

Improved Water Solubility of Neohesperidin Dihydrochalcone in Sweetener Blends

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Significant technological advantages in terms of sweetness synergy and hence cost-saving can be obtained if neohesperidin dihydrochalcone (NHDC) is used in sweetener blends with other intense or bulk sweeteners. The combination of NHDC with sodium saccharin or sodium cyclamate is an excellent method to improve the water solubility at room temperature of NHDC. In the case of NHDC–sodium saccharin, two different methods for blend preparation, a simple mixture and a cosolubilized mixture, can be used, with similar results obtained for solubility and solution stability properties. To improve temporarily the water solubility of NHDC in combination with sodium cyclamate, it is absolutely necessary to prepare cosolubilized blends.

Keywords: Neohesperidin dihydrochalcone; NHDC; sweetener blends; sodium saccharin; sodium cyclamate

INTRODUCTION

Neohesperidin dihydrochalcone (NHDC) (Figure 1) is an intense sweetener with excellent properties as a bitterness suppressor (1) and flavor modifier, which are apparent even at concentrations at which there is no significant contribution to sweetness (1–5 ppm) (2).

NHDC was discovered by Horowitz and Gentili (3) in their studies on the taste/structure relationship of different citrus flavonoids. Initial evaluations of its taste revealed that it elicits menthol- or licorice-like aftertastes, which were considered to be serious defects when evaluated as the sole sweetener in foods and beverages (4).

Nowadays, there is an increasing trend toward using sweetener blends in beverages and foods, not only to improve the sweetness profile and decrease undesirable aftertastes or bitter flavor but also to reduce the daily intake of sweeteners such as saccharin and cyclamate (5, 6). In addition, significant technological advantages in terms of sweetness synergy and hence cost-saving can be obtained if NHDC is used in sweetener blends (at a level of <10 ppm) with other intense or bulk sweeteners (2, 7). Synergy has been reported in blends with saccharin (8), sucralose (9), cyclamate (10), acesulfame-K (11), and polyols (12).

Sweetener blends are usually prepared with a simple mixture of sweeteners or with cosolubilized and codried mixtures, and both methods have specific advantages and disadvantages. With respect to NHDC, its water solubility and solubilization rates at room temperature are low, and these make it impossible to prepare syrups with NHDC, thus limiting its use for water-soluble applications. In this paper we studied the preparation, behavior, and solubility properties of several NHDC sweetener blends by enhancing its water solubility at room temperature.

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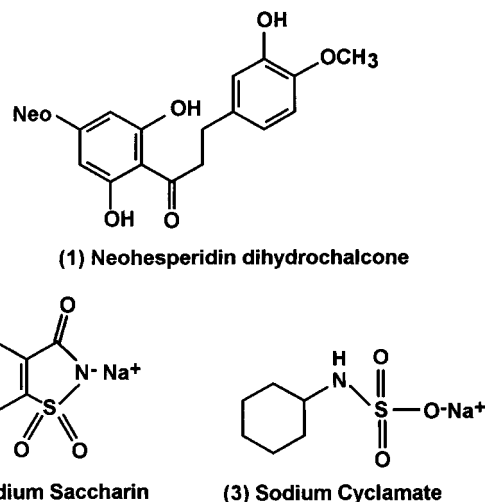


Figure 1. Molecular structures of NHDC, sodium saccharin, and sodium cyclamate.

MATERIALS AND METHODS

Chemicals. Sodium saccharin and sodium cyclamate were obtained from Sigma Chemical Co. (Madrid, Spain). NHDC was obtained from Furfural Español S.A. (Alcantarilla, Spain). HPLC grade water and ethanol was obtained from Merck (Darmstadt, Germany).

Blend Preparation. Single mixtures of sodium saccharin or sodium cyclamate with NHDC in a weight ratio (90:10) were performed by milling the individual ingredients in an electric mill. Cosolubilized blends of sodium saccharin or sodium cyclamate and NHDC in a weight ratio (90:10) were performed in a stirring stoppered flask, and a water/ethanol mixture was added at 50:50 for sodium saccharin and at 75:25 for sodium cyclamate. The suspension was heated to 50 °C and stirred until total solution, and then the solvent was evaporated to dryness at 50 °C in a vacuum in a Heidolph Laborota 4000 model rotavapor.

Solubility Test. The solubility test was performed in a stirring stoppered flask thermostated at different temperatures. Successive additions of 10, 100, or 1000 mg of substances (depending on solubility range) to 100 mL of solvent were

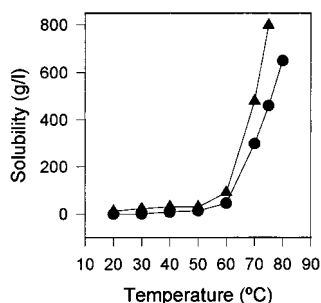


Figure 2. Water solubility (●) and ethanol solubility (▲) of NHDC versus temperature.

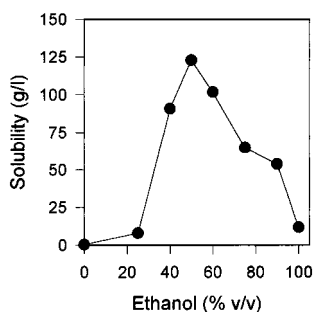


Figure 3. NHDC solubility in ethanol/water mixtures (% v/v) at 20 °C.

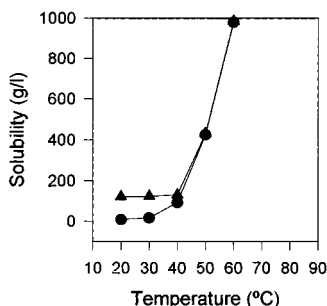


Figure 4. NHDC solubility in ethanol/water (25:75) (●) and ethanol/water (50:50) (▲) mixtures versus temperature.

performed. The final point of solubility for single and mixed compounds was determined in a Unicam UV 530 spectrophotometer with a cell temperature controller, measuring the absorbance of aliquots of the different solutions at 660 nm at different intervals (1, 5, 15, 30, 60, and 120 min) and the temperature required. The initial absorbance after each addition was always >1.0 UA, and the final point was considered to be when the absorbance dropped under 0.010 UA. The solubility time is registered, and a new addition is performed.

RESULTS AND DISCUSSION

The solubility curves of NHDC in water and ethanol at different temperatures are shown in Figure 2. The solubilities of NHDC in water and in ethanol at 20 °C are 0.4 and 12 g/L, respectively. This solubility rises sharply with temperature to 650 g/L at 80 °C in water and to 790 g/L at 75 °C in ethanol. NHDC, as occurs with other citrus flavonoids such as naringin, exhibits a higher solubility in the water/ethanol mixture than in water or ethanol alone (Figure 3). The solubility of NHDC in water/ethanol (50:50, v/v) at 20 °C, 123 g/L, is 10 and 300 times higher than that in ethanol or water alone and rises to 985 g/L when the temperature increases to 60 °C (Figure 4). In the water/ethanol mixture (75:25, v/v) the solubility at 20 °C drops sharply to 8 g/L. This value is 20 times higher than that in water

Table 1. Water Solubility at Room Temperature of Individual Ingredients, a Simple Mixture of Ingredients (A), and a "Cosolubilized Mixture" (B)

mixture	solution time ^a (min)	water solubility (g/L)	equivalent NHDC water solubility (g/L)
sodium saccharin	1	833	
sodium cyclamate	1	210	
NHDC	1	0.4	0.4
sodium saccharin/NHDC (A)	1	20	2.4
sodium saccharin/NHDC (B)	30	840	84
sodium cyclamate/NHDC (A)	5	840	84
sodium cyclamate/NHDC (A)	1	10	1.0
sodium cyclamate/NHDC (A)	30	10	1.0
sodium cyclamate/NHDC (B)	5	214	21

^a Time after the last addition.

and 1.5 times lower than that in ethanol. However, when the temperature rises to 50 °C, the solubility increases rapidly to 425 g/L, close to that in the water/ethanol (50:50, v/v) mixture.

We used the enhanced solubility of NHDC in the water/ethanol mixture to cosolubilize NHDC with sodium saccharin or sodium cyclamate in the weight ratio 10:90. Sodium saccharin is soluble in desired quantity (450 g/L) in the water/ethanol (50:50, v/v) mixture; however, the sodium cyclamate solubility in this water/ethanol (50:50, v/v) mixture is <400 g/L. NHDC and sodium cyclamate cosolubilization was performed in a water/ethanol (75:25, v/v) mixture. The solutions were evaporated under vacuum at the same temperature to dryness to obtain a homogeneous powder.

Table 1 shows the water solubility at room temperature of individual ingredients, a simple mixture of ingredients, and the mixture described above (cosolubilized mixture). The main characteristic of the cosolubilized mixtures is that their solubility and dissolution rates at room temperature are close to those of the main component on its own (sodium saccharin or sodium cyclamate) and remarkably higher than those of NHDC by itself, over 207 and 52 times for sodium saccharin and sodium cyclamate mixtures, respectively. It is important to consider the solubility of the simple mixtures according to the time process.

The solution time data in Table 1 show the different behaviors of cosolubilized and simple mixtures of sodium saccharin and sodium cyclamate with respect to the complete solution time. Cosolubilized mixtures with sodium saccharin show a quick dissolution rate, with a complete and clear solution (0.005 UA at 660 nm) obtained 5 min after addition. When the simple mixture NHDC–sodium saccharin is used, there is a small opalescence (0.055 UA at 660 nm) after the initial instant (1 min), which remains for ~30 min. After this time, the solution is complete and clear (0.009 UA at 660 nm), close to the above-mentioned aspect for the cosolubilized mixture. Both of these solutions, simple mixture and cosolubilized mixture, show a very high stability versus time, without opalescence or sediment after 15 days at room temperature.

The behavior of NHDC–sodium cyclamate blends is different from that of sodium saccharin blends. The cosolubilized blend shows a dissolution rate similar to that of saccharin blends; however, its stability versus time is lower than that of saccharin blends because NHDC precipitation begins after 10–12 h. On the other hand, the simple mixture NHDC–sodium cyclamate initially shows an important opalescence (2.474 UA at 660 nm) for quantities >10 g/L that does not disappear

completely at 120 min, thus proving the low solubility of this simple blend.

To complete the study of the possible mechanism that allows the NHDC solubility to increase by means of the simple and cosolubilized blend preparations, we made an experiment with the previous solution of the mean ingredients, in other words, saccharin or cyclamate. A water solution with 756 g/L of sodium saccharin (the same saccharin concentration as that of a saturated solution of cosolubilized blend with NHDC) was prepared, and then the quantity of NHDC needed to obtain a NHDC concentration of 84 g/L was added (see Table 1). After 15 min, the NHDC solution was complete and clear (0.009 UA at 660 nm). It is very important to note that the stability versus time of this solution is close to that of the cosolubilized and simple mixtures of sodium saccharin–NHDC.

In the case of sodium cyclamate, a solution with 193 g/L of this ingredient (the same cyclamate concentration as that of a saturated solution of cosolubilized blend with NHDC) was prepared. The quantity of NHDC needed to obtain an NHDC concentration of 21 g/L was slowly added (see Table 1), and the mixture showed an initial significant sediment and opalescence (3.670 UA at 660 nm) that remained with time, thus proving the low solubility of NHDC in this simple combination with sodium cyclamate.

Obviously, this difference in behavior between NHDC combinations with sodium saccharin or sodium cyclamate is markedly affected by the molecular structure of these compounds (Figure 1). The molecular structure and absolute configuration of NHDC have been determined by single-crystal X-ray analysis (13). The crystal structure consists of two crystallographically independent molecules that mainly differ in the orientation of the isovanillyl B-ring. The molecules are linked together by an intricate arrangement of intermolecular hydrogen bonds formed between the host and solvent water molecules (13, 14).

The molecular structures of sodium saccharin and sodium cyclamate have been recognized and can be consistent with the behavior in aqueous solution of their respective NHDC blends. The sodium saccharin structure presents three oxygen atoms with a high electronic density, mainly in oxygen atoms linked to a sulfur atom. This structure and its stereospatial arrangement, with the sulfoxide and carbonyl links in the same plane, suggest a potential linkage through hydrogen bonds with several functional groups in the NHDC skeleton: (I) the enolic form of the 4-keto group; (II) the 5-hydroxyl group in the A-ring; and (III) the hydroxyl groups of rhamnose and glucose molecules in the neohesperidosyl structure. It has been reported that the electron-donating power and the location of substituents in the sweetener molecule (15) control the strength of the hydrogen bond. The minor number of oxygen atoms and the different conformation of functional groups with regard to sodium saccharin can reduce the hydrogen bond strength in the NHDC–sodium cyclamate molecular interaction, being responsible for the minor increase of NHDC solubility and the lower stability of NHDC–sodium cyclamate cosolubilized blends.

In conclusion, the combination of NHDC with sodium saccharin or sodium cyclamate is an excellent method to improve the water solubility of NHDC at room temperature. In the case of NHDC–sodium saccharin, two different methods for blend preparation, a simple mixture and a cosolubilized mixture, can be used with similar results in their solubility and solution stability properties, apart from a small difference in their dissolution rates. Likewise, it is possible to obtain similar results with a previous solution of sodium saccharin and with later NHDC dissolution. However, to improve the water solubility of NHDC temporarily in combination with sodium cyclamate, the preparation of cosolubilized blends is absolutely necessary.

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Received for review February 11, 2000. Accepted October 4, 2000.

JF000186L