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Stability of neohesperidine dihydrochalcone in a lemonade system

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The stability of the intense sweetener neohesperidine dihydrochalcone (DC) has been assessed during storage of a carbonated lemonade formulation. High-performance liquid chromatographic (HPLC) analysis showed no change in neohesperidine DC levels after beverage samples had been stored for up to 1 year at room temperature in the light and in the dark, or after a 3 month storage period at 40°C. Sensory data and model system predictions corroborated the HPLC results. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Neohesperidine dihydrochalcone (neohesperidine DC) is an intense sweetener and flavour modifier first prepared more than 30 years ago by Horowitz & Gentili (1963). Neohesperidine DC is several hundred times as sweet as sucrose (Horowitz & Gentili, 1991; Borrego *et al.*, 1995) and recent developments have shown that it is most effective when used at low concentrations in combination with other intense or bulk sweeteners (Lindley *et al.*, 1991, 1993).

With the recent adoption and publication of the EU Sweeteners Directive (Anonymous, 1994), neohesperidine DC is now authorized for use in a wide range of foods and beverages.

The stability of neohesperidine DC has previously been studied in aqueous model systems (Inglett *et al.*, 1969; Crosby & Furia, 1980; Canales *et al.*, 1993). While data obtained from model systems are useful, they indicate only a general trend as they do not measure potential interactions between neohesperidine DC and common food components. Therefore, confirmation of model system data in actual food and beverage formulations is desirable in assessing the stability of new ingredients (Tomás-Barberán *et al.*, 1995).

There is no published information on the stability of neohesperidine DC during normal storage conditions of beverages. Therefore, the objective of this study was to measure the stability of neohesperidine DC in a typical lemonade soft-drink system which was stored under conditions designed to encompass the normal shelf-life of soft drinks. Stability under some extreme conditions was also measured.

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MATERIALS AND METHODS

Reagents

Commercial neohesperidine DC was from Zoster, SA (Murcia, Spain). High-performance liquid chromatography (HPLC) grade acetonitrile and acetic acid were from Merck (Darmstadt, Germany). All other reagents were analytical grade.

Beverage formulations

The composition (w/w) of the lemonade syrup used to assess the chemical stability was: citric acid, 1.560%; trisodium citrate, 0.300%; juicy lemon flavour, 0.650%; sodium benzoate (20% solution), 0.625%; aspartame, 0.195%; neohesperidine DC (0.1% solution), 13.000%; water to 100%. To produce the carbonated beverage, 1 part of syrup was mixed with 5.5 parts of carbonated water. Control samples without neohesperidine DC were also prepared. The nominal neohesperidine DC concentration in the final product was 20 mg litre^{-1}.

For sensory tests, sweetener compositions were adjusted to optimize sensory quality; neohesperidine DC concentration was decreased to 10 mg litre^{-1}, as this level avoided the liquorice aftertaste characteristic of the sweetener. Aspartame and acesulfame K levels were accordingly adjusted to 325 and 460 mg litre^{-1},

respectively. All lemonade products were placed in sterile glass bottles and capped with metal caps. The pH was 3.3.

Chemical stability tests

Storage conditions and sampling intervals are indicated in Table 1. For the accelerated kinetic study, aliquots (10 ml) of carbonated lemonade were placed in a temperature-controlled circulating polyethylene glycol 400 bath (±0.1°C) (Grant W 14) at 90°C.

In the studies conducted at room temperature and 40°C, 200 ml glass capped bottles were used. Samples at room temperature were stored both in the dark and exposed to light under uncontrolled temperature conditions (20–25°C).

Blanks without neohesperidine DC were also stored under identical conditions for use in the HPLC analysis performed at each sampling point.

In all cases, samples were removed at predetermined time intervals and frozen at −20°C until HPLC analyses were performed.

Analysis

Test and control lemonade samples (10 ml) were degassed in beakers using an ultrasonic bath for 30 min.

Neohesperidine DC was determined in each sample by HPLC. Analysis was performed against an external neohesperidine DC standard (20 mg litre^{-1} dissolved in water). A Merck Hitachi liquid chromatograph, equipped with a L-6200 pump, a L-4000 UV detector, a D-2500 integrator and an AS-2000A autosampler, was used under the following conditions: Novapack RP C_{18} column (4 μm particle size, 4 mm×150 mm); flow rate 1 ml min^{-1}; column temperature 30°C; UV detection at 282 nm; mobile phase 20% acetonitrile (HPLC grade, Merck) and 80% acidified water (containing 5 ml litre^{-1} acetic acid). Injection volume was 10 μl. With this procedure neohesperidine DC eluted at about 12 min.

Qualitative sensory assessment of stored lemonade partially sweetened with neohesperidine DC

As a supplement to the analytical assessment of neohesperidine DC stability, qualitative sensory assessment of stored samples was carried out.

Table 1. Storage conditions and sampling intervals

Storage conditions	Storage time	Sampling intervals
Room temperature in the light	12 months	0, 3, 6, 9, 12 months
Room temperature in the dark	12 months	0, 3, 6, 9, 12 months
40°C in the dark	3 months	0, 1, 2, 3 months
90°C in the dark	58 h	0, 6, 9, 24, 33, 48, 58 h

Test samples were fully sweetened with either a blend of acesulfame K and neohesperidine DC, or a blend of aspartame and neohesperidine DC; blank samples were partially sweetened with the same acesulfame K or aspartame concentrations as in the test samples. The concentrations of sweeteners used in lemonade were chosen to given equi-sweet products.

Assessments were made at time 0 and after 2, 4, 8, 12, 24 weeks and 1 year of storage.

To determine the effects of storage on neohesperidine DC stability, the blank and test samples were stored at room temperature (20–25°C). At each sampling time, blank samples were spiked with the appropriate amount of neohesperidine DC to yield a final concentration of 10 mg litre^{-1}. Sweetness intensity, sweetness quality and any flavour changes in the two samples were compared by a small panel ($n = 5$) of experienced tasters. Panel sensitivity was such that a 5% loss of neohesperidine DC could be detected.

RESULTS AND DISCUSSION

A linear correlation ($r = 0999$) between neohesperidine DC concentration and detector response was observed in the studied range (1–30 mg litre^{-1}). The HPLC method allowed the quantitation of neohesperidine DC, without any analytical interference, throughout the whole storage period.

The results from the lemonade storage test at room temperature in the dark and in the light are reported in Table 2. The neohesperidine DC concentrations represent mean values from replicate analyses (two samples at each point and three injections per sample).

No loss of neohesperidine DC was found after a 1 year storage period. In addition, exposure to light had no influence on neohesperidine DC stability in this particular system.

Similarly, on storage of samples at 40°C for up to 3 months, there was so significant change in neohesperidine DC concentration (19.3 ± 0.85 mg litre^{-1}).

Stability of neohesperidine DC in lemonade has also been examined under the more drastic conditions of prolonged storage at 90°C. The hydrolysis may be

Table 2. Recovery of neohesperidine DC in lemonade stored at room temperature

Time (months)	Neohesperidine DC (mg litre^{-1})	
	Light	Dark
0	19.5	19.5
3	18.3	19.4
6	17.9	20.4
9	18.4	19.9
12	19.3	19.8

Original concentration of neohesperidine DC, 20 mg litre^{-1}; all samples ± 0.85 mg litre^{-1} at 95% confidence level.

Table 3. Predicted and actual concentrations (%) of neohesperidine DC remaining after storage at different temperatures

	Data in lemonade	Model prediction[a]
Neohesperidine DC remaining (%) after:		
1 year at 20–25°C	98.9	94.8–97.0[b] 95.9–97.2[c]
3 months at 40°C	105.5	94.5–96.8[d]
Half-life at 90°C (days)	9.1	9.9[e]

[a]Data taken from Canales *et al.* (1993).
[b]Extrapolated values at pH 3; lower limit at 20°C, upper limit at 25°C.
[c]Extrapolated values at pH 4; lower limit at 20°C, upper limit at 25°C.

represented as a pseudo-first-order reaction (data not shown) in which a logarithmic plot of percentage remaining neohesperidine DC versus time is a straight line with slope rate constant (K_{obs}) (95% confidence level) of 0.00318 ± 0.000413 h^{-1} ($t_{1/2} = 9.1$ days).

The stability of neohesperidine DC in an aqueous model system (pH 1–7; temperature, 30–60°C) has been reported (Canales *et al.*, 1993). These data may be used to estimate losses during processing and storage of water-based foods containing neohesperidine DC. The results obtained in the present work correlate well with prediction of stability at pH 3 and different temperatures (Table 3).

Although data at 90°C have no relevance to industrial situations, they demonstrate the consistency of the results of neohesperidine DC behaviour in the beverage studied and the model system used.

Sensory analysis confirmed the HPLC results, that there were no detectable differences in sweetness quality, intensity or flavour profiles between the test samples or spiked blank at any sampling occasion throughout the complete storage test period.

CONCLUSIONS

Long-term stability is an important factor for the use of neohesperidine DC in soft-drinks. HPLC analysis showed that there was no change in neohesperidine DC level in a lemonade system under storage conditions relevant for soft-drinks.

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