alzheimer's disease, 6-OHDA model of Parkinson's disease or scopolamine-induced amnesia, and to prevent oxidative damage in various pathophysiological conditions due to its ability

to penetrate into the brain (Pratico, 2008); Kakkar & Dahiya, 2015; Noble & Burns, 2010).

For its part, hesperidin also has antioxidant, bone-sparing and lipid-lowering effects and plays a significant role in inflammation and cancer inhibition. Its antioxidant property, together with the ability to cross the blood–brain barrier, seems to contribute to neuroprotection against oxidative damage (Salas, Céliz, Geronazzo, & Daz, 2011).

As a result, there is a growing interest in using flavonoids to enrich food products in order to enhance their health functions, as well as in developing flavonoid-based nutraceutical and pharmaceutical products. However, the very poor water solubility of these flavanones imposes considerable limitations to their use (Guo, Tang, Lu, Fang, Tu & Zheng, 2018). So, it is necessary to develop new formulations that incorporate naringein and hesperetin in foods to improve their solubility and *in vivo* bioavalilability. Nanoencapsulation (formation of molecular complexes) can overcome the low water solubility and bioavailability of poor water-soluble compounds, and the high demand to produce foods with additional nutritional value led to many applications of nanoencapsulation in functional food development.

In recent years, complexation with cyclodextrins (CDs) has been successfully used to improve the solubility, chemical stability and

bioavailability of a number of poorly soluble compounds, including, flavonoids (Kin, Kim & Jung, 2008; Lucas-Abellán, Fortea, Gabaldón-Hernández, & Núñez-Delicado, 2008a, Lucas-Abellán, Fortea, Gabaldón-Hernández, & Núñez-Delicado, 2008b; López-Nicolás, Núñez-Delicado, Pérez-López, Carbonell-Barrachina & Cuadra-Crespo, 2006). The formation of molecular complexes with CDs could be one way to improve the water solubility of flavonoids. CDs are cyclic oligosaccharides consisting of six (α-CDs), seven (β-CDs), eight (γ-CDs) or more glucopyranose units linked by α (1-4) bonds and adopting a truncated cone structure with an internal hydrophobic cavity (Martín Del Valle, 2004). They are produced as a result of intramolecular transglycosylation reactions through the degradation of starch by cyclodextrin glucanotransferase (CGTase) (Szetjili, 1998). Compared with water, the internal cavity of CDs is relatively hydrophobic, while the outer surface is hydrophilic. This characteristic enables CDs to form inclusion complexes with a variety of compounds (Loftsson & Brewster, 1996; Szetjli, 1998; Zyzelewicz, Oracz, Kaczmarska, Budryn & Grzelczyk, 2018). Indeed, CDs have been used as complexing agents to increase the water solubility of many compounds, such as drugs, vitamins or food colorants (Fujishima, Kusaka, Umino, Urushinata & Terumi, 2001; Bhardwaj, Dorr & Blanchard, 2000), and it has been demonstrated that complexation can considerably increase the water solubility, stability and bioavailability of many guest molecules.

The objective of this paper was to study the aqueous solubility and stability of two flavanones, naringenin and hesperetin in different conditions of temperature, pH and also in the presence of natural complexing agents as CDs. The effect of temperature, pH and complexation with β-cyclodextrins (β-CDs) or hydroxy-propyl-β-cyclodextrins (HP-β-CDs) on the aqueous solubility and stability of these flavanones were studied in an attempt to develop a new system in which they could be solubilized in a stable way for use in food or pharmaceutical applications. In order to characterize the complexation process and determine the best cyclodextrin type to complex each flavanone, complexation constant (Kc), complexation efficiency (CE) and molar ratio were calculated in each case.

2. Materials and methods

Naringenin and hesperetin (assay > 90% purity) were kindly provided by Nutrafur S.A. (Murcia, Spain). β-CDs and HP-β-CDs were purchased from Henan Puertai Animal Medicine Co. (Zhengzhou, China). HPLC-grade acetonitrile and double-distilled HPLC-grade water was supplied from Aldrich (Madrid, Spain). All other chemicals used were of analytical grade.

2.1. Effect of temperature on aqueous solubility of the flavonones

An excess amount of naringenin (30 mg) or hesperetin (30 mg) was added to 10 mL of water. The mixtures were shaken in an ultrasonic bath at different temperatures (20, 25, 30, 35, 40, 50, 70 and 90 ºC) and for different

times (5, 15, 30, 45 and 60 min). Undissolved naringenin or hesperetin was removed by filtering through 0.45 μm acetate cellulose filters. The concentration of naringenin or hesperetin in the clear filtrate was determined spectrophotometrically at 290 (ε_{290}=17.51 $M^{-1}cm^{-1}$) or 292 (ε_{292}=34.38 $M^{-1}cm^{-1}$) nm, respectively.

2.2. Effect of pH

An excess amount of naringenin (30 mg) or hesperetin (30 mg) was added to 10 mL of 100 mM sodium acetate buffer pH 3.5, 100 mM sodium phosphate buffer pH 6.5, or 100 mM sodium borate buffer pH 8.5. The mixtures were shaken in an ultrasonic bath for 60 min. After that, undissolved naringenin or hesperetin was removed by filtration thought 0.45 μm acetate cellulose filter and the flavanone concentrations were determined as described in section 2.1. In order to improve the assay accuracy and standardizing the measure procedure, in all cases, the concentration of naringenin or hesperetin were quantified at 290 or 292 nm with samples diluted with ethanol 80%, minimizing thus the possible variation in the absorbance due to the pH effect.

2.3. Effect of CDs on aqueous solubility of flavanones.

Phase solubility diagrams were constructed according to Higuchi & Connors (1965) with some modifications. Excess amounts of each flavanone were added to aqueous solutions with increasing concentrations of β- or HP-

β-CDs up to 13 or 100 mM, respectively, in 10 mL of 100 mM sodium acetate buffer (pH 3.5), 100 mM sodium phosphate buffer (pH 6.5) or 100 mM sodium borate buffer (pH 8.5). The samples were maintained in an ultrasonic bath at 25 ºC for 60 min to reach equilibrium. The aqueous solutions were then filtered through 0.45 µm acetate cellulose membrane filter and diluted in 80% ethanol before flavanone quantification. Naringenin or hesperetin concentration in each clear filtrate was spectrophotometrically determined by measuring at their wavelength maximum.

The complexation constant (Kc) between naringenin or hesperetin and β- or HP-β-CDs were calculated by using the equation [1] (Higuchi & Connors 1965):

$$K_c = \frac{slope}{S_0(1 - slope)} \qquad [1]$$

where S_0 is the water solubility of the flavanone, and "slope" the slope of the phase solubility diagram.

2.4. Stability tests

The stability of naringenin and hesperetin in aqueous solution at different pHs (3.5, 6.5 and 8.5) was studied at 25 ºC. For this purpose, a 1 ml aliquot of naringenin or hesperetin solution (1 mg/mL in 80 % ethanol) was mixed with 5 ml of buffer solution (13 % ethanol) containing (or not) 6 mM of CDs (β- or HP-β-CDs). At different time intervals, the remaining

naringenin or hesperetin concentration was determined by HPLC (Hewlett-Packard Series HP 1100 equipped with a diode array detector). The stationary phase was a C18 LiChrospher 100 analytical column (250 x 4 mm i.d.) with a particle size of 5 µm (Merck, Darmstadt, Germany) thermostatic at 30 ºC. The mobile phase consisted of acetonitrile/water (60/40) with a 1 mL/min flow rate, and the injection volume was 20 µL. Naringenin or hesperetin was detected at 290 or 292 nm, respectively and they were quantified buy using a calibration curve from 0.005 to 0.05 mM prepared in ethanol 80%.

3. Results and discussion

The purpose of this paper was to study the solubility and stability of naringenin and hesperetin in aqueous solution at different pHs and temperatures and the effect of their inclusion in native β- or their modified HP-β-CDs on the aqueous solubility and stability of these compounds at different pH values.

3.1. Effect of temperature on aqueous solubility

Because naringenin and hesperetin are poorly soluble in water, the effect of temperature on their aqueous solubility was studied by means of a solubility test at 20, 25, 30, 35, 40, 50, 70 and 90 ºC in sodium phosphate buffer 100 mM pH 6.5.

As can be seen in Figure 1, the solubility of naringenin and hesperertin at 25 ºC was only 0.14±0.03 mM (Figure 1A) and 0.03±0.01 mM (Figure 1B), respectively, indicating their poor aqueous solublility. However, their solubility increased exponentially with temperature, suggesting that the process is thermodynamically favorable, at 90 ºC reaching a concentration of 1.5±0.04 mM for naringenin (10-fold higher than at 25 ºC) and 0.6±0.02 mM for hesperetin (20-fold higher than its aqueous solubility at 25 ºC).

The lower aqueous solubility of hesperetin at all the temperatures assayed was due to the presence of the $–CH_3$ group in 4'C position.

3.2 Effect of pH in aqueous solubility

The solubility of naringenin and hesperetin was also affected by the pH of the medium used, increasing from $9 \cdot 10^{-3} \pm 2 \cdot 10^{-3}$ mM at pH 3.5 to 2.83±0.18 mM at pH 8.5 (314-fold) in the case of naringenin; and from 0.02±0.01 mM at pH 3.5 to 0.07±0.02 at pH 8.5 (3.5 fold) for hesperetin (Table 1).

The more pronounced increase in the solubility of naringenin with increasing pH was due to the chemical structure associated with the deprotonated form of the three –OH groups. However, in the case of hesperetin, the presence of the $–CH_3$ group in 4´C position leads to the formation of a less resonant structure at pH 8.5 and thus a less soluble form.

3.3. Effect of complexation in CDs

After studying the effect of temperature and pH on the solubility of naringenin and hesperetin, the effect of the presence of β- or their modified HP-β-CDs at 25 ºC was also investigated by developing phase solubility diagrams.

As can be seen in Figure 2 either flavanones concentration increased linearly with β- (Figure 2A) or HP-β-CDs (Figure 2B) concentration. In the case of β-CDs, due to their low aqueous solubility, the maximum CDs concentration used was 13 mM (Figure 2A). Naringenin solubility increased

from 0.14±0.03 (S_0) to 1.3 mM with 13 mM β-CDs (9.3-fold) (Figure 2A, ●) and hesperetin from 0.03±0.01 (S_0) to 0.75 mM with 13 mM β-CDs (25-fold) (Figure 2A, ○). In the case of HP-β-CDs, due to their higher aqueous solubility, the CDs concentration used could reach 100 mM (Figure 2B). Naringenin solubility increased from 0.14±0.03 to 23±0.1 mM with HP-β-CDs at 100 mM (164-fold) (Figure 2B, ●) and from 0.03±0.01 to 14±0.05 mM in the case of hesperetin (467-fold) (Figure 2B, ○).

Comparing the results obtained by using different CDs with those obtained by increasing temperature or pH (Figure 3), the increase in the solubility of both flavanones obtained with 100 mM HP-β-CDs was much greater than that obtained by increasing the temperature from 20 to 90 °C (164-fold *vs.* 10.7-fold for naringenin and 467-fold *vs.* 20 fold for hespertin). In the case of β-CDs the increase in aqueous solubility was similar to that obtained by increasing the temperature from 20 to 90 ºC for both types of flavanones (9.3-fold *vs.* 10.7-fold for naringenin and 25-fold *vs.* 20-fold for hesperetin).

Increasing the pH from 3.5 to 8.5 led to a 314-fold increase in naringenin solubility, a greater increase than was obtained by using 100mM HP-β-CDs (143-fold). However, in the case of hesperetin, the increase in solubility due to de pH change was only 3.5-fold *vs* 467-fold observed with 100 mM HP-β-CDs (Figure 3).

Note that at the same concentration (13 mM), HP-β-CDs increased the aqueous solubility of both flavanones to a greater extent than β-CDs (Figure 3).

The phase solubility plots for the flavanone-CDs complexes displayed typical A_L diagrams, that is to say, a linear increase in flavanone solubility with increasing CDs concentration (β- and HP-β-CDs) (Figures 2A and 2B). The slope values lower than 1 point to a 1:1 stoichiometry for the complexes formed between naringenin or hesperetin and β- or HP-β-CDs.

The Kc values obtained for both naringenin and hesperetin were higher in the case of HP-β-CDs (1,786±185 M^{-1} for naringenin and 4,651±525 M^{-1} for hesperetin) (Pérez-Abril et al. 2017) than in the case of β-CDs (681±77 M^{-1} for naringenin and 1,123±101 M^{-1} for hesperetin), at pH 6.5 (Table 1). These results indicate that the complexes formed between each naringenin or hesperetin, and HP-β-CDs were much more stable than those formed with β-CDs. The addition of hydroxypropil groups to native β-CDs favored the entrapment of naringenin or hesperetin in the hydrophobic cavity of the CDs. This phenomenon has also been demonstrated in the case of other flavonoids such as resveratrol (Lucas-Abellán, Fortea, Gabaldón-Hernández, & Núñez-Delicado, 2008a; Lucas-Abellán, Fortea, Gabaldón-Hernández, & Núñez-Delicado, 2008b; Mercader-Ros, Lucas-Abellán, Gabaldón, Fortea, Martínez-Cachá, & Núñez-Delicado, 2010).

The Kc values obtained represented the strength of the interaction between each flavanone and β- or HP-β-CDs, making it possible to compare the affinity between each one and native or modified CDs. However, to study the solubility effect of CDs on naringenin and hesperetin in greater depth, their complexation efficiency (CE) values were calculated. The CE represents the molar ratio between complexed and the free CDs concentration (Loftsson & Duchêne., 2007). Taking into account both the aqueous solubility of the flavanone and the Kc value, for 1:1 stoichiometry complexes, the CE was calculated from the slope of the phase solubility diagram using equation [2] (López-Miranda, Guardiola, Hernánrdez-Sánchez & Núñez-Delicado, 2017)

$$CE = S_0 * Kc = \frac{slope}{1-slope} = \frac{[F-CDs]}{[CDs]} \qquad [2]$$

where [F-CDs] is the soluble complex concentration, [CDs] is the dissolved free CDs concentration and slope is the slope of the phase solubility diagram.

The CE values obtained for naringenin and hesperetin with β- or HP-β-CDs are shown in Table 1. Comparing the CE values obtained for each flavanone rather than the Kc values is more convenient when different types of CDs and experimental conditions are being compared. As can be seen in Table 1, the CE values obtained at pH 6.5 for both naringenin and hesperetin

were higher with HP-β- than for β-CDs. In the case of naringenin, the CE value obtained for β-CDs was 9.5% and for HP-β-CDs 25% (2.6-fold higher). In the case hesperetin, the CE value obtained for β-CDs was 3.4% while for HP- β-CDs it was 13.9% (4.1-fold higher).

The CE values were also used to calculate the molar ratio in solution of naringenin:CDs or hesperetin:CDs, the value of which would reflect the expected increase in naringenin or hesperetin solubility with different types of CDs (Equation [3]) (Loftson & Duchêne, 2007):

$$Flavanone{:}\,CDs = 1{:}\left(1 + \frac{1}{CE}\right) \quad [3]$$

The values of the molar ratio obtained for naringenin and hesperetin with β- or HP-β-CDs are shown in Table 1. At pH 6.5, the molar ratio of the solution showed that HP-β-CDs was the most effective because about one in 5 CDs molecules in solution formed water soluble complexes with naringenin, whereas in the case of β-CDs the ratio was about one in 11 (Table 1). In the case of hesperetin, the results obtained were similar to those obtained for naringenin, HP-β-CDs being more effective than β-CDs (1:31 for β-CDs and 1:8 for HP-β-CDs) (Table 1).

In summary, HP-β-CDs were more effective for the complexation of naringenin and hesperetin than β-CDs at pH 6.5, as indicated by the CE values and molar ratio (Table 1). Moreover, complexes formed by HP-β-CDs

were more stable, as indicated by the Kc values (Table 1). Of the two flavanones, hesperetin formed more stable complexes with β- or HP-β-CDs than naringenin (Kc hesperetin >Kc naringenin, for both β- and HP-β-CDs) (Table 1), probably because the presence of the -CH_3 group in 4´position makes hesperetin more hydrophobic than naringenin, thus increasing the Kc values for both types of CDs (Table 1).

However, naringenin was the flavanone most effectively complexed by both types of CDs (CE naringenin>CE hesperetin, for both β- and HP-β-CDs) at pH 6.5 and 8.5 (Table 1), because this parameter represented the ratio between complexed and free CDs. Moreover, CE is obtained by the product between Kc value and S_0. Hence, a decrease in S_0 would be expected to involve a lower CE value, as was the case with hesperetin.

3.3. Effect of pH on the complexation process

The effect of acid (3.5) or basic (8.5) pH on the complexation of the flavanones (naringenin and hesperetin) with β- or HP-β-CDs was also studied.

The pH value determines the presence (or not) of protonated flavanone forms in the solution, influencing their aqueous solubility and also the complexation process. At both pH values studied (3.5 and 8.5), the phase solubility diagrams obtained were A_L type with a slope of less than 1, indicating the 1:1 stoichoimetry of the complexation formed between

naringenin or hesperetin and β- or HP-β-CDs (Figure 4). The Kc values obtained from the phase solubility diagrams for both, naringenin or hesperetin were higher at acid or neutral pH than at basic pH (Table 1). These results indicated that the protonated forms of each flavanone formed more stable complexes with β- or HP-β-CDs than the corresponding deprotonated forms. The significant decrease in the Kc value at basic pH (8.5) was also due to the characteristic balance existing in these compounds between the flavanone (closed C ring) and chalcone (open ring C) forms begins to move significantly towards the chalcone form, at basic pH with higher aqueous solubility S_0 (from 9.10^{-3}±2.10^{-3} mM at pH 3.0 to 2.83±0.18 mM at pH 8.5 in the case of naringenin and from 0.02±0.02 mM at pH 3.0 to 0.07±0.02 mM at pH 8.5 in the case of hesperetin) (Table 1). The pronounced increase in the aqueous solubility of naringenin as the pH increased from pH 3.0 to 8.5 implied a drastic decrease in the Kc value, both in the presence of β- (from 1085±108 M^{-1} at pH 3.5 to 184±44 M^{-1} at pH 8.5) and HP-β-CDs (from 1515±223 M^{-1} at pH 3.5 to 225±37 M^{-1} at pH 8.5) (Table 1). The Kc value obtained for naringenin and β-CDs at pH 3.0 was in the same order to that obtained by Yang et al. in 2013 (Yang, Ma, Zhou, Chen, Yuan, Yin & Yang, 2013).

As can be seen in the results presented in Table 1, the Kc values obtained for naringenin or hesperetin at both pH 3.5 and 6.5 were higher

following complexation with modified HP-β-CDs than with the parental β-CDs, indicating the formation a more stable complex with HP-β-CDs. However, at basic pH (8.5), the increase in solubility of flavanones due to the presence of the chalcone form, masked the effect of interaction between HP-β-CDs and the flavanone, so that the Kc value did not increase so much as might be expected.

As the aqueous solubility of flavanones and Kc values varied with the pH of the medium, both the CE and molar ratio were also affected by this parameter. In the case of naringenin, the CE clearly increased with the pH for both β- (from 0.9 % at pH 3.5 to 52 % at 8.5) and HP-β-CDs (from 1.4 % at pH 3.5 to 63.7 % at pH 8.5) (Table 1). In the case of hesperetin, the CE values increased but to a lesser extent than with β-CDs (from 1.9 % at pH 3.5 to 22.9 % at pH 8.5) and decreased for HP-β-CDs (from 7.1 % at pH 3.5 to 2.8 % at pH 8.5) (Table 1). The highest CE values obtained were 63.7 % and 52 % for naringenin complexation at pH 8.5 with HP-β- and β-CDs, respectively, indicating that at this basic pH, one in every 3 β- or HP-β-CDs molecules in solution forms water soluble complexes with naringenin (molar ratio 1:3) (Table 1).

3.3. Stability of naringenin and hesperetin in aqueous solution

The stability of naringenin and hesperetin in aqueous solution at different pHs (3.5, 6.5 and 8.5) was also studied.

As can be seen in Figure 5A, the naringenin concentration in aqueous solution remained stable for 48 hours at all the pHs studied (3.5, 6.5 and 8.5). In the case of hesperetin (Figure 5B), its stability was affected with time at pH 3.5 and pH 6.5, but remained constant at pH 8.5. In order to establish whether the loss of hesperetin with time at pH 3.5 and 6.5 was due to its transformation into another compound or to the precipitation of the flavanone, the chromatographic profile of the solution was analyzed. As can be seen in Figure 6, the hesperetin chromatogram profile did not change with time at pH 3.5 or 6.5, indicating that the loss of the compound was due to its precipitation because of its low solubility at both pHs. At basic pH (8.5), hesperetin solubility increased significantly due to a change in the chalcone-flavanone structure with higher aqueous solubility increasing its stability.

The stability of naringenin and hesperetin were also studied in buffered media at different pHs (3.5, 6.5 and 8.5) in the presence of β- or HP-β-CDs. In the case of naringenin, it was stable for 48 h in the presence of CDs (Figure 5C and 5E), as in the absence (Figure 5A). With respect to hesperetin, the presence of β- or HP-β-CDs permitted the stabilization of the flavanone at pHs 3.5 and 6.5 (Figures 5D and 5F) due to the complexation of hesperetin in the hydrophobic cavity of native or modified CDs, increasing the aqueous solubility.

4. Conclusion

This paper shows that flavanones aqueous solubility and stability are affected by temperature, pH and the presence of complexating agents as CDs in the media, being HP-β-CDs the most effective agents to increase these properties. Moreover, β- and HP-β-CDs form 1:1 complexes with naringenin and hesperetin. The results obtained indicated that the highest Kc value did not always correspond to the highest CE because of the influence of the aqueous solubility (S_0) of the flavanone on both values. For this reason, it is more accurate to use the CE value to study the effect of pH on the complexation process of flavanones. In the case of naringenin and hesperetin, the deprotonated form of naringenin at pH 8.5 was the most efficiently encapsulated by β- and HP-β-CDs, whereas the protonated form of hesperetin at pH 6.5 formed the strongest complexes with HP-β-CDs, and had the highest Kc value. Moreover, the complexation process improved the stability of hesperetin at pH 3.5 and 6.5.

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	Flavonoids	S_0 (mM) pH 3.5	Kc mM	CE % pH 3.5	Molar Ratio	S_0 (mM) pH 6.5	Kc mM	CE % pH 6.5	Molar Ratio	S_0 (mM) pH 8.5	Kc mM	CE % pH 8.5	Molar ratio
β-CDs	**Naringenin**	$9 \cdot 10^{-3} \pm 2 \cdot 10^{-3}$	1085±108	0.9	1:103	0.14 ± 0.03	681 ± 77	9.5	1:11	2.83 ± 0.18	184 ± 44	52	1:3
	Hesperetin	0.02 ± 0.01	932 ± 189	1.9	1:55	0.03 ± 0.01	1123 ± 101	3.4	1:31	0.07 ± 0.02	410± 41	22.9	1:36
HP-β-CDs	**Naringenin**	$9 \cdot 10^{-3} \pm 2 \cdot 10^{-3}$	1515 ± 223	1.4	1:74	0.14 ± 0.03	1786 ± 385	25	1:5	2.83 ± 0.18	225 ± 37	63.7	1:3
	Hesperetin	0.02 ± 0.01	3546 ± 216	7.1	1:15	0.03 ± 0.01	4651 ±525	13.9	1:8	0.07 ± 0.02	403 ± 29	2.8	1:36

Table 1

Aqueous solubility, Kc, CE and Molar Ratio of flavanones at different pH values with β- and HP-β-CDs

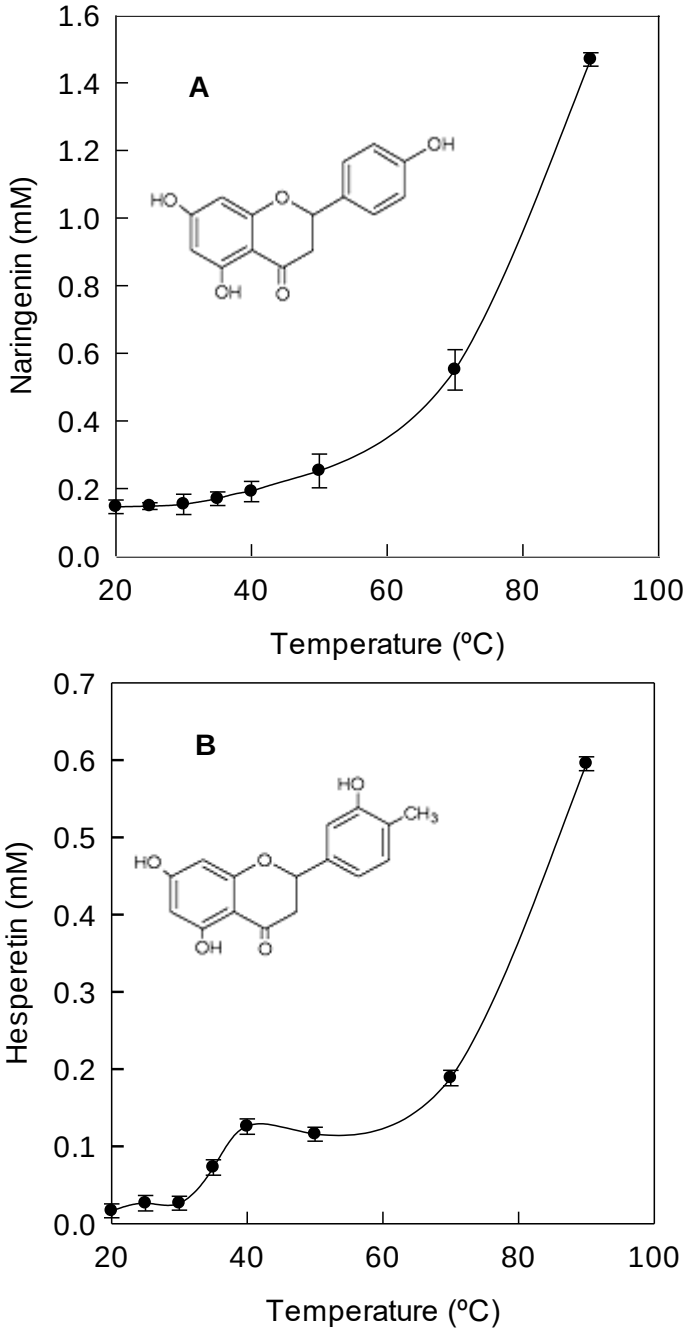

Figure 1: Effect of temperature on the solubility of the flavanones naringenin (A) and hesperetin (B)

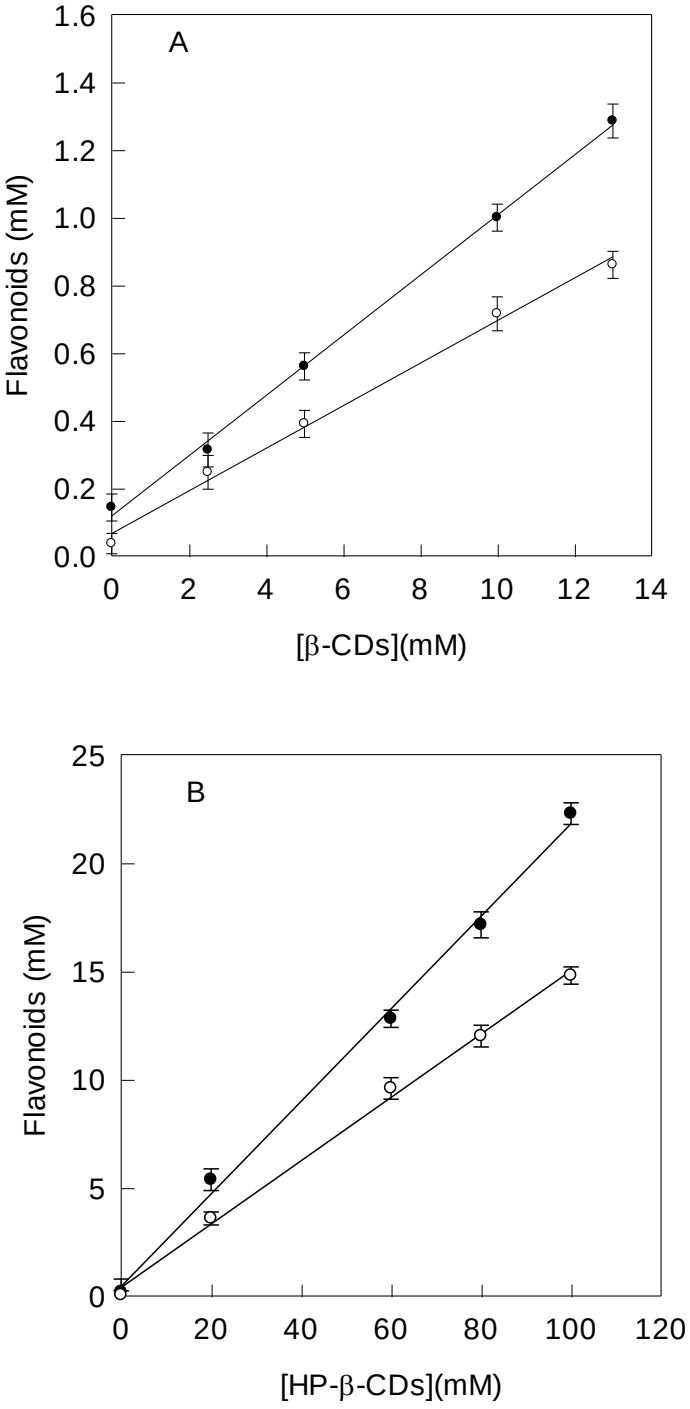

Figure 2: Phase of solubility diagram of naringenin (●) and hesperetin (○) sodium phosphate buffer 100 mM (pH 6.5) with β-CDs (A) and HP-β-CDs (B).

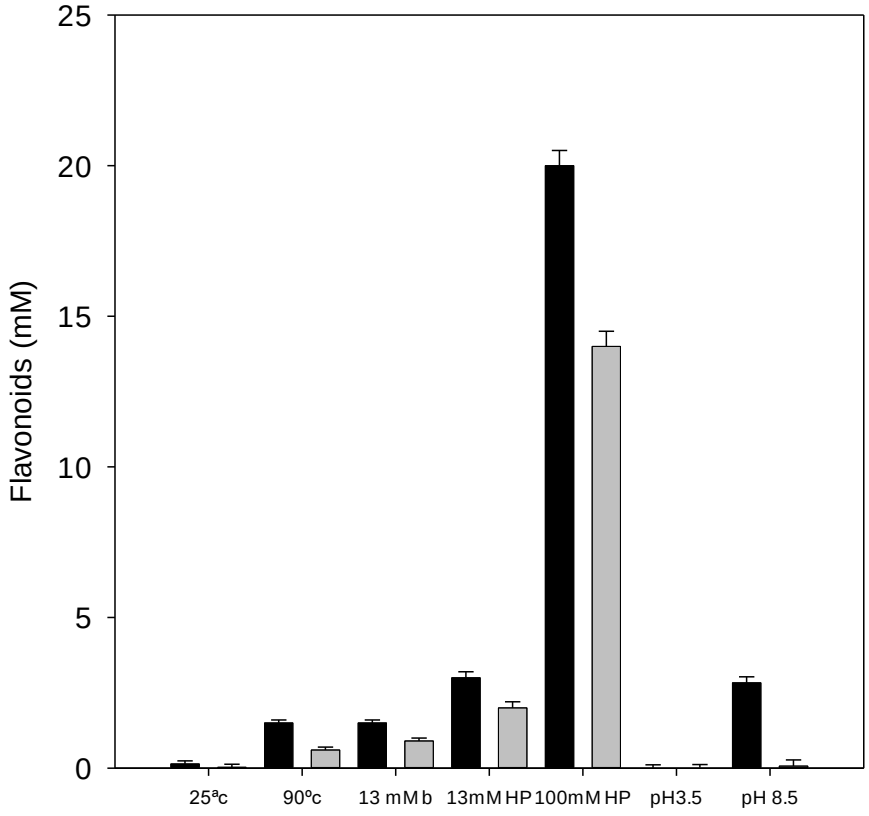

Figure 3: Solubility of naringenin and hesperetin at different temperatures and with different types of CDs

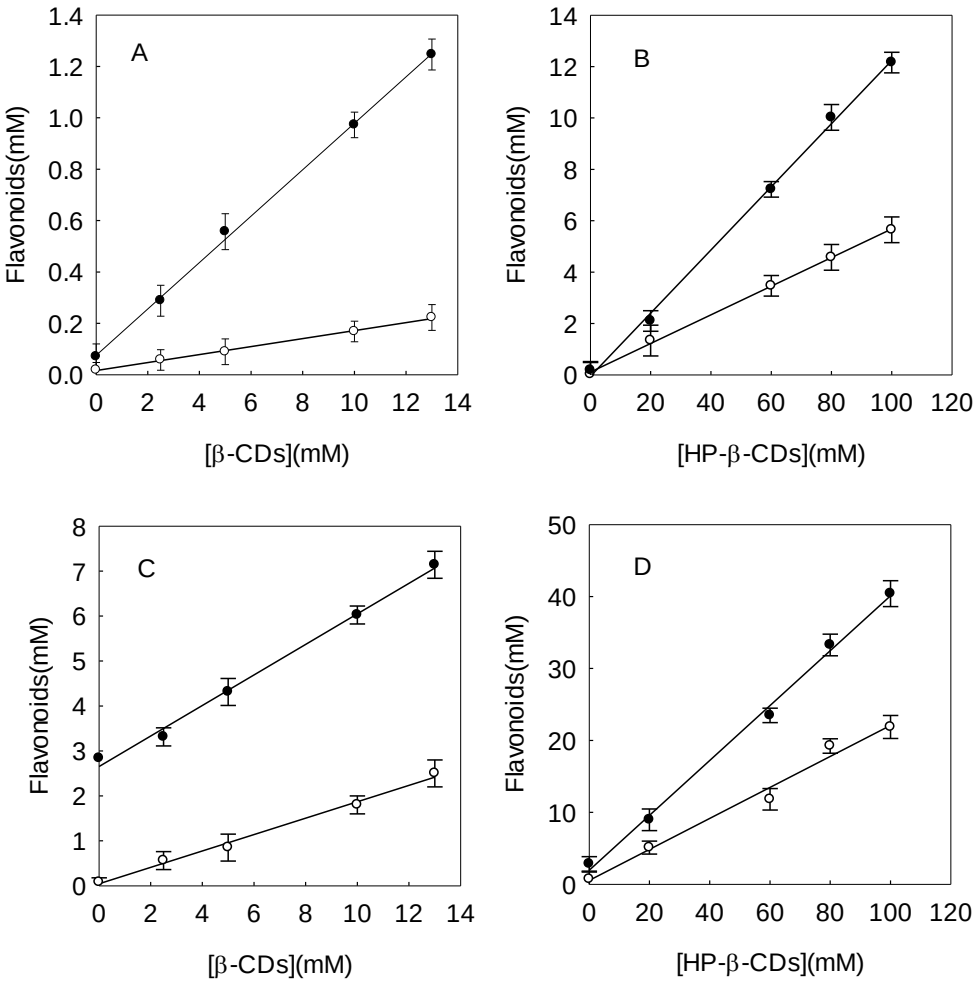

Figure 4: Phase of solubility diagrams of naringenin (●) and hesperetin (○) in 100 mM sodium acetate buffer (pH 3.5) with β- (A) or HP-β-CDs (B) and 100 mM sodium borate buffer (pH 8.5) with β- (C) or HP-β-CDs (D).

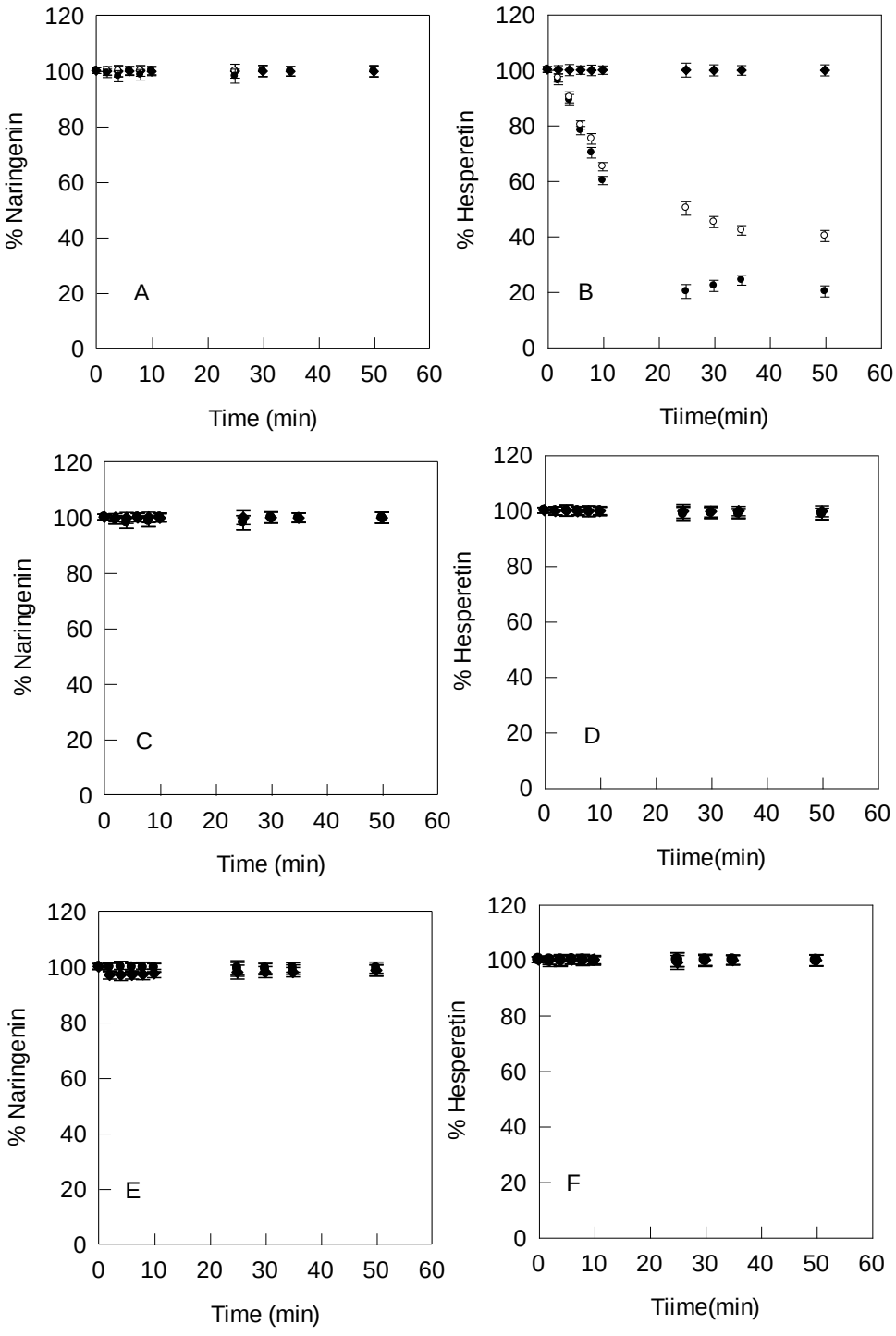

Figure 5: Time course for loss of naringenin (A) and hesperetin (B), naringenin with β-CDs (C), hesperetin with β-CDs (D), naringenin with HP-β-CDs (E) and hesperetin with HP-β-CDs (F) in different solution media 100 mM sodium acetate buffer (pH 3.5) (●), 100 mM sodium phosphate buffer (pH 6.5) (○) and 100 mM sodium borate buffer (pH 8.5) (◆).

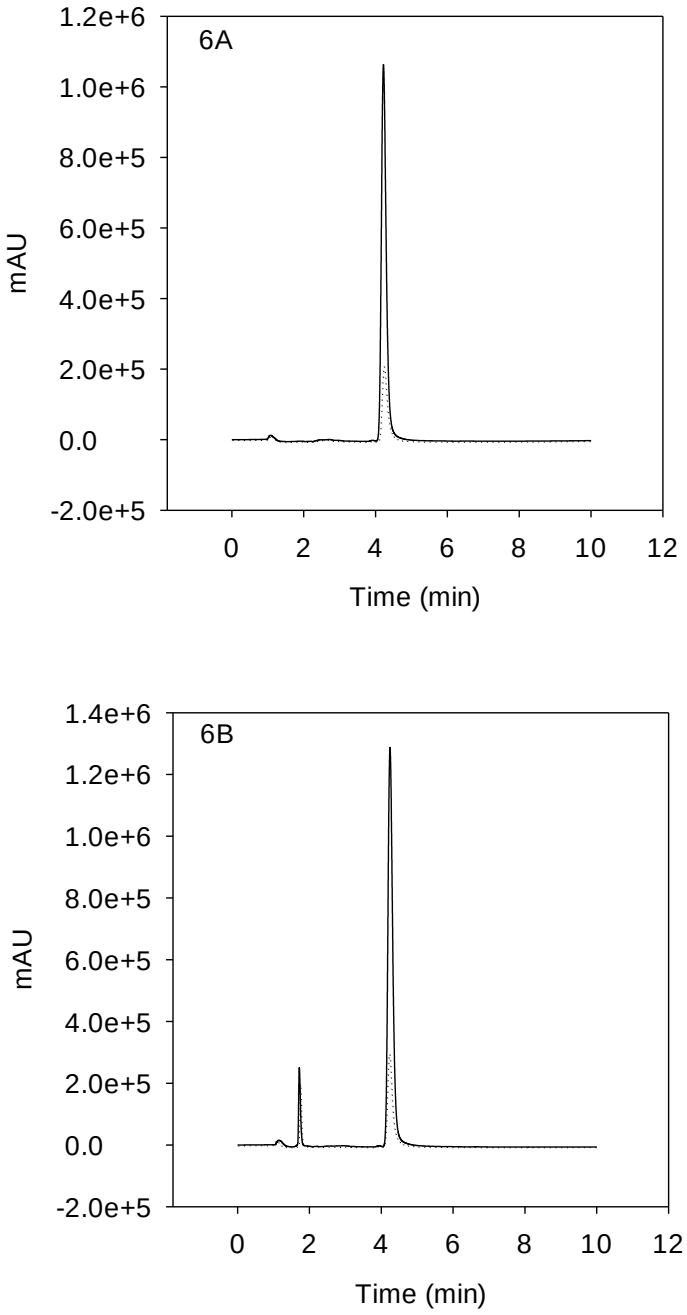

Figure 6: (A) Chromatogram of hesperetin in aqueous solution 100 mM sodium acetate buffer (pH 3.5) at 1h (-) and 48h (...). (B) The chromatogram of hesperetin in aqueous solution 100 mM sodium phosphate buffer (pH 6.5) at 1h (-) and 48h (...).