ELSEVIER

Food Chemistry 52 (1995) 263–265

0308-8146/95/$9.50

Stability of the intense sweetener neohesperidine dihydrochalcone in blackcurrant jams

F. A. Tomás-Barberán,[a] F. Borrego,[b]* F. Ferreres[a] & M. G. Lindley[c]

[a]*CEBAS (CSIC), PO Box 4195, Murcia, 30800, Spain*

[b]*Zoster, S. A. (Grupo Ferrer), Raiguero 143, 30588 Zeneta, Murcia, Spain*

[c]*Lintech, Reading University Innovation Centre, PO Box 68, Whiteknights, Reading RG6 2BX, UK*

(Received 23 March 1994; accepted 9 May 1994)

An HPLC technique has been used to quantitate neohesperidine DC in blackcurrant jams and evaluate stability of this sweetener over manufacture and storage of jams. Results showed non-significant degradation (a) under the temperature conditions prevailing during the manufacturing process and (b) after 18 months storage at room temperature. Sensory data and model system extrapolations confirmed HPLC results.

INTRODUCTION

Neohesperidine dihydrochalcone (neohesperidine DC) is an intense sweetener and flavour modifier first prepared in 1963 (Horowitz & Gentili, 1963). The Scientific Committee for Foods of the European Community determined in 1987 that neohesperidine DC was safe for human consumption and allocated it an ADI of 5 mg/kg/bw (Bär *et al.*, 1990). Currently, the sweetener is included in the EC Proposal for a Council Directive on Sweeteners, where its use in a wide range of foodstuffs is considered (EC, 1992).

Stability of food ingredients under normal conditions of use and storage is a critical determinant of suitability for any particular product application. However, in spite of its relatively old history, current information is limited to a number of studies in aqueous buffered solutions (Inglett *et al.*, 1969; Crosby & Furia, 1980; Canales *et al.*, 1993). While assessment of sweetener ingredient stability in controlled buffer solutions is a useful way to predict whether it would be suitable for a specific application, it is generally accepted that data obtained in such simplified systems indicate general trends only and do not consider potential interactions between a sweetener, such as neohesperidine DC, and other typical compounds in foods (Canales *et al.*, 1993). Confirmation of model system results in real complex foods is then a desirable practice in assessing the stability properties of new ingredients. The results of a series of such evaluations concerning neohesperidine DC stability are presented in this paper.

*To whom correspondence should be addressed.

In the analysis of fruit jam components, one of the main problems arises during the extraction of the compounds to be analysed, since the high amount of sugars and pectins and the complexity of this type of food create process and analytical problems when classical extraction with organic solvents is attempted (Tomás-Barberán *et al.*, 1992). Recently the use of the non-ionic polymeric resin Amberlite XAD has been described for the extraction of flavonoids and other phenolic compounds from fruit jams (Tomás-Lorente *et al.*, 1992) and from honey (Ferreres *et al.*, 1991). This technique has been used to quantitate the sweet flavonoid neohesperidine DC, in blackcurrant jam, and this method has been applied to the evaluation of stability of this ingredient over manufacture and storage of jams during 18 months.

MATERIALS AND METHODS

Preparation of blackcurrant jams with neohesperidine DC

The composition of jams was the following (% w/w): blackcurrants, 25; sorbitol, 45; pectin solution (5%), 2·25; water, 27·709; acesulfame K, 0·040; neohesperidine DC, 0·001. For preparation of the jam all the ingredients — except the pectin — were placed in a saucepan, and the mixture was boiled to 65° Brix for 35–40 min (temperature of jam at 65° Brix was 106°C). Pectin was added and homogenized. The pH of the final product was 3·08.

The final product was packed in sterile jars and stored at ambient temperature for 18 months. Samples were taken at 0, 3, 6 and 18 months and stored at –20°C until analysis was performed.

Sensory stability of blackcurrant jams containing neohesperidine DC

Test samples were fully sweetened with a blend of acesulfame K and neohesperidine DC, while blank samples were partially sweetened with the same acesulfame K level as found in the test.

To determine the effects of storage on the stability of neohesperidine DC, the blank and test samples were stored at ambient temperature. At each sampling time, the blank was spiked with the appropriate amount of neohesperidine DC (to yield a final concentration of 10 ppm) and the sweetness intensity and quality and any flavour changes in the two samples were compared by a panel of tasters.

Extraction of neohesperidine DC

A 10 g jam sample was suspended in 100 ml of 80% methanol, extracted during 60 min, and vacuum-filtered through filter paper. The clear alcoholic layer was transferred to a volumetric flask, and the residual material was similarly extracted with another 100 ml of 80% methanol. The combined extracts were vacuum-concentrated (40°C) until complete methanol removal. The resulting aqueous solution was diluted with distilled water to 100 ml. This solution was mixed with the amount of the non-ionic polymeric resin Amberlite XAD-2 (Sigma) necessary to fill a column of 25 × 2 cm, and stirred for 30 min to allow the adsorption of the extracted neohesperidine DC on the resin particles (Tomás-Barberán *et al.*, 1992).

The resin suspension was poured into a column (25 × 2 cm) and washed with 400 ml distilled water. Phenolic compounds (i.e. neohesperidine DC) remained in the column while sugars, pectins and other polar compounds eluted with the aqueous solvent. Neohesperidine DC was then eluted with 400 ml of methanol. The methanol extract was concentrated under reduced pressure (40°C) and fractionated on a Sephadex LH-20 column (Pharmacia) (13 × 1 cm) elution with methanol and monitoring the elution of the different fractions under UV light (360 nm). The different fractions eluting from the column were chromatographed on HPLC to detect in which fraction neohesperidine DC was present (this was only achieved in the first analysis). The neohesperidine DC fraction was taken to dryness, redissolved in 1 ml of methanol and analysed by HPLC.

HPLC analysis of neohesperidine DC

HPLC analyses were performed using a LiChrospher 100 RP-C_{18} column (Merck, Darmstadt, Germany) (12·5 × 0·4 cm; particle size 5 μm). Twenty μl of each sample were injected in triplicate. Elution was performed with water + 5% formic acid (solvent A) and methanol (solvent B), with a solvent flow rate of 1 ml/min, starting with 20% methanol isocratically until 10 min, then installing a solvent gradient to reach 40% methanol in 40 min. Detection was carried out with a diode array detector and the chromatograms were recorded at 285 nm.

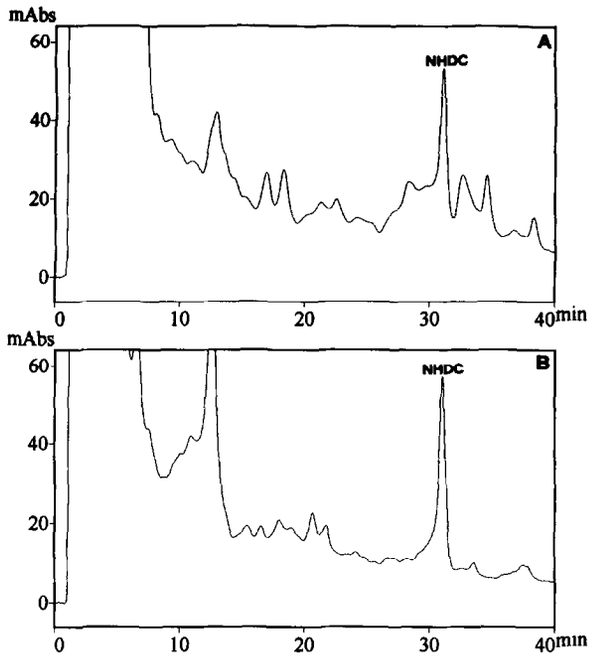

Fig. 1. Extract chromatograms after (A) filtration through Amberlite XAD-2 and elution of phenolic compounds with methanol and (B) fractionation on Sephadex LH-20. NHDC: Neohesperidine DC.

Quantitation of neohesperidine DC in blackcurrant jams

Quantitation of neohesperidine DC in test samples was performed by the method of area under the neohesperidine DC peak in the test sample relative to the neohesperidine DC peak in the blank samples submitted to the same extraction procedure. Blank samples of blackcurrant jams containing 10·0 ppm of neohesperidine DC were prepared in triplicate. The error associated with this method was estimated to be *c.* ± 10%.

RESULTS AND DISCUSSION

Adsorption of neohesperidine DC onto Amberlite XAD and subsequent fractionation on Sephadex were judged to be essential steps for successful quantitation of neohesperidine DC in blackcurrant jam. Figure 1 shows chromatograms of jam extracts after (a) Amberlite adsorption and (b) Sephadex fractionation. As can be seen, neohesperidine DC could not be accurately

Table 1. Stability of neohesperidine DC in stored jam

Storage time	Neohesperidine DC (ppm)
Zero time	10·0 ± 0·42
3 months	9·8 ± 0·47
6 months	9·2 ± 0·77
18 months	8·9 ± 0·55

Table 2. Predicted and experimental neohesperidine DC remaining (%) after processing and storage of blackcurrant jam

	Experimental	Extrapolated
Processing		
(105°C, 35 min)	100	99·7
Storage		
(25°C, 18 months)	89	92·4

quantitated in a jam extract without a previous selective separation of phenolic compounds.

The results from the jam storage trial are summarized in Table 1. Neohesperidine DC concentrations represent mean values from replicate analyses (two samples at each point and three HPLC injections per sample).

No neohesperidine DC decomposition was noted under the temperature conditions prevailing during the fruit jam manufacturing process (102–106°C during 35–40 min), as judged by the identical values of nominal concentration included in the formulation and the neohesperidine DC concentration in jam, after processing, at storage time zero. A statistically non-significant degradation of 11% of the initial neohesperidine DC was observed after 18 months storage at room temperature.

The stability of neohesperidine DC in an aqueous model system (pH: 1–7, temperature: 30–60°C) has been previously studied (Canales *et al.*, 1993). Such data could then be used to estimate losses during processing and storage of water-based foods containing neohesperidine DC. Results obtained in the present work correlate well with model predictions for processing at 105°C for 35 min and storage during 18 months at 25°C (Table 2). This clearly indicates that potential interactions between neohesperidine DC and other jam ingredients have no impact on the stability of this sweetener.

There were no detectable differences in sweetness quality, intensity or flavour profiles between the test sample or spiked blank on each sampling occasion throughout the whole storage test period.

CONCLUSIONS

Long-term and heat stability are important factors for the use of intense sweeteners in foods and beverages. The HPLC results showed a slight, non-significant change in the neohesperidine DC level. These results were corroborated by the sensory data demonstrating that there had been no loss of sweetness nor any interaction with other sample ingredients during storage. The fact that no loss of neohesperidine DC was detected during processing at high temperatures indicates its potential in fruit jams.

REFERENCES

Bär, A., Borrego, F., Benavente, O., Castillo, J. & del Rio, J. A. (1990). Neohesperidin dihydrochalcone: properties and applications. *Lebens. Wiss. Technol.*, **23**, 371–6.

Canales, I., Borrego, F. & Lindley, M. G. (1993). Neohesperidin dihydrochalcone stability in aqueous buffer solutions. *J. Food. Sci.*, **58**, 589–91, 643.

Crosby, G. A. & Furia, T. E. (1980). New sweeteners. In *CRC Handbook on Food Additives*, ed. T. E. Furia. CRC Press, Boca Raton, CA, p. 203.

EC (1992). Proposal for a council directive on sweeteners for use in foodstuffs. *Official J. European Communities* (13.8.92). No C 206/3-11.

Ferreres, F., Tomás-Barberán, F. A., Gil, M. I. & Tomás-Lorente, F. (1991). An HPLC technique for flavonoid analysis in honey. *J. Sci. Food Agric.*, **56**, 49–56.

Horowitz, R. M. & Gentili, B. (1963). Dihydrochalcone derivatives and their use as sweetening agents. U.S. Patent 3,087,821.

Inglett, G. E., Krbechek, L., Dowling, B. & Wagner, R. (1969). Dihydrochalcone sweeteners: sensory and stability evaluation. *J. Food Sci.*, **34**, 101–3.

Tomás-Barberán, F. A., Blázquez, M. A., García-Viguera, C., Ferreres, F. & Tomás-Lorente, F. A. (1992). Comparative study of different Amberlite-XAD resins in flavonoid analysis. *Phytochem. Anal.*, **3**, 178–81.

Tomás-Lorente, F., García-Viguera, C., Ferreres, F. & Tomás-Barberán, F. A. (1992). Phenolic compounds analysis in the determination of fruit jam genuineness. *J. Agric. Food Chem.*, **40**, 1800–4.