Chromatographic Determination of D-Amino Acids as Native Constituents of Vegetables and Fruits[a)]

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Key Words

Column liquid chromatography
Gas chromatography
Chirasil-Val
Amino acid enantiomers
N-Isobutyryl-cysteine

Summary

Free D-amino acids (D-AA) were detected as native constituents in juices of vegetables (cultivars of cabbage, tomato, carrot, garlic) and fruits (oranges, clementine, grapefruit, lemon, apples, pear, grapes) using gas chromatography (GC) or high-performance liquid chromatography (LC). For investigation by GC, AA enantiomers were converted into their *N*(*O*)-pentafluoropropionyl 2-propyl esters and resolved on a Chirasil-L-Val capillary column. For determination by LC, precolumn derivatization of AA enantiomers using *o*-phthaldialdehyde together with the chiral thiols *N*-isobutyryl-L-cysteine or *N*-isobutyryl-D-cysteine and fluorescence detection of the diastereomeric isoindole derivatives, resolvable on an octadecylsilyl stationary phase, were used. D-Ala (0.6–3.8 %) was detected in all freshly pressed plant juices usually in the highest relative amounts. Other D-AA detected were D-Asx (0.1–1.9 %), D-Glx (0–1.3 %), D-Ser (0–1.7 %), D-Arg (0.4–1.2 %, in grapes, orange, grapefruit, and clementine) and D-Leu and D-Val (1 % in cabbage). Absolute amounts of native D-AA were totally 28–57 μmol L^{-1} in fruit juices, 14.5 μmol L^{-1} in a tomato juice and 8.5 μmol L^{-1} in a carrot juice.

Introduction

Using gas chromatography (GC) with the chiral stationary phase Chirasil-Val [1], orange juices had been investigated for the presence of D-amino acids (D-AA) [2–4]. It was shown that several processed juices contained D-AA and that controlled fermentation of orange juice with *Lactobacillus plantarum*, serving as model for bacterial contamination, led to the formation of relatively high amounts of various D-AA. It was concluded that bacterial growth in fruit juices in general will lead to the formation of D-AA and that they might serve as markers for microbial contamination, together with established methods such as determination of lactic acid. This was recently confirmed by others [5].

Based on our results we have also critically discussed the use of small amounts of D-AA as markers for fruit juice adulteration. Moreover, from reports on the presence of free or derivatized D-AA in various plants, the question was addressed as to whether free or conjugated D-AAs permanently occur in fruits and lead to detectable amounts of D-AA [2].

In continuation of our gas and liquid chromatographic work on fruit juices [2–4, 6], we now present further data on the occurrence of D-AA as native constituents of fruit and vegetable juices. Furthermore, we show that precolumn derivatization of AA with *o*-phthaldialdehyde together with *N*-isobutyryl-L-cysteine (IBLC) or *N*-isobutyryl-D-cysteine (IBDC), followed by liquid chromatographic resolution of the resulting, highly fluorescent, diastereomeric isoindole derivatives on an octadecylsilyl stationary phase [3, 6] is a fast and reliable method for the detection and determination of amino acid enantiomers occurring in plant juices.

Experimental

Gas Chromatography

For the determination of D-AA in vegetable juices (cf. Table I) a Carlo Erba Series HRGC 5160 instrument

[a)] Presented in part as lecture at 3rd International Congress on Amino Acids, Peptides and Analogues, August 23–27, 1993, Vienna; and as posters at 31st Scientific Meeting of German Society of Nutrition, Giessen, March 17th and 18th, 1994 [19]; and at Analytica Conference, April 19–22, 1994, Munich [20].

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Table I. Relative amounts[1] (% D) of most abundant native D-amino acids (D-AA) in cabbage and garlic, and relative[1] and absolute[2] amounts of D-AA in pickled cabbage.

D-AA[3]	Red cabbage[4] % D	Green cabbage[5] % D	Green cabbage[5] % D (Stem)	Green cabbage[6] % D	White cabbage[7] % D	White cabbage[8] % D	Pickled cabbage[9] % D	Pickled cabbage[9] mg L^{-1}	Garlic[10] % D
D-Ala	2.5	3.8	0.8	2.6	0.8	1.7	9.4	51	1.3
D-Asx	1.5	0.9	0.9	0.4	1.6	1.3	3.7	17	0.8
D-Glx	0.3	–	0.5	0.8	0.6	0.4	11.0	35	0.5
D-Leu	–	1.0	–	–	–	–	5.2	6	0.6
D-Lys	–	–	–	–	–	–	8.7	4	–
D-Val	1.0	–	0.9	–	–	–	–	–	1.4

[1]Determined by GC on Chirasil-Val, % D = 100 · D/(D + L); [2]determined using automated amino acid analyzer and ninhydrin derivatization; [3]Asx = Asp + Asn; Glx = Glu + Gln; [4]cultivar (cv) 'Wanner'; [5]cv. 'Winterboor'; [6, 10]cv. not known; [7]cv. 'Quisto'; [8]cv. 'Filder Spitzkraut'; [9]D-AA 0.11gL^{-1}; AA totally 3.43 gL^{-1}; (–) = not detected (≤ 0.1 %) or not determinable

(Carlo Erba, Milano, Italy) with flame ionization detector and Chirasil-L-Val column (25 m × 0.25 mm I.D.; C.G.C. Analytic, Mössingen, Germany) were used. AA were isolated by cation-exchange treatment, converted into pentafluoropropionyl amino acid 2-propyl esters and analyzed as described in detail previously [2].

High-Performance Liquid Chromatography

For HPLC an HP 1090 Series L instrument was used consisting of a binary solvent delivery system, autoderivatizer, heatable column compartment and an HP 1046 programmable fluorescence detector operated at an excitation wavelength of 230 nm and emission wavelength of 445 nm. Standards and juice samples were measured at photomultiplier tube (PMT) gain 7 to 9. Data were processed using a Series HP 79994A ChemStation computer. The instruments were supplied by Hewlett-Packard GmbH (Waldbronn Analytical Division, Waldbronn, Germany). For details of the instrument and its unique derivatization device we refer to previous publications [3, 6]. Stainless-steel columns (250 × 4 mm I.D.) and guard columns (20 × 2.1 mm I.D.) (Hewlett-Packard) were used. The stationary phase was Hypersil ODS, particle size 5 µm (Shandon Scientific, Runcorn, UK). Columns packed with this stationary phase are obtainable from Hewlett-Packard GmbH, on request. Eluent A was prepared by dissolving 3.13 g (23 mmol) sodium acetate trihydrate in 990 ml doubly distilled water and adjustment to pH 5.95 by addition of 10 % (v/v) acetic acid. Eluent B consisted of 474 g methanol and 39 g acetonitrile. Helium was passed through the eluents. A linear gradient was applied for 75 min at a flow rate 1 ml min^{-1} from 0 to 53.5 % B and then equilibrated with 100 % A for 10 min.

The derivatization procedure for amino acids was as follows: 260 mM IBLC (or IBDC) and 170 mM OPA in 1 M potassium borate buffer, pH 10.4 (Pierce, Rockford, IL, USA) were used as derivatizing reagents. For automated derivatization, amounts of 5 µl 0.4 M sodium borate buffer, pH 10.4 (Hewlett-Packard), 1 µl OPA/IBLC (or IBDC) reagent and 2 µl aliquots of juices or samples to be analyzed were drawn up and mixed in the derivatization device of the instrument [3]. For calibration a standard mixture containing 100 pmol L-AA and 5 pmol D-AA in 2 µl 0.1 M HCl was injected.

Reagents and Chemicals

IBLC and IBDC are obtainable from Calbiochem-Novabiochem, (Läufelfingen, Switzerland) from Calbiochem Comp., (La Jolla, CA, USA) and from Fluka AG, (Buchs, Switzerland) and their subsidiaries. The reagents we used had an optical purity of 99.91 ± 0.01 % (IBLC) and 99.78 ± 0.02 % (IBDC). Chemicals and solvents were of analytical or chromatographic grade from Merck (Darmstadt, Germany). Amino acids used for the standard were from Sigma, (St. Louis, MO, USA) and Fluka and were tested for their optical purity by HPLC or GC analogous to the procedures described in this paper; L-*homo*-arginine (L-*homo*-Arg) was from Serva, (Heidelberg, Germany).

Sources and Treatment of Fruits, Vegetables and Processed Juices

Investigated were freshly harvested, perfect apples [*Malus sylvestris* var. *domestica* (Borkh.) Mans.] (for names of cultivars of fruits and vegetables see Table I and Table II, for Latin names see [21]), pear (*Pyrus communis* L.), orange (*Citrus sinensis* (L.) Pers.), clementine (*Citrus reticulata* Blanco), grapefruit (*Citrus* × *paradisi* Macf.), lemon (*Citrus medica* L. var. *limon* L.), grapes used for wine making (*Vitis vinifera* L.), carrots (*Daucus carota* var. *sativus* L.), and tomato (*Lycopersicon lycopersicum* Karst.) The cabbages investigated were red cabbage (*Brassica oleracea* L. convar. *capitata* var. *capitata* f. *rubra* DC.), green cabbage (*Brassica oleracea* L. convar. *acephala* var. *sabellica* DC.), white cabbage (*Brassica oleracea* L. convar. *capitata* var. *capitata* f. *alba* DC.), Wirsing (*Brassica oleracea* L. convar. *capitata* var. *sabauda* DC.). Cabbages were grown on farms close to the University campus; garlic (*Allium sativum* L.) was purchased in a grocery.

Table II. Relative amounts [% D = 100 · D/(D + L)] and absolute amounts ($\mu mol L^{-1}$) of native D-amino acids (D-AA) in fruits and vegetables determined by derivatization with OPA-IBLC and HPLC; for IBDC see text.

D-AA[1]	Apple[2]		Apple[3]		Apple[4]		Apple[5]		Apple[6]		Pear[7]	
	% D	$\mu mol L^{-1}$	% D	$\mu mol L^{-1}$	% D	$\mu mol L^{-1}$	% D	$\mu mol L^{-1}$	% D	$\mu mol L^{-1}$	% D	$\mu mol L^{-1}$
D-Asp	0.4	3.2	0.5	21.2	0.3	6.9	0.4	4.6	0.4	5.9	0.5	4.7
D-Glu	0.5	3.4	0.5	1.9	0.4	3.0	0.2	1.0	1.0	2.7	0.9	3.6
D-Asn	0.7	14.6	0.2	18.3	0.3	27.3	0.4	29.6	0.4	14.6	0.4	9.1
D-Ser	1.7	3.4	0.6	2.1	1.1	2.6	1.1	2.2	1.2	3.5	1.1	5.4
D-Gln	–	–	–	–	–	–	–	–	–	–	–	–
D-Ala	2.7	2.9	2.1	3.2	1.8	3.8	2.1	2.9	1.7	2.9	2.1	3.5
D-Arg	–	–	–	–	–	–	–	–	–	–	–	–

D-AA[1]	Grape[8]		Grape[9]		Tomato[10]		Carrot[10]		Orange[11]		Clementine[10,12]	Grapefruit[10,13]	Lemon[10,14]
	% D	$\mu mol L^{-1}$	% D	$\mu mol L^{-1}$	% D	$\mu mol L^{-1}$	% D	$\mu mol L^{-1}$	% D	$\mu mol L^{-1}$	% D	% D	% D
D-Asp	1.9	7.6	0.9	1.6	0.2	1.7	0.2	1.5	0.4	8.4	1.0	0.7	0.5
D-Glu	0.8	8.9	0.7	3.9	0.1	1.9	–	–	1.2	13.7	1.3	1.1	1.1
D-Asn	–	–	–	–	0.2	1.8	0.1	1.3	0.5	9.6	0.5	3.4	0.7
D-Ser	0.6	4.3	1.2	1.9	0.1	0.7	0.1	0.7	0.2	4.3	0.3	0.3	0.4
D-Gln	–	–	–	–	0.2	6.3	–	–	–	–	–	–	–
D-Ala	0.6	10.5	1.0	5.4	0.7	2.1	0.6	5.0	1.3	14.3	0.8	1.4	0.9
D-Arg	0.6	13.9	1.2	8.2	–	–	–	–	0.4	7.1	0.8	1.0	–

[1]The LC method distinguishes between Asp/Asn and Glu/Gln; [2]cultivar (cv.) 'Golden Delicious'; [3]cv. 'Goldparmäne'; [4]cv. 'Jonathan'; [5]cv. 'Jonagold'; [6]cv. 'Granny Smith'; [7]cv. 'Alexander Lukas'; [8]cv. 'Kerner'; [9]cv. 'Trollinger'; [10]cv. not known; [11]cv. 'navel orange'; [12–14]absolute amounts not determined; (–) = not detectable (≤ 0.1 %)

Fruits and carrots were carefully washed and brushed with water and 70 % ethanol, then peeled, sliced and subjected to an automatic juicer based on the centrifugal principle (Multipress MP 50, Type 4154, from Braun Comp. (Frankfurt, Germany). In the case of apples, the cores were removed and discarded. From blue and white cabbages inner leaves were sliced and juiced. From green cabbage, leaves and stem were washed and investigated separately. The juices were filtered using a fluted filter-paper and the filtrates were centrifuged at 1650 g. All procedures were carried out as fast as possible and 2 µl aliquots of the juices investigated immediately. Samples for repetitive analyses were stored at – 30 °C. In some cases (apple, pear, grape) 1 ml aliquots of the juices were adjusted to pH 2 by addition of 2 M HCl and 1.6 mM L-*homo*-Arg (31.3 µl) and the mixtures were subjected to Dowex 50 WX8 cation-exchange treatment (bed size 5 × 1.0 cm). AA were desorbed with 4 M aqueous ammonia as described previously [2, 7]. The effluents were evaporated to dryness, the residues dissolved in 0.1 M HCl (1 ml), and aliquots of 2 µl analysed by LC. For quantification of AA by LC 2 µl aliquots of juices were also directly derivatized with IBLC and IBDC, respectively.

Results and Discussion

The relative amounts of D-AA determined in vegetables by chiral phase GC are displaced in Table I (for chromatograms illustrating the GC determination of D-AA in foods, beverages and microorganisms we refer to previous publications [7–10]). In the case of the juice of pickled cabbage, as an example of a lactic acid fermented vegetable juice, the relative and the absolute amounts of D-AA are also given in Table I.

In the juices of freshly pressed cabbages and garlic D-Ala (0.8–3.8 %), D-Asx (0.4–1.6 %), D-Glx (0–0.8 %) and, in few cases, D-Val (0.9–1.4 %) and D-Leu (0.6–1.0 %)were determined. The amounts of D-AA drastically increased in the case of the juice of pickled cabbage in which D-Ala (9.4 %, 51 mg L^{-1}), D-Asx (3.7 %, 17 mg L^{-1}), D-Glx (11.0 %, 35 mg L^{-1}), D-Leu (5.2 %, 6 mg L^{-1}), D-Lys (8.7 %, 4 mg L^{-1}) were determined (relative and absolute amounts are given).

D and L-amino acids in freshly pressed juices of fruits, tomato and carrot were investigated by precolumn derivatization with OPA-IBLC and OPA-IBDC, respectively, and resolution by HPLC of the diastereomeric isoindole derivatives which were formed (since in all cases IBLC or IBDC has to be used together with OPA, we omit the latter in the following).

The elution profile of a standard composed of L-AA and the respective D-AA, derivatized with IBLC and IBDC, respectively, is shown in Figures 1a and 1b. As can be seen, derivatization with IBDC instead of IBLC leads to the opposite elution order of diastereomers formed from AA-enantiomers [6]. This elegant approach of reversing the elution order of amino acids just by change of reagent under otherwise constant chromatographic conditions was routinely used for establishing the presence of native D-AA in plant juices. The relative and absolute amounts of D-AA determined are displayed in Table II.

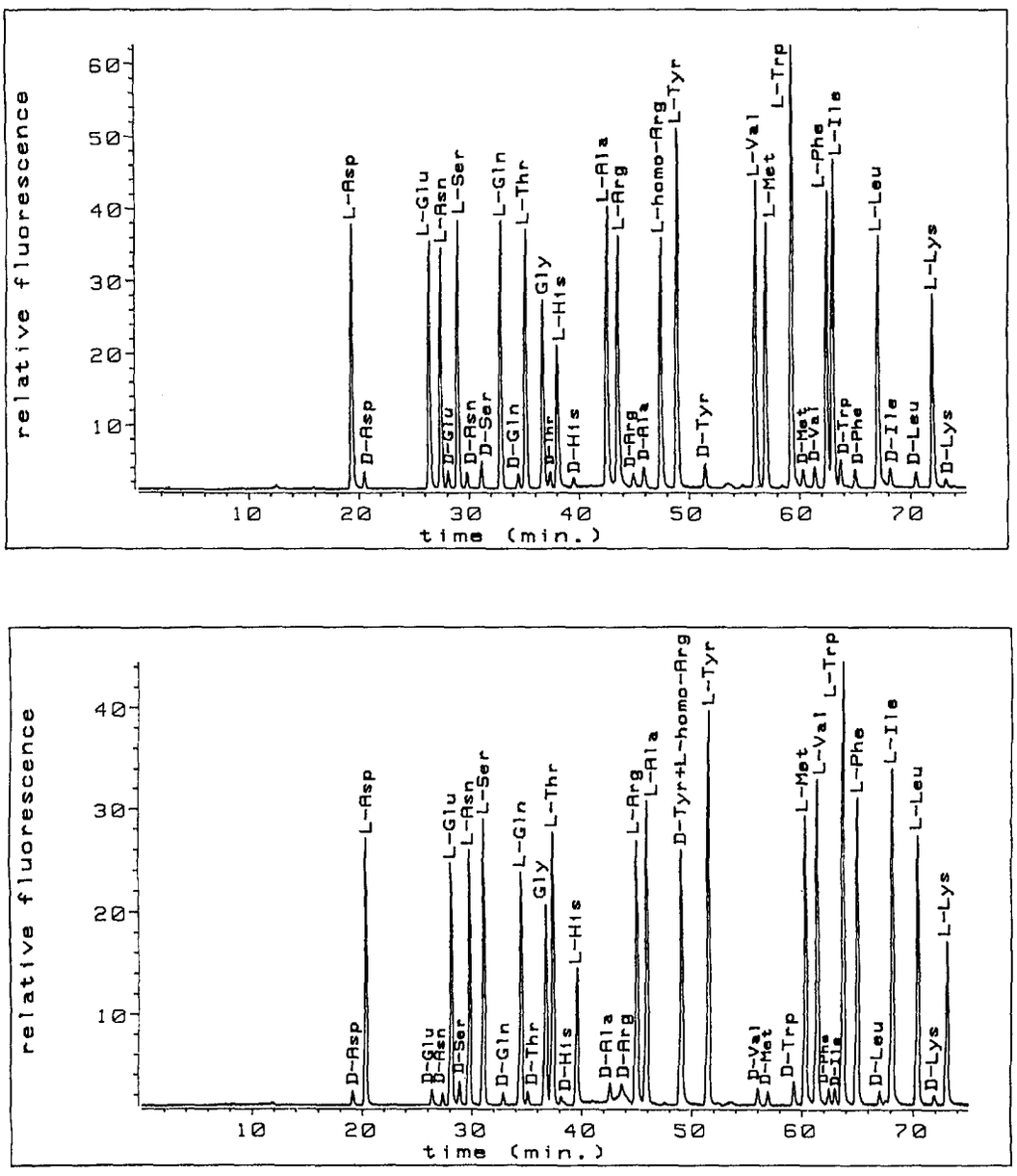

Figure 1

Elution profile of amino acid standard (100 pmol L- and 5 pmol D-enantiomer), derivatized with (a) IBLC and (b) IBDC chromatographic conditions of Figures 1–7 see Experimental).

The detection of free native D-AA in the cytoplasm of a citrus fruit is demonstrated by the elution profiles ("aminograms") of derivatized amino acids in the fresh pressed juice from navel oranges (Figures 2a, b). The aminogram of a processed orange juice from a retail outlet, which attracted attention as a result of unpleasant taste and brownish color, is shown in Figure 2c. In the freshly pressed orange juice the following amounts of D-Asp (0.4 %, 0.5 %), D-Glu (1.2 %, 0.8 %), D-Asn (0.5 %, 0.3 %), D-Ser (0.2 %, 0.2 %), D-Arg (0.4 %, 0.3 %), and D-Ala (1.3 %, 1.6 %) were determined (the first value in parentheses refers to quantitation with IBLC, the second to that with IBDC). It should be mentioned that γ-aminobutyric acid (GABA) gives rise to two derivatives in aminograms which coelute together with L-Tyr and L-Phe, respectively, by derivatization with IBLC, but not by derivatization with IBDC. In the spoiled orange juice (Figure 2c) the following amounts of D-Asp (0.6 %), D-Glu (0.5 %), D-Asn (1.7 %), D-Ser (3.9 %), D-Ala (10.2 %), and D-Arg (0.6 %) were found. The chromatograms also demonstrate that the HPLC method allows us to distinguish between the enantiomers of Asp/Asn, and Glu/Gln and to determine routinely D-Arg if present (D-His, also determinable by the method, was not detected in the juices). This is an advantage over GC using fused silica columns coated with chiral stationary phases where Asn, Gln, Arg and His are not determinable routinely. D-AA as native constituents were detected in all freshly pressed fruit juices investigated

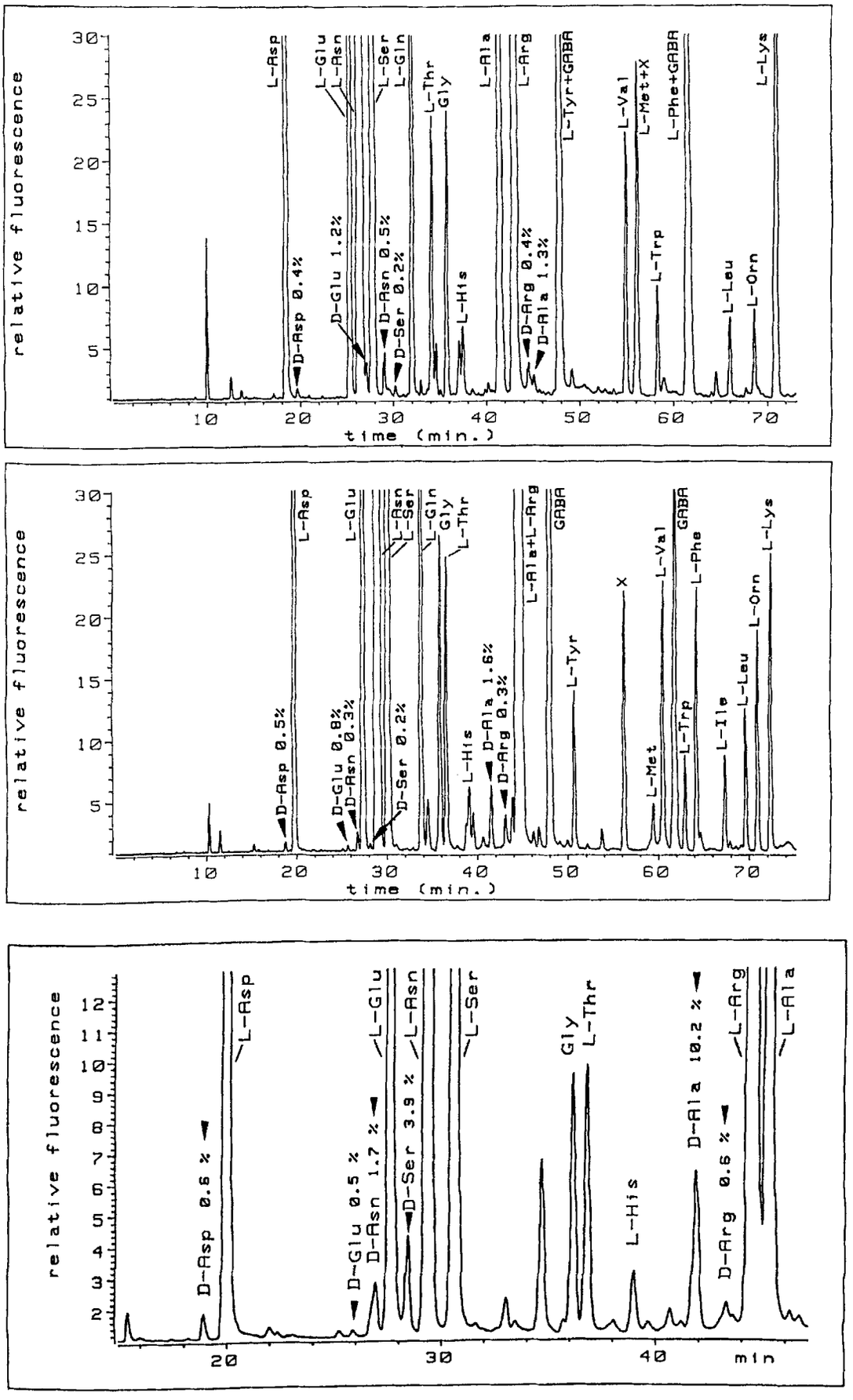

Figure 2

Elution profile of amino acids ("aminogram") from freshly pressed orange juice, derivatized directly with (a) IBLC and (b) IBDC, and (c) section of aminogram of processed orange juice with unpleasant taste and brownish color; juice directly derivatized with IBDC.

(Table II). Representative aminograms are shown for apple (Figure 3), pear (Figure 4), grape (Figure 5), tomato (Figure 6) and carrot (Figure 7).

Taking the data of Table II together, in the fruit juices the stated amounts of D-Ala (0.8–2.7 %), D-Asp (0.3–1.9 %), D-Asn (0–0.7 %), D-Ser (0.2–1.7 %) and, in several cases, D-Glu (0.2–1.0 %) and D-Arg (0.4 %–1.2 %) were detectable. In the juices of tomato and carrot, relatively lower amounts of D-AA (0.1–0.2 %) were determined, with the exception of D-Ala (0.7 % and 0.6 %, respectively). The data reveal that total amounts of 28–57 μmol L^{-1} of certain D-AA in their free form are true native constituents of the cytoplasm of the fruits investigated. Amounts of 14.5 μmol L^{-1} and 8.5 μmol L^{-1} of D-AA were determined in tomatoes and carrots, respectively, serving as examples for unprocessed vegetable beverages. Notably, no native D-AA (with the exceptions of those which were added) were detected in fruit juices investigated by others using various gas and liquid chromatographic methods [11–14]. This is attributed to the methodologies applied and the focus on the relatively high amounts of D-AA which are to be expected from adulterations of juices by addition of racemic AA. The relatively high amounts of D-Ala found in certain processed fruit juices [5] are attributed to massive bacterial contamination.

This is also obvious from the D-AA determined in the juice of pickled cabbage as an example of the control-

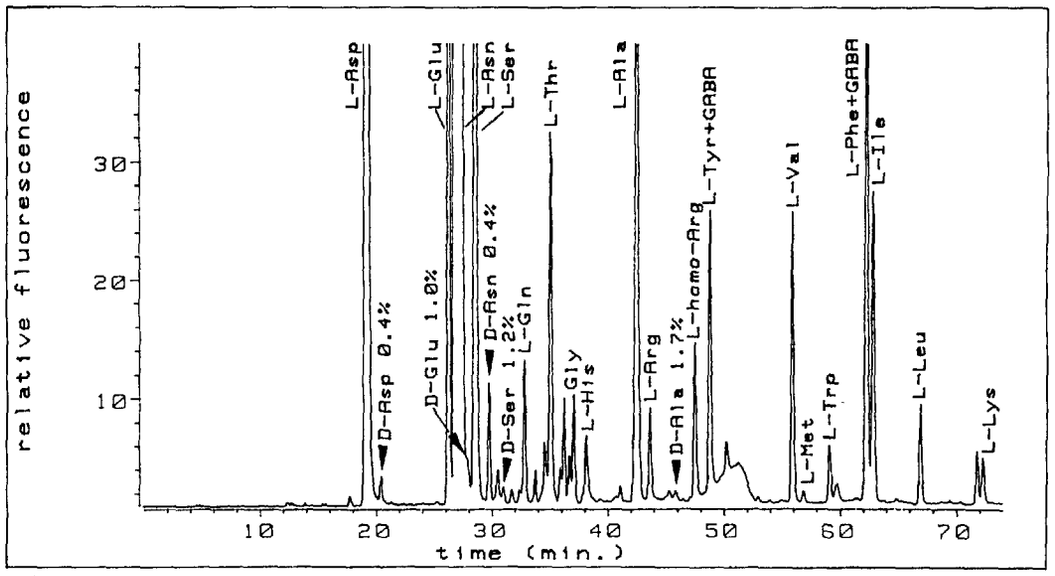

Figure 3

Aminogram of freshly pressed apple juice (cv. 'Granny Smith'); derivatization with IBLC.

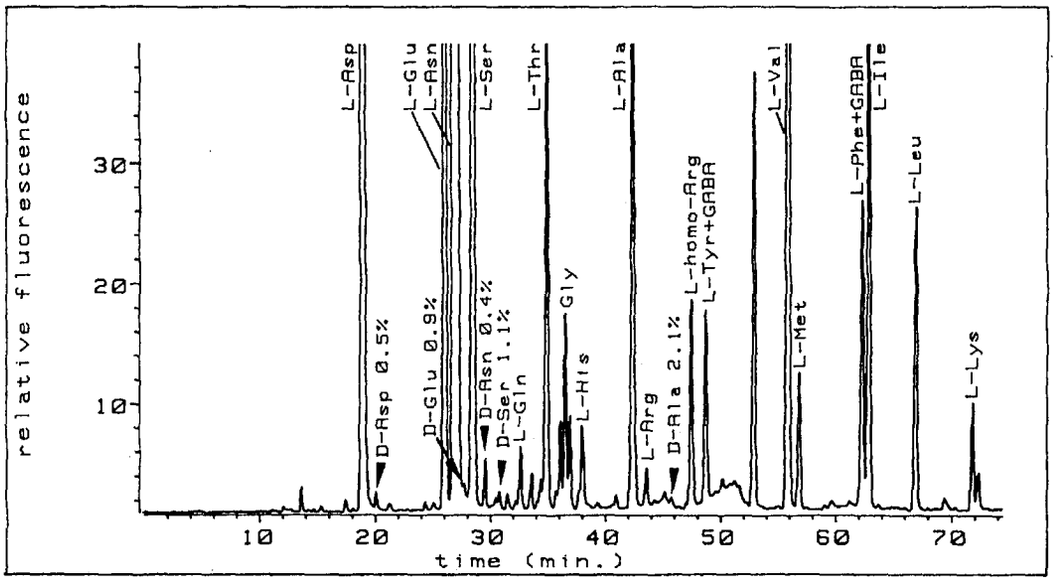

Figure 4

Aminogram of freshly pressed pear juice (cv. 'Alexander Lucas'); derivatization with IBLC.

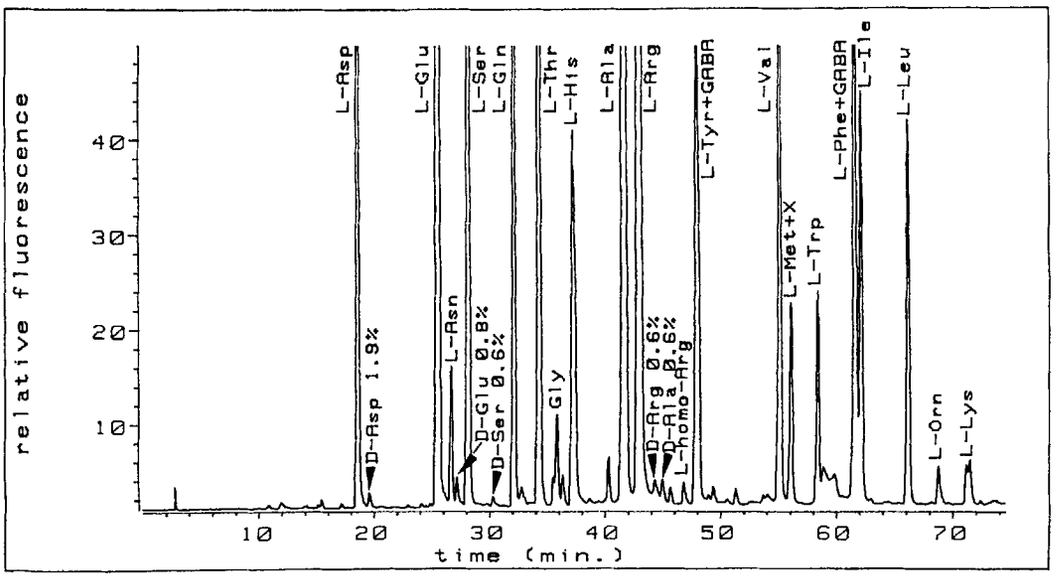

Figure 5

Aminogram of freshly pressed grape juice (cv. 'Kerner'); derivatization with IBLC.

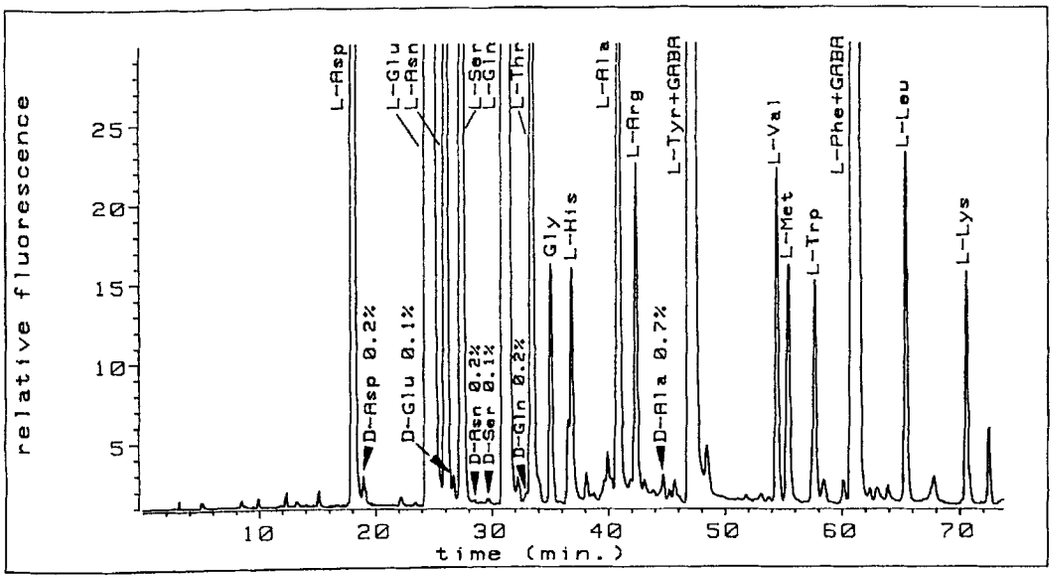

Figure 6

Aminogram of freshly pressed tomato juice directly derivatized with IBLC.

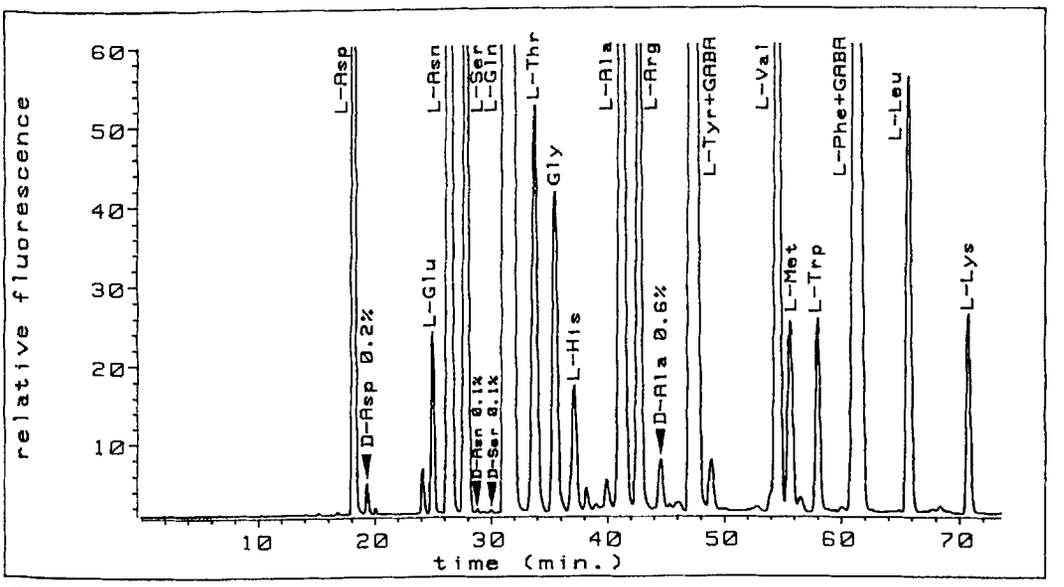

Figure 7

Aminogram of freshly pressed carrot juice directly derivatized with IBLC.

led fermentation of vegetables (Table I). The amount and type of D-AA increases as a result of the fermentation process caused by microorganisms (Table I). Typically Lactobacilli, such as *Lactobacillus* (*Lb.*) *mesenteroides, Lb. plantarum, Lb. brevis,* and also *Pediococcus cerevisiae*, capable of producing D-AA [2, 6, 7] are involved in the fermentation process of white cabbage. For D-AA determined in other lactic fermented vegetable juices see references [8–10].

Since the above results prove the occurrence of D-AA in native, unfermented juices, the possible origin of D-AA in plant juices may be briefly discussed. Taking literature data into account, it is concluded that D-AA are common in plants and that therefore common mechanisms for their uptake and biosynthesis exist. Since D-AA are readily taken up by plants, it is assumed that this is also the case with D-AA occurring as a result of the activity of soil microorganisms, as well as D-AA originating from the use of organic fertilizers or liquid manure. Detection of D-amino acid aminotransferases in pea seedlings, capable of transferring amino groups from various D-AA to pyruvate or α-ketoglutarate with formation of D-Ala and D-Glu, respectively, has been reported [15]. Conjugated D-AA such as γ-L-glutamyl-D-alanine, *N*-malonyl-D-Ala, D-Ala-D-Ala and *N*-malonyl-D-Trp have been detected in higher plants [16–18]. These compounds would go undetected using methods for the determination of free D-AA, but could lead to the release and increase of free D-AA after, for example, being enzymatically or otherwise hydrolyzed in plant tissues. Consequently, from a food analyst's point of view, the use of small amounts of D-AA as markers for the microbial status of processed juices is limited since the amounts of native D-AA already present in the respective raw materials are usually not known.

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References

[1] *H. Frank, G. J. Nicholson, E. Bayer*, J. Chromatogr. Sci. **15,** 174 (1977).
[2] *H. Brückner, M. Lüpke*, Chromatographia **31,** 13 (1991).
[3] *H. Brückner, R. Wittner, H. Godel*, Chromatographia **32,** 383 (1991).
[4] *M. Langer, R. Wittner, P. Jaek, H. Godel, H. Brückner*, in: "Strategies for Food Quality Control and Analytical Methods in Europe", Proceedings of Euro Food Chem VI, Hamburg, September 22–26, 1991, Vol. 1, W. Baltes, T. Eklund, R. Fenwick, W. Pfannhauser, A. Ruiter, H. P. Thier, Eds., Behrs Verlag, Hamburg, 1991; p. 385.
[5] *I. Gandolfi, G. Palla, R. Marchelli, A. Dossena, S. Puelli, C. Salvadori*, J. Food Sci. **59,** 152 (1994).
[6] *H. Brückner, S. Haasmann, M. Langer, T. Westhauser, R. Wittner, H. Godel*, J. Chromatogr. **666,** 259 (1994).
[7] *H. Brückner, D. Becker, M. Lüpke*, Chirality **5,** 385 (1993).
[8] *H. Brückner, M. Hausch*, J. High Resol. Chromatogr. **12,** 680 (1989).
[9] *H. Brückner, M. Hausch*, Chromatographia **28,** 487 (1989).
[10] *H. Brückner, M. Hausch*, in: "Amino Acids: Chemistry, Biology and Medicine", G. Lubec, G. A. Rosenthal, Eds., Escom Science, Leiden, The Netherlands, 1990: p. 1172.
[11] *W. Ooghe, H. Kasteleyn, I. Temmerman, P. Sandra*, J. High Resol. Chromatogr., Chromatogr. Commun. **7,** 284 (1984).
[12] *D. W. Kuneman, J. K. Braddock, L. L. McChesney*, J. Agric. Food Chem. **36,** 64 (1988).
[13] *H. J. Hofsommer, I. Klein, J. Grüning, H.-R. Höpker*, Flüssiges Obst **56,** 646 (1989).
[14] *A. Dyremark, M. Ericsson*, Chromatographia **29,** 51 (1990).
[15] *T. Ogawa, M. Fukuda*, Biochem. Biophys. Res. Commun. **52,** 998 (1973).
[16] *M. Fukuda, A. Tokumura, T. Ogawa, K. Sasaoka*, Phytochemistry **12,** 2593 (1973).
[17] *M. H. Zenk, H. Scherf*, Planta **62,** 350 (1964).
[18] *J. L. Frahn, R. J. Illman*, Phytochemistry **14,** 1464 (1975).
[19] *T. Westhauser, S. Aßfalg, H. Brückner*, Ernährungsumschau **41,** 122 (1994).
[20] *T. Westhauser, H. Brückner*, in: "Analytica 94", Abstracts of Analytica Conference, April 19–22, 1994, Munich, Gesellschaft Deutscher Chemiker, Ed., Frankfurt, Germany, 1994; p. 285.
[21] „Lexikon Lebensmittel und Ernährung", Fa. Dr. A. Oetker, Ed., Ceres-Verlag, Bielefeld, 1983.

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