Food Chemistry **45** (1992) 283–287
Analytical Methods Section

Analysis of organic acids in potato wastewater

Peter J. W. Rovers & Teresa Kasprzycka-Guttman*

University of Warsaw, Department of Chemistry, 02–093 Warsaw, ul. Pasteura 1, Poland

(Received 12 July 1991; revised version received and accepted 9 September 1991)

Quantitative and qualitative analyses of organic acids, especially citric acid, in potatoes are reported. It appears that potato juice could be used as a source for the recovery of citric acid. This raw material is fully renewable, while being a troublesome waste in the production of glue and potato flour.

INTRODUCTION

Wastewater from potato processing industries contains several carboxylic acids, such as citric, malic, fumaric and oxalic acid. Citric acid would be expected to be the predominant acid among these and, from a commercial point of view, to be a likely candidate for separation from such wastewaters.

Within the framework of a programme aiming to improve the recovery of citric acid from aqueous solutions, including fermentation broths and potato wastewaters, a qualitative and quantitative method of analysis is currently being developed. This research is needed in order to monitor the effectiveness of the sequential purification steps of citric acid, as well as to determine and eventually to eliminate impurities caused by other acids.

Hitherto, in the literature, no method has been presented for the simultaneous determination of citric acid and allied Krebs-cycle acids in any type of wastewater. However, a considerable number of papers report methods of analysing those acids in potatoes, fruits and their juices, and dairy products. All such general research works in this field are considered here, and the advantages and disadvantages of each of the analytical procedures are discussed.

ORGANIC ACIDS IN POTATOES

The first workers to report the presence of non-volatile organic acids in potatoes used a method of distillation based on their ethyl esters (Curl & Nelson, 1940). In this way they isolated citric and L-malic acids as the major acids and isocitric and oxalic acids in trace amounts.

* To whom correspondence should be addressed.

Food Chemistry 0308-8146/92/$05.00

Secondly, ion-exchange column chromatography in combination with paper chromatography indicated citric, malic and oxalic acids, as well as glutamic, pyroglutamic (possibly an artifact) and aspartic acids (Schwartz *et al.*, 1962). Citric acid was quantified by spectrophotometry after extraction with trichloroacetic acid (Hughes *et al.*, 1962).

Then, in the cell juice of different varieties of Belorussian potatoes, the acid constituents were reported to be, in increasing order of magnitude, malic, oxalic and citric acids (Vecher & Masny, 1965). By employing an identical ion-exchange column, as done previously, the same acids of potato extracts were determined by subsequent titration of the eluates (Schwartz *et al.*, 1968). In a study of the changes in the content of organic acids during storage of potatoes, ion-exchange column chromatography was also applied (Sweeney *et al.*, 1969). The major components investigated were malic and citric acids.

Later, after extraction from potatoes, non-volatile organic acids were analysed by paper chromatography and gas chromatography in conjunction with mass spectrometry (Jadhav & Andrew, 1977). The gas-chromatographic procedure involved the preparation of trimethylsilyl (TMS) derivatives (see also, for more information on TMS derivatization, Fernandez-Flores *et al.*, 1970; Englmair, 1980), and showed that citric and malic acids accounted for more than 90% of the total acidity. Another investigation followed the changes in malic and citric content of tubers during growth and storage (Shekhar & Iritani, 1979). After extracting the material with ethanol, concentrations of citric and malic acids were determined by enzymatic analysis. They showed that the period when malic acid was observed to be low coincided with the optimum time of harvest.

Trimethylsilylation also formed the basis of a gas-chromatographic analysis of citrate and malate contents of stored tubers (Sherman & Ewing, 1982).

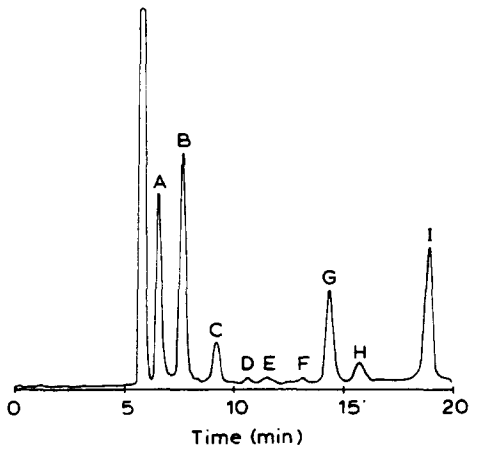

Fig. 1. A typical HPLC chromatogram of the separation of potato organic acids from Cobbler tubers: oxalic (A), citric (B), malic (C), unidentified (D), succinic (E), unidentified (F), unidentified (G), fumaric (H) and pyroglutamic (I) acids.

Later, a high-performance liquid chromatographic (HPLC) method was reported to quantify oxalic, fumaric, malic and citric acids (Bushway *et al.*, 1984). The authors claimed that the earlier procedures were time-consuming, non-specific or insensitive. They quantified the acids using ion-exclusion HPLC, which does not require a lengthy ion-exchange clean-up step. Besides the major acids, they showed the occurrence of succinic, lactic, pyroglutamic, tartaric, aconitic, quinic, and glutamic acids.

A typical HPLC chromatogram depicting the separation of potato organic acids is shown in Fig. 1. Of nine chromatographic peaks, six were identified as succinic, oxalic, citric, malic, fumaric and pyroglutamic acids. All organic acids were identified by comparing retention times with standards. This technique was developed by Yost *et al.* (1977) and is a widely used LC peak identification procedure. This method was used to determine the quantity of oxalic, citric, malic and fumaric acids in nine different potato varieties (Table 1). Citric and malic acids, as others have observed (Jadhav & Andrew, 1977), were present in the highest concentration in the tubers.

Another study was carried out on nutrient variation of Australian potatoes (Wills *et al.*, 1984). Applying HPLC too, this study revealed that citric acid comprised 90% of total organic acids. Malic acid was the other major acid, while oxalic acid could also be detected in trace amounts. In a short communication, the relationship between the contents of citric acid and after-cooking darkening was investigated (Lynch & Kaldy, 1985) by enzymatic analysis.

Finally, the effects of the physiological age of tubers on growth and yield have been studied using malic and citric acids as indicators (Reust & Aerny, 1985). After extraction on a strongly basic anion-exchange resin and elution, the methyl esters of the acids were prepared by reaction with diazomethane and analysed by gas–liquid chromatography (GLC).

In summary, it can be concluded that a wide variety of methods have been applied to analyse organic acids in potatoes: ion-exchange column chromatography, thin-layer and paper chromatography, spectrophotometry, titrimetric and enzymatic analysis, ion-exclusion HPLC, GLC and mass spectrometry.

The content of citric acid mentioned in the articles above—including potatoes from America, Russia, Europe and Australia—averages 0·35 ± 0·05 g per 100 g on a fresh weight basis. The content of malic acid is approximately 10–20% that of citric acid; oxalic acid is next in importance.

FUNDAMENTAL AND APPLIED RESEARCH ON ANALYSIS OF ORGANIC ACIDS

Developments clearly indicate greater use of GLC and HPLC than other methods of analysis (AOAC, 1980). Also, in investigations on the presence of non-volatile organic acids in food products, fruit and its juices, vegetables, dairy products and roots, this greater use of either GLC or HPLC is evident.

The remainder of this paper, therefore, focuses on the determination of organic acids by GLC and HPLC.

Determination of organic acids by GLC

Since the Krebs-cycle acids are not sufficiently volatile for direct gas-chromatographic analysis, the prepara-

Table 1. Content of organic acids in several potato varieties[a]

Variety	Oxalic acid	Citric acid	Malic acid	Fumaric acid
Allagash Russet	37·5	414·2	68·4	1·2
Atlantic	33·8	435·9	86·1	1·3
Monona	26·1	438·4	62·3	1·2
Red Pontiac	30·3	503·7	33·3	0·4
Russet Burbank	25·7	374·5	108·7	0·4
Norchip	35·6	570·5	107·8	1·8
Superior	27·7	274·4	29·2	0·8
Green Mountain	28·3	506·6	68·1	0·6
Cobbler	22·9	348·6	25·2	0·5

[a] Milligrams per 100 g of tuber (wet weight).

tion of suitable volatile derivatives is required. This is usually achieved by esterification (i.e. alkylation) or etherification (silylation). The former procedure concerns the conversion of carboxyl groups to alkyl esters, while the latter involves the conversion of both carboxyl groups to TMS esters and hydroxyl groups to TMS ethers. The esterifying agents commonly used were diazomethane/ether or boron trifluoride/methanol for methyl esters and trimethychlorosilane (TMCS) and hexamethyldisilazane (HMDS) in pyridine for TMS derivatives. The use of these and other derivatives, e.g. propyl esters and butyl esters, for their GLC determination, has been extensively demonstrated in a wide variety of samples (Turkelson & Richards, 1978).

Although diazomethane proved to be an effective esterifying agent, it is not discussed here. Due to its high reactivity and toxicity, it cannot be applied without meeting strict safety requirements (Fabig *et al.*, 1989).

Despite the fact that organic acids in peaches have been studied extensively, reports of their concentrations have often been contradictory (Li & Woodroof, 1968). With the advent of GLC, contradictions concerning the identification of Krebs-cycle acids in any type of fruits could be avoided. Until then, esterification for analysis had been scarcely reported, but methods for converting acids to their methyl esters, by boron-fluoride/methanol reagent, were developed.

For the last 20 years, a shift has been observed towards the use of higher alcohols, especially propanol and butanol, as esterifying agents, using a strong acid which serves as catalyst (sulphuric acid or hydrochloric acid). Simultaneous analysis of a mixture of volatile and non-volatile organic acids as their butyl esters gave good separations (Saito *et al.*, 1984). The increasing use of butyl esters and propyl esters can be explained by their lower volatility (Molnár-Perl & Morvai, 1987).

For the evaluation of the esterification yield of aliphatic hydroxy acids in aqueous media, their *n*-propyl esters with free hydroxy groups were measured (Fig. 2).

For the simultaneous determination of D- and L-malic acids in apple juice, esterification was achieved with butanol/hydrochloric acid as the derivatizing agent under anhydrous conditions (Agarwal, 1988). Results obtained by GLC and enzymatic analysis were in close agreement.

Most of the procedures described consider reaction to take place under strictly anhydrous conditions, though many practical samples contain water. Therefore, in order to avoid time-consuming separation processes, direct esterification in aqueous solutions is attempted using butanol with concentrated sulphuric acid (Molnár-Perl & Pintér-Szakács, 1986). During derivatization the addition of anhydrous sodium sulphate allows reaction to be almost complete in the presence of a reasonable water quantity. However, in retrospect no data are available on the application to samples other than model solutions, except for citrus

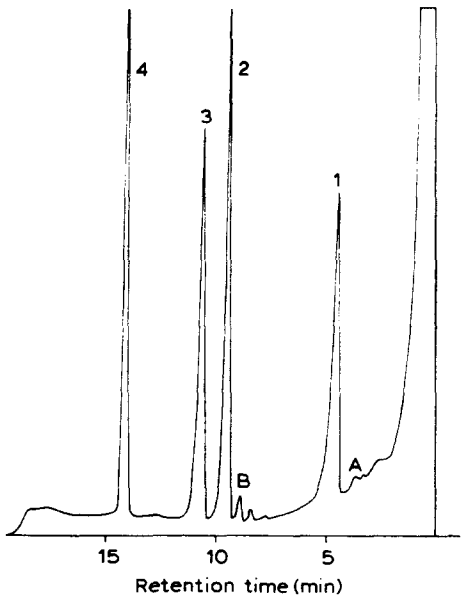

Fig. 2. Chromatogram of the mixture of *n*-propyl esters of 1,lactic, 2,tartronic, 3,malic and 4,citric acids having free (underivatized) hydroxy group. Peaks A and B correspond to dipropyl ether and monoproply sulfate, respectively.

fruit juice examined by the same institute (Perl-Molnár *et al.*, 1988).

A wide range of silylating agents is used for the preparation of TMS derivatives of organic acids. A major disadvantage of this method is the chemical instability and, hence, the decomposition of the silylated acids, resulting in multiple peaks (Englmair, 1980). Some of the problems were resolved through modification of the typical procedures.

At the initial stage the separation of acids as their TMS esters by GLC involved treatment with TMCS and HMDS in pyridine, causing instant silylation of both carboxylic and hydroxyl groups (Horii *et al.*, 1965). This basic report was shortly followed by a frequently cited article considering the determination in various fruits and fruit products (Fernandez-Flores *et al.*, 1970). Making use of the same silylating reagent mixture, a wider range of organic acids in fruits could be identified and quantified than by GLC of the corresponding methyl esters (Heatherbell, 1974).

A fundamental study on the kinetics of silylation revealed that acetone is advantageous over other solvents such as pyridine (Englmair, 1980). *N,O*-Bis(TMS)-acetamide was preferred as the silylating agent, while butylmalonic acid served as an internal standard, compensating for variable reaction conditions and decomposition of substances.

Later, other workers used a mixture of HMDS and *N,O*-bis(TMS)-trifluoroacetamide for the general detection of metabolites of the Krebs cycle in microgram amounts (Förster *et al.*, 1986). Yet another report described a modified procedure based on previous research for the chemical characterization of citrus

oil-mill effluent (Parish *et al.*, 1986). An analytical technique has been developed to determine the non-volatile acids in fruit and sweet-potato extracts (Chapman & Horvat, 1989). The organic acids were converted by *N,O*-bis(TMS)-trifluoroacetamide and TMCS reagents, and identification was obtained by GLC and mass spectrometry. The quantitative results agreed well with previous values in the literature.

Finally, the results obtained by silylation and alkylation are combined in a critical publication on the GLC separation of organic acids in VA Mycorrhizal roots (Fabig *et al.*, 1989). Several (but not all) silylating and alkylating agents were tested, as well as five columns of different polarity, including packed and capillary columns. All procedures showed overlapping peaks in a mixture comprising 34 acids. Evidently, improvements are still needed in the GLC separation of Krebs-cycle acids by any of the derivatization procedures described, but some of the procedures would probably meet the demands. Despite the disadvantages of TMS derivation, gradually more scientists have committed themselves to this method of derivatization, e.g. for organic acids in apples (Morvai & Molnár-Perl, 1989).

Determination of organic acids by HPLC

With the use of HPLC, a lengthy esterification step is not necessary. The most important issue is the selection of the proper column and liquid phase.

Four main methods in HPLC are commonly used: ion-exchange, ion-exclusion, ion-pair and reversed-phase chromatography. The choice of method depends on the nature of the organic acids to be analysed and on the matrix in which they are present, as well as their relative concentration in the sample.

For the determination of organic acids in dairy products, the column contained a strong cation-exchange resin, Aminex HPX-87 (Biorad Laboratories), and a mobile phase of 0·0090 M sulphuric acid (Marsili *et al.*, 1981).

Organic acids in guava (*Psidium guajava* L.) cultivars have been separated on a 10-cm radial compression C_{18} column (Wilson *et al.*, 1982). The mobile phase contained 2% aqueous ammonium phosphate.

An ion-exclusion HPLC method has been presented for the major organic acids in potatoes (Bushway *et al.*, 1984). Tuber extracts were injected on an Aminex HPX-87 column with a mobile phase of 0·018 M sulphuric acid.

Subsequently, in sweet potatoes, determination on an Aminex HPX-87H column was also applied with 0·0008 M sulphuric acid as mobile phase (Picha, 1985).

Organic acids in apple extracts were analysed on a column packed with octadecyl silica (C_{18}) using a phosphate buffer as an eluent (Blanco Gomis *et al.*, 1988).

An HPLC glass column with diethylaminoethyl-Si 200 Polyol packing was applied with 0·28 M ammonium sulphate for the general analysis of organic acids (Gey, 1988).

Finally, again for analysis of dairy products, a reversed-phase Beckman C8 ultrasphere octyl column was used (Bevilacqua & Califano, 1989). The mobile phase consisted of a phosphate buffer with acetonitrile.

The principle of ion-exclusion HPLC has some significant features to recommend it above other HPLC methods, namely that no elaborate sample preparation is needed and that detection limits of less than 15 ppm for citric acid can be achieved (Turkelson & Richards, 1978). The use of dilute mineral acid as eluent facilitates separation of strong to moderately strong organic acids, which could not be clearly separated by water alone.

DISCUSSION AND CONCLUSION

Many analytical methods are reported in the literature for the separation of organic acids, and a number of these have been devoted to the determination of acids of the Krebs cycle. Omitting non-specific and less sensitive ones, these methods involve either tedious extraction and concentration procedures, to increase detection sensitivity in liquid chromatography, or time-consuming extraction and derivatization, to enhance volatilization for gas chromatography. However, through experience and modification, these disadvantages have been partly abolished. Consequently, simultaneous qualitative/quantitative analysis of Krebs-cycle acids in practical samples is made possible by HPLC as well as by GLC.

For the present purpose, samples from potato wastewaters and others are assumed to be best compared with fruit juices. Both are aqueous solutions with similar (citric) acid concentrations. Therefore, the following is proposed:

(1) ion-exclusion HPLC; or
(2) GLC as butyl esters or as TMS esters.

With reference to GLC analysis, it should be mentioned that a clear separation of acids present in potato juice is possible by both esterification techniques, on condition that the proper gas-chromatographic conditions are selected. Citric acid can also be quantified after choosing an appropriate internal standard for compensating fluctuation in operating conditions and taking into account the possible decomposition of esters.

In spite of the fact that GLC is more laborious than HPLC, both techniques are approximately equally sensitive and accurate. Finally, the choice for any of these procedures depends upon the technological arsenal of the laboratory and the personal preference of the investigator in question.

REFERENCES

Agarwal, V. K. (1988). Simultaneous determination of D- and L-malic acids in apple juice by gas chromatography. *J. AOAC*, **71**, 466–8.

AOAC (1980). *Official Methods of Analysis* (13th edn). Association of Official Analytical Chemists, Washington, DC, USA.

Bevilacqua, A. E. & Califano, A. N. (1989). Determination of organic acids in dairy products by high performance liquid chromatography. *J. Food Sci.*, **54**, 1076–7, 1079.

Blanco Gomis, D., Morán Gutiérrez, M. J., Gutiérrez Alvarez, M. D. & Mangas Alonso, J. J. (1988). Application of HPLC to the characterization and control of individual acids in apple extracts and ciders. *Chromatographia*, **25**, 1054–8.

Bushway, R. J., Bureau, J. L. & McGann, D. F. (1984). Determinations of organic acids in potatoes by high performance liquid chromatography. *J. Food Sci.*, **49**, 75–7, 81.

Chapman Jr, G. W. & Horvat, R. J. (1989). Determination of nonvolatile acids and sugars from fruits and sweet potato extracts by capillary GLC and GLC/MS. *J. Agric. Food Chem.*, **37**, 947–50.

Curl, A. L. & Nelson, E. K. (1940). The nonvolatile acids of potatoes. *Am. Potato J.*, **17**, 328–30; *Chem. Abstr.*, **35**, (1941), 1091, No. 4.

Englmair, P. (1980). Trimethylsilyl-Ester pflanzlicher Saüren und ihre Anwendung in der Gaschromatographie. Darstellung, Kinetik der Silylierung und Einflüsse verschiedener Lösungmittel auf Ausbeute und Stabilität der Derivate. *J. Chromatogr.*, **194**, 33–42.

Fabig, B., Vielhauer, K., Moawad, A. M. & Achtnich, W. (1989). Gas-chromatographic separation of organic acids and electrophoretic determination of phosphatases from VA Mycorrhizal roots. *Z. Pflanzenerähr. Bodenk.*, **152**, 261–5.

Fernandez-Flores, E., Kline, D. A. & Johnson, A. R. (1970). GLC determination of organic acids in fruits as their trimethylsilyl derivatives. *J. AOAC*, **53**, 17–20.

Förster, H.-G., Thomas, C., Rathmann, J. & Müller, M. (1986). Die quantitative gaschromatografische Bestimmung ausgewählter Krebszylklusmetabolite im Mikrogramm-Bereich. *Z. Chem.*, **26**, 413–4.

Gey, M. (1988). Analyse organischer Säuren an HPLC-Glassäulen mit Diethylaminoethyl-Si 100 Polyol-Füllung. *Z. Chem.*, **28**, 377–8.

Heatherbell, D. A. (1974). Rapid concurrent analysis of fruit sugars and acids by gas–liquid chromatography. *J. Sci. Food Agric.*, **25**, 1095–107.

Horii, Z., Makita, M. & Tamura, Y. (1965). Gas–liquid chromatographic separation of acids of Krebs cycle as trimethylsilyl derivatives. *Chem. Ind.*, **34**, 1494.

Hughes, J. C., Ayers, J. E. & Swain, T. (1962). After-cooking blackening in potatoes. I. Introduction and analytical methods. *J. Sci. Food Agric.*, **13**, 224–9.

Jadhav, S. J. & Andrew, W. T. (1977). Effects of cultivars and fertilizers on nonvolatile organic acids in potato tubers. *Can. Inst. Food Sci. Technol. J.*, **10**, 13; *Chem. Abstr.*, **86** (1977) 138211b.

Li, K. C. & Woodroof, J. G. (1968). Gas chromatographic resolution of nonvolatile organic acids in peaches. *J. Agric. Food Chem.*, **16**, 534–5.

Lynch, D. R. & Kaldy, M. S. (1985). Citric acid and potassium contents of Russet Burbank potato in Alberta. *Can. J. Plant Sci.*, **65**, 793–5.

Marsili, R. T., Ostapenko, H., Simmons, R. E. & Green, D. E. (1981). High performance liquid chromatographic determination of organic acids in dairy products. *J. Food Sci.*, **46**, 52–7.

Molnár-Perl, I. & Morvai, M. (1987). Esterification of aliphatic hydroxy acids to *n*-propyl esters in aqueous solutions for their gas chromatographic analysis. *Chromatographia*, **23**, 760–3.

Molnár-Perl, I. & Pintér-Szakács, M. (1986). Modifications in the chemical derivatization of carboxylic acids for their gas chromatographic analysis. *J. Chromatogr.*, **365**, 171–82.

Morvai, M. & Molnár-Perl, I. (1989). Simultaneous GLC quantification of the characteristic organic acids and sugars in apples. Proceedings of the 5th European Conference on Food Chemistry, (Vol. 2), pp. 497–502; *Chem. Abstr.*, **112** (1990) 53838s.

Parish, M. E., Braddock, R. J. & Graumlich, T. R. (1986). Chemical and microbial characterization of citrus oil-mill effluent. *J. Food Sci.*, **51**, 431–3.

Perl-Molnár, I., Morvai, M. & Szakács-Pintér, M. (1988). Gas chromatographic analysis for isocitric acid in the presence of citric acid. *Magy. Kem. Foly.*, **94**, 520–3; *Chem. Abstr.*, **110** (1989) 191338p.

Picha, D. H. (1985). Organic acid determination in sweet potatoes by HPLC. *J. Agric. Food Chem.*, **33**, 743–5.

Reust, W. & Aerny, J. (1985). Determination of physiological age of potato tubers using sucrose, citric and malic acid as indicators. *Potato Res.*, **28**, 251–61.

Saito, S., Miki, T., Ito, H. & Kamoda, M. (1984). Studies on the separation of organic acid butylesters by GLC. *Bunseki Kagaku*, **33**, 209–12; *Chem. Abstr*, **101** (1984) 47898t.

Schwartz, J. H., Greenspun, R. B. & Porter, W. L. (1962). Identification and determination of the major acids of the white potato. *J. Agric. Food Chem.*, **10**, 43–6.

Schwartz, J. H., Fow, M. I., Raskin, R. L. & Porter, W. L. (1968). Chemical composition of potatoes. VI. Effect of variety and location on acid concentrations. *Am. Potato J.*, **45**, 81–92.

Shekhar, V. C. & Iritani, W. M. (1979). Changes in malic and citric acid contents during growth and storage of *Solanum tuberosum* L. *Am. Potato J.*, **56**, 87–94.

Sherman, M. & Ewing, E. E. (1982). Temperature, cyanide, and oxygen effects on the respiration, chip color, sugars, and organic acids of stored tubers. *Am. Potato J.*, **59**, 165–78.

Sweeney, J. P., Hepner, P. A. & Libeck, S. Y. (1969). Organic acid, amino acid, and ascorbic acid content of potatoes as affected by storage conditions. *Am. Potato J.*, **46**, 463–9.

Turkelson, V. T. & Richards, M. (1978). Separation of the citric acid cycle acids by liquid chromatography. *Anal. Chem.*, **50**, 1420–3.

Vecher, A. S. & Masny, M. M. (1965). Organic acids in the cell juice of different varieties of potatoes of the Belorussian SSR. *Ser. Biyal. Navuk.*, **4**, 26–30; *Chem. Abstr*, **64** (1966) 18033d.

Wills, R. B. H., Lim, J. S. K. & Greenfield, H. (1984). Variation in the nutrient composition of Australian retail potatoes over a 12-month period. *J. Sci. Food Agric.*, **35**, 1012–7.

Wilson, C. W., Shaw, P. E. & Campbell, C. W. (1982). Determination of organic acids and sugars in guava (*Psidium guajava* L.) cultivars by high-performance liquid chromatography. *J. Sci. Food Agric.*, **33**, 777–80.

Yost, R., Stoveken, J. & MacLean, W. (1977). Positive peak identification in liquid chromatography using absorbance ratioing with a variable-wavelength spectrophotometer detector. *J. Chromatogr.*, **134**, 75–8.