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Mass Spectrometric Detection and Formation of D-Amino Acids in Processed Plant Saps, Syrups, and Fruit Juice Concentrates

RALF PÄTZOLD AND HANS BRÜCKNER*

Interdisciplinary Research Center, Department of Food Sciences, Heinrich-Buff-Ring 26-32, University of Giessen, 35392 Giessen, Germany

Liquid and syrupy dietary saps and juices of plant origin, characterized by the presence of large quantities of saccharides (glucose, fructose, or sucrose) and containing amino acids, were analyzed for the presence of D-amino acids using enantioselective gas chromatography−mass spectrometry. D-Amino acids were detected in processed saps and juices of trees (maple, palm, birch), fruits (grape, apple, pear, pomegranate, date), and various other plants (agave, beetroot, sugar cane, carob). D-Ala was detected in all plant products and amounted to ∼34% D-Ala (relative to L-Ala + D-Ala) in Canadian maple syrups, to ∼13% in palm saps, and to 48 and 13% D-Ala, respectively, in concentrated grape juices (Spanish Arrope and Turkish Pekmez). Varying amounts and kinds of other D-amino acids were also detected. To test the hypothesis that racemization, that is, partial conversion of L-amino acids into their corresponding D-enantiomers, occurs at reversible stages of the Maillard reaction, the Amadori compound fructose-L-phenylalanine was synthesized. On heating at 200 °C for 5 (20) min, release of 10.8% (24.2%) D-Phe was detected. From the data it is concluded that the Amadori compounds formed in the course of the Maillard reaction are pecursors of D-amino acids in foodstuffs.

KEYWORDS: Maillard reaction; Amadori compounds; amino acid racemization, dietary plant saps; GC-SIM-MS; Chirasil-Val

INTRODUCTION

Owing to their use as building blocks for peptides and proteins, the naturally occurring α-amino acids in plants are predominantly of the L-configuration. Consequently, the ∼20 genetically encoded L-amino acids used for the ribosomal synthesis of plant proteins are commonly called proteinogenic or protein amino acids. However, despite the paramount abundance and importance of protein L-amino acids in plants, their mirror images (optical antipodes or enantiomers), named D-amino acids, are commonly also present in plants, albeit in the low percentage range (*1*−*3*). As to the genesis of D-amino acids in plants it is assumed that endogenous transaminases and racemases (*4*, *5*) play a role in the conversion of L-amino acids into D-amino acids (or vice versa), a process usually referred to as racemization (or epimerization as one of several stereogenic centers is concerned). Although in the strictest sense an amino acid racemate consists of equal amounts of the corresponding enantiomers, the partial conversion of one enantiomer into the other is frequently also named racemization. The generation of free or protein-bound D-amino acids in the course of food processing under harsh alkaline or acidic treatment, together with increased heat and pressure, has attracted some attention (*6*−*9*). This is attributed to the change of nutritional value of essential amino acids, proteolytic resistance of protein-bonded D-amino acids, and formation of amino acid enantiomers to which some specific physiological and pharmacological activities have been attributed. In recent years, however, it has been fully realized that the well-known and established presence of free and peptidoglycan-bonded D-amino acids, in particular in bacteria, is an important source of D-amino acids detectable in foodstuffs (*10*, *11*). Consequently, the use of D-amino acids as chemical markers for food processing in terms of good manufacturing practice, nutritional value, maturation, food quality and authenticity, shelf life, food hygiene, and microbial contamination resulting in spoilage requires thorough knowledge on the occurrence and genesis of D-amino acids in raw materials and processed foodstuffs. This point was, and still is, the subject of intensive investigations (*12*−*17*). We were puzzled by the fact that the abundance and diversity of D-amino acids in certain foods and beverages such as dried fruits, honey, or fortified wines, in comparison to table wines, could not be satisfactorily and unambiguously rationalized by applying established chemical or enzymatic racemization mechanisms or the action of bacteria (*15*, *16*, *18*−*20*). It was realized, however, that heating of saccharides together with L-amino acids led to the formation of D-amino acids (*21*). The interaction of reducing sugars and amino components is known as nonenzymic browning or the Maillard reaction and is of paramount importance in food chemistry and life sciences (*22*−*26*). On the basis of the hypothesis that foodstuffs rich in saccharides and containing amino acids should contain D-amino acids as a result of the

* Author to whom correspondence should be addressed (e-mail Hans.Brueckner@ernaehrung.uni-giessen.de).

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Maillard reaction, selected edible plant saps, syrups, and concentrated fruit juices were analyzed for relative quantities of amino acid enantiomers by enantioselective gas chromatography.

MATERIALS AND METHODS

Solvents and Chemicals. Dichloromethane (DCM), 2-propanol (2-PrOH), methanol (MeOH), aqueous ammonia (25%), aqueous HCl (36%), and acetyl chloride (AcCl) were purchased from Merck, Darmstadt, Germany. Cation exchanger Dowex 50W-X8, practical grade, 200−400 mesh (0.037−0.075 mm particle size) was from Sigma, Deisenhofen, Germany. Pentafluoropropionic acid anhydride (PFPAA) was from Pierce, Rockford, IL; antioxidant 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) was from Fluka, Buchs, Switzerland. D- and L-amino acids were purchased from Sigma and Fluka.

For testing the column and optimization of chiral resolution, a mixture of D- and L-amino acids (ratio approximately 1:2) including the internal standard L-Nle (norleucine) and with the addition of achiral Gly and γ-aminobutyric acid (GABA) was prepared and analyzed (cf. **Figure 1a**).

Sources of Foodstuffs. Foods and beverages were purchased from local retail outlets or specialty shops. Maple syrup A (no. 1) and maple syrup B (no. 2) were products of Canada; birch sap (no. 3) was a commercial product from Russia; palm syrup Miel de Palma (no. 4) was from Gomera, Canary Islands, Spain; Toddy palm sap (no. 5) was a beverage from Thailand; grape syrup Pekmez (no. 6) was a product of Turkey; grape concentrate Arrope (no. 7) was a product used for coloring and flavoring Sherry wine and was acquired in a bodega in Jerez, Spain; ~7-fold concentrated syrupy juices of apple (no. 8) and pear (no. 9) were German products; pomegranate syrup Grenadine (no. 10), mulberry syrup (no. 11), and date syrup (no. 12) were Turkish products; sugar beet syrup (no. 13) was from Germany; sugar cane syrup (no. 14) containing partly inverted sucrose was a product of the United Kingdom; agave syrup (no. 15) was imported from Mexico; and carob syrup (no. 16) was from Turkey. Numbers in parentheses correspond to those of **Table 1**.

Treatment of Samples, Isolation, and Derivatization of Amino Acids. Aliquots (1 g) of foodstuffs were diluted with water (5 mL) and adjusted to pH 2.3 by the addition of 0.01 M HCl. Samples were passed through glass columns (Pasteur pipets) packed with Dowex 50W-X8 cation exchanger (H^+-form) having a bed volume of 3.5 cm length × 0.5 cm diameter. After washing with distilled water (10 mL), amino acids adsorbed were eluted with 4 M aqueous ammonia (5 mL). Effluents were evaporated to dryness using a vacuum evaporator. Then 0.1 M HCl (0.5 mL) was added, the solute transferred to 1 mL glass vials, and the solvent removed with a stream of nitrogen.

To the remaining residue was added a mixture (500 μL) of acetyl chloride in 2-propanol, prepared with a mixture of AcCl (1 mL) and 2-PrOH (4 mL), and this mixture was chilled with ice. Vials were tightly closed with Teflon-lined screw caps and heated for 1 h at 100 °C. Solvents were removed in a stream of nitrogen, and DCM (500 μL) and pentafluoropropionic anhydride (100 μL) were added. The mixture was heated for 20 min at 100 °C, and solvents were removed at ambient temperature in a stream of nitrogen. To the residue was added DCM (500 μL), and aliquots of 0.5−1 μL were analyzed by GC-MS.

Quantification of Amino Acid Enantiomers. Relative quantities of amino acid enantiomers were calculated from peak areas of derivatives according to the equation %D = 100D/(D + L), where %D represents relative amounts of D-amino acids with regard to the sum of (D + L) amino acids and D and L represent the peak areas of the respective enantiomer determined by GC-SIM-MS. Ions were selected for PFP−amino acid−(2)-propyl esters.

Note that enantiomers of basic amino acids (His, Arg) cannot be determined routinely using this approach and that Gln and Asn are converted to Glu and Asp under derivatization conditions. The sums of (Asp + Asn) and (Glu + Gln) are defined as Asx and Glx, respectively, in the table.

Quantification of Saccharides and 5-(Hydroxymethyl)furfural (HMF) in Analytes. Sucrose, D-glucose, and D-fructose were determined using an enzymatic assay (test kit catalog no. 10716260035, Boehringer Mannheim, R-Biopharm, Germany). HMF was determined colorimetrically in selected samples after derivatization with barbituric acid/*p*-toluidine (*28*).

Synthesis of Fructose-L-phenylalanine (Fru-L-Phe) and Heating Experiment. *N*-(1-Deoxy-D-fructosyl)-L-phenylalanine (Fru-L-Phe) was synthesized according to the literature (*29*) by refluxing D-glucose (30 mmol) and L-Phe (30 mmol) in MeOH (250 mL) for 8 h. Crude compounds containing unreacted amino acids were purified by preparative HPLC according to a protocol similar to that reported (*30*). Fractions containing the pure fructose-amino acids were combined, partly evaporated, and freeze-dried. The compound was characterized by ESI-MS (Thermo-Finnigan LCQ instrument) in negative and positive ion modes. Fru-L-Phe (molecular weight calculated as 327.0 g/mol): *m*/*z* 325.8 $(M - H)^-$, most intensive; 308.1 $(M - H_2O - H)^-$; 350.6 $(M + Na)^+$, most intensive; 328.4 $(M + H)^+$. Aliquots of 5 mg of Fru-L-Phe were heated in the dry state in closed vials for various periods of time (5, 10, 20, and 40 min). The configuration of Phe released was determined by GC-SIM-MS as described below.

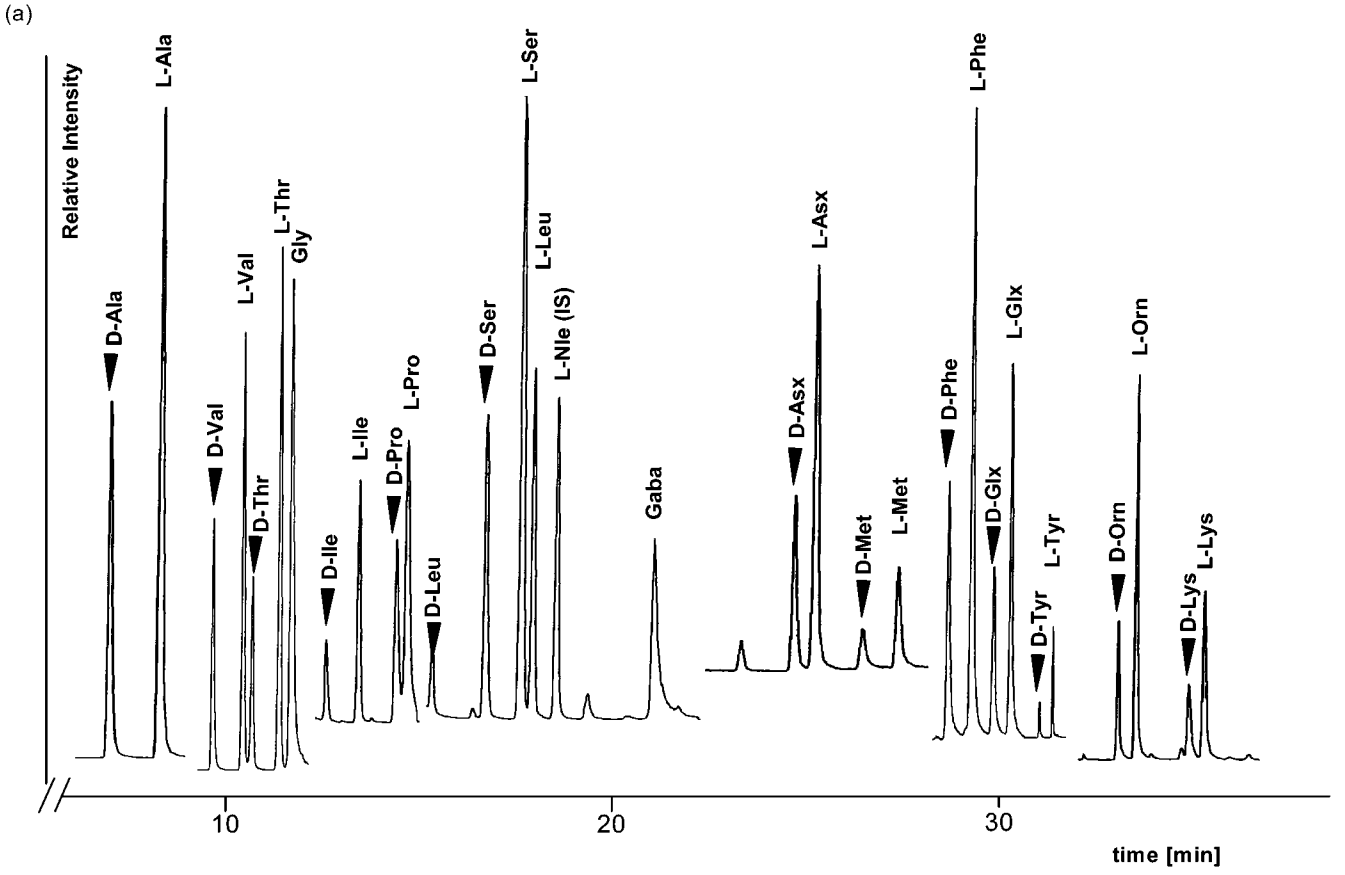
Gas Chromatography−Mass Spectrometry. For enantioselective separations of derivatized amino acids a fused silica capillary column Chirasil-Val (*N*-propionyl-L-valine *tert*-butylamide polysiloxane) (*27*) of 25 m length × 0.25 mm i.d. and film thickness pf 0.12 μm of the stationary phase (from Varian Inc., Darmstadt, Germany) was used together with a model A17 gas chromatograph coupled to a model QP5000 mass spectrometer (Shimadzu, Kyoto, Japan).

The carrier gas was helium set at an inlet pressure of 5.0 kPa, a flow rate of 0.5 mL/min, and a purge flow of 3 mL/min. Injector and interface temperatures were 250 °C, and 0.5−1 μL aliquots of analytes were injected at a split ratio of 1:30. The temperature program was 70 °C for 1 min, increased at 2.5 °C/min to 100 °C, then 2 min isothermal, increased at 2.5 °C/min to 150 °C, increased at 5 °C/min to 150 °C, then increased at 20.0 °C/min to 190 °C, then for 8 min isothermal at 190 °C. The pressure program of the carrier gas was 5.0 kPa for 1 min, increased at 0.2 kPa/min to 7.0 kPa, then 2 min isobaric, increased at 0.3 kPa/min to 10.8 kPa, at 1.4 kPa/min to 13.0 kPa, and at 2.4 kPa/min to 15.0 kPa, and then 5 min isobaric. For selected ion monitoring appropriate ion sets were selected and characteristic mass fragments (*m*/*z*) of the PFP/2-Prp esters of the amino acids were used: Ala (190, 191); Val (218, 203); Thr (203, 202); Gly (176, 177); Pro (216); Leu (190, 232); Ser (188, 189); Asx = Asp + Asn (234, 216); Met (263, 203); Phe (91, 148); Glx = Glu + Gln (202, 203); Tyr (253, 266); Orn (216); Lys (230); GABA (232, 176).

RESULTS

Relative quantities of D-amino acids in the samples determined by GC-MS are compiled in **Table 1**. Whereas in Arrope and agave syrup only D-Ala could be determined, varying kinds and quantities of other D-amino acids were detected in the remaining samples. D-Ala was the most abundant among D-amino acids and was detected in all analytes. Quantities exceeding 30% D-Ala were detected in Arrope (47.8%) and maple syrups (34.1 and 33.2%, respectively). More than 10% D-Ala was detected in pomegranate syrup (15.5%), agave and palm syrup (13.5%), Pekmez (13.4%), and sugar cane syrup (11.2%). Quantities of 1.7−7.6% D-Ala were determined in the other samples. GC-SIM-MS of a derivatized standard mixture of DL-amino acids resolved on Chirasil-Val and illustrative chromatograms of maple syrup, palm syrup Miel de Palma, and grape syrup Pekmez are presented in **Figure 1**.

Quantities of glucose, fructose, and sucrose were also determined, and data are compiled in **Table 2**. The major saccharide was either sucrose (ranging from 23.9 to 60.7 g/100 g of sample), a mixture of glucose and fructose, or a mixture of all three saccharides. HMF was also determined in selected samples (cf. **Table 2**). Exceptionally high amounts were found in Arrope (14.5 g/100 g of sample), whereas amounts from as low as 7 mg/100 g (sample 15) to as high as 338 mg/100 g (sample 9) were detected in the remaining foods investigated.


(a)
Relative Intensity
D-Ala
L-Ala
D-Val
L-Val
D-Thr
L-Thr
Gly
D-Ile
L-Ile
D-Pro
L-Pro
D-Leu
D-Ser
L-Ser
L-Leu
L-Nle (IS)
Gaba
D-Asx
L-Asx
D-Met
L-Met
D-Phe
L-Phe
D-Glx
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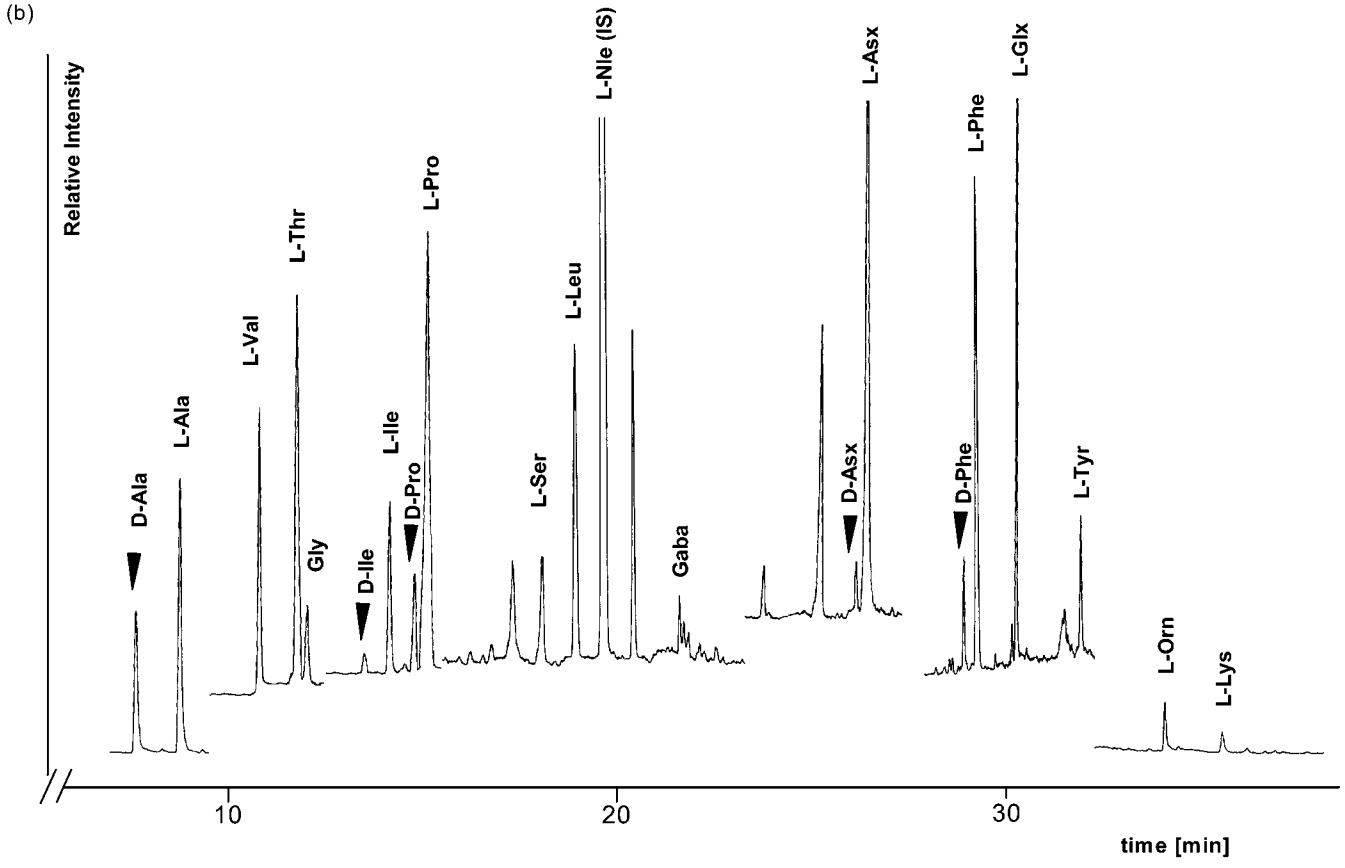
D-Lys
L-Lys
10
20
30
time [min]


(b)
Relative Intensity
D-Ala
L-Ala
L-Val
L-Thr
Gly
D-Ile
L-Ile
D-Pro
L-Pro
L-Ser
L-Leu
L-Nle (IS)
Gaba
D-Asx
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L-Phe
L-Glx
L-Tyr
L-Orn
L-Lys
10
20
30
time [min]

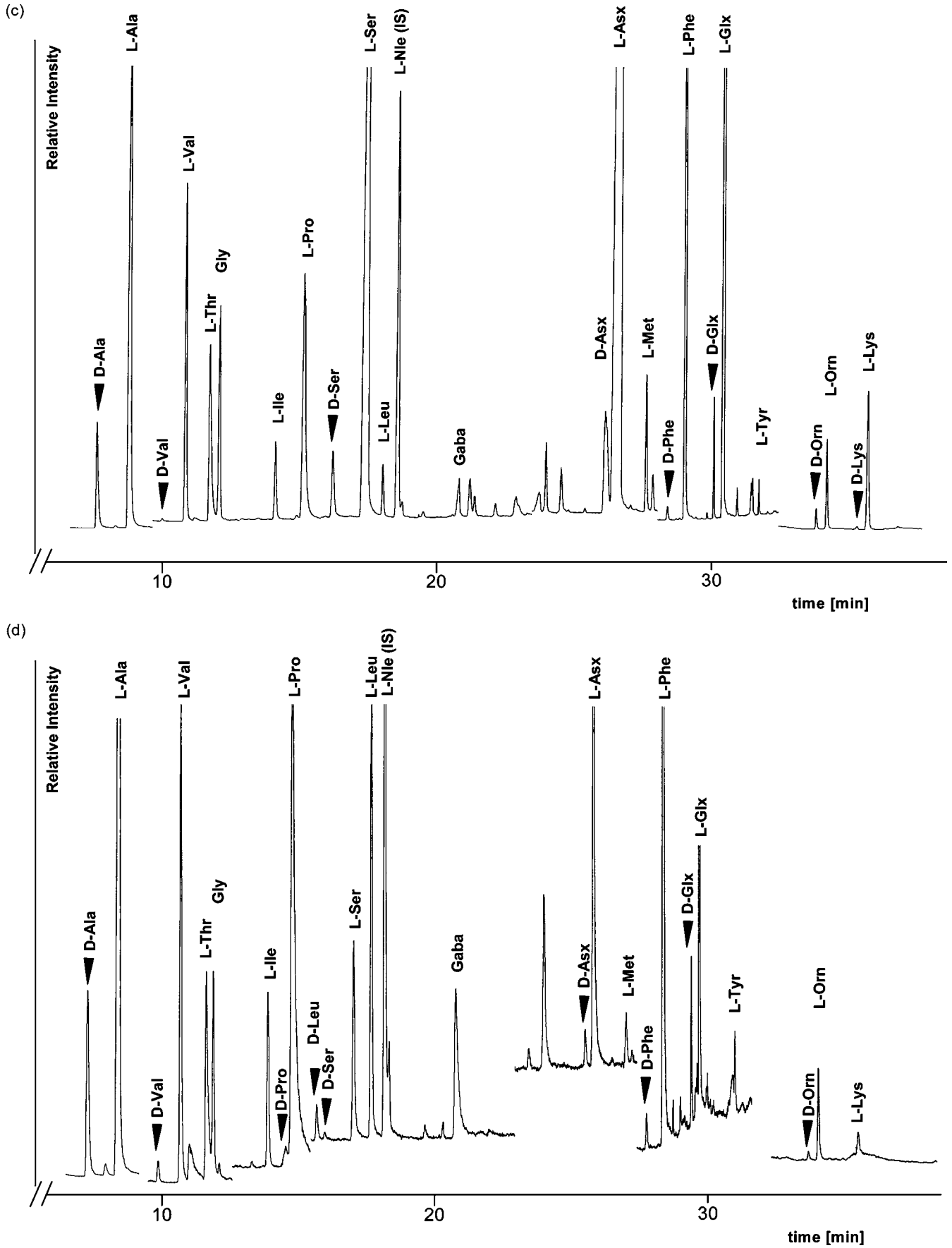

Figure 1. GC-SIM-MS of amino acid derivatives (PFP/2-Prp esters) on Chirasil-Val of (**a**) a standard mixture of DL-amino acids (ratio D:L about 1:2), (**b**) maple syrup A (no. 2), (**c**) palm syrup (no. 4, Miel de Palma), and (**d**) grape syrup (no. 6, Pekmez); numbers correspond to those of **Table 1**; arrows indicate D-amino acids. Note that varying baseline levels are the results of varying amplifications within the ion sets selected and that minor shifts of retention times are due to varying concentrations of analytes.

Table 1. Relative Quantities of D-Amino Acids [%D = D/(D + L)] in Plant Syrups, Saps, and Juice Concentrates[a]

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
D-Ala	33.2 ± 0.2	34.1 ± 0.3	5.6 ± 0.4	13.5± 0.2	13.6 ± 0.3	13.4 ± 0.3	47.8± 0.6	1.8 ± 0.1	2.5 ± 0.1	15.5 ± 0.2	3.4 ± 0.1	1.7 ± 0.1	2.3 ± 0.2	11.2 ± 0.3	13.5 ± 0.2	1.9 ± 0.1
D-Val	–	4.4 ± 0.1	–	0.7 ± 0.1	1.0 ± 0.1	4.3 ± 0.2	–	–	–	–	0.4 ± 0.1	–	–	–	–	0.3 ± 0.0
D-Pro	8.9 ± 0.4	1.8 ± 0.2	–	–	–	1.4 ± 0.0	–	–	–	–	0.7 ± 0.1	0.3 ± 0.0	–	–	–	0.6 ± 0.1
D-Ser	–	11.3 ± 0.3	–	4.7 ± 0.1	1.0 ± 0.1	2.2 ± 0.2	–	–	–	–	–	–	3.3 ± 0.2	–	–	–
D-Leu	–	–	–	–	–	6.4 ± 0.2	–	–	–	–	–	–	–	–	–	–
D-Asx	3.4 ± 0.2	8.0 ± 0.1	–	4.6 ± 0.1	1.6 ± 0.1	4.5 ± 0.1	–	1.2 ± 0.2	2.4 ± 0.1	–	2.4 ± 0.4	3.2 ± 0.2	4.3 ± 0.2	10.2 ± 0.1	–	1.7 ± 0.2
D-Phe	6.4 ± 0.2	7.0 ± 0.3	–	1.1 ± 0.1	–	2.9 ± 0.2	–	–	–	–	2.4 ± 0.3	–	1.5 ± 0.1	–	–	2.3 ± 0.2
D-Glx	–	11.8 ± 0.4	–	3.0 ± 0.3	1.6 ± 0.2	8.8 ± 0.3	–	1.5 ± 0.1	2.2 ± 0.2	4.5 ± 0.1	2.8 ± 0.4	–	4.6 ± 0.1	–	–	1.5 ± 0.1
D-Orn	–	–	–	16.7 ± 0.3	8.8 ± 0.1	8.1 ± 0.2	–	–	–	–	–	–	–	–	–	–
D-Lys	–	–	–	1.7 ± 0.1	2.9 ± 0.3	–	–	–	–	–	–	–	–	–	–	–
D-Ile	16.8 ± 0.3	12.7 ± 0.1	–	–	–	–	–	–	–	–	–	–	5.9 ± 0.2	–	–	1.1 ± 0.1

[a] 1, maple syrup A; 2, maple syrup B; 3, birch sap; 4, palm syrup (Miel de Palma); 5, palm sap; 6, grape syrup (Pekmez); 7, grape concentrate (Arrope); 8, apple syrup; 9, pear syrup; 10, pomegranate syrup; 11, mulberry syrup; 12, date syrup; 13, sugar beet syrup; 14, sugar cane syrup; 15, agave syrup; 16, carob syrup; –, not detectable or not determinable; Asx = Asp + Asn; Glx = Glu + Gln. Data are averages of two analyses of two separate sample treatments.

To establish a racemization mechanism for amino acids in the foodstuffs Fru-L-Phe was synthesized and heated at 200 °C for various periods of time. Hydrolysis (6 M HCl, 100 °C, 18 h) released 99.2% L-Phe and 0.8% D-Phe, indicating that the L-configuration of Phe was almost completely retained in the fructose-amino acid. Quantities of 10.8 and 24.2% D-Phe could be analyzed after 5 and 10 min, respectively, of heating. After 40 min, neither L- nor D-Phe could be detected, indicating that the amino acid was destroyed at advanced stages of the Maillard reaction. This experiment indicates that the Amadori compounds (fructose-amino acids) are the key compounds for amino acid racemization.

DISCUSSION

As plants, including their saps and juices, contain D-amino acids in the low percentage range (*2*, *3*) the presence of relatively high amounts in some of the food samples investigated had to be rationalized. It was assumed that the composition of raw materials of plants and the interaction of constituents, together with specific technological treatments as outlined before, must be responsible for the partial conversion of L-amino acids into the corresponding D-enantiomers. Therefore, the origin and processing of the plant products investigated are briefly described in the following.

Maple syrup is produced from the sap of the sugar maple tree (*Acer saccharum* Marsh.) of North America and Canada. Traditionally the sap is concentrated by boiling in open pans over a wood fire (*31*). Birch sap is obtained by tapping birch trees (*Betula* spp.) in the spring, similar to sugar maple trees. The sap, however, is not concentrated by evaporation and represents a colorless sweet beverage (*32*).

Traditional palm honey (Miel de Palma) is obtained from the palm tree (*Phoenix canariensis* Hort. ex Chabaud) of the Canary Islands, Spain, by cutting the top of the palm tree, collecting the sap, and concentrating by boiling. The resulting product is a dark brown honey-like syrup used as spread or sweetener. Juice from the cut stalk of the male inflorescence of palm trees such as Toddy palm (*Borassus flabellifer* L.) is a colorless, sweet beverage (*33*). The sap provides also brown palm sugar on concentration by boiling. Agave syrup, representing a light brown product, is obtained from various species of *Agave* (Liliaceae) growing natively in Mexico and Latin America. A syrupy sap, rich in fructose, is obtained from the stems of mature plants by steam heating or autoclaving (*34*).

Turkish grape syrup (Pekmez) is produced from the must of cultivars of vine grapes (*Vitis vinifera* L. subsp. *vinifera*), which is concentrated by boiling until a dark syrup is obtained (*35*). Similarly, almost black Spanish Arrope is manufactured from grape must. The other syrups were products from juices obtained by squeezing fruits of cultivars of silkworm mulberries (*Morus alba* L.), apples (*Malus domestica* Borkh.), pears (*Pyrus communis* L.), and grenadine from pomegranate (*Punica granatum* L.). For the preparation of syrups of sugar beet (*Beta vulgaris* L. subsp. *vulgaris* var. *altissima* Döll) and carob from fruit pods of St. John's bread tree (*Ceratonia siliqua* L.) or dates (*Phoenix* spp. L.) (*36*) aqueous extracts were prepared, followed by concentration of extracts. Sugar cane syrup is a concentrate from the sap of *Saccharum officinalis* L.

The edible plant juices and saps investigated and the concentrates prepared therefrom had the following features: (i) they were obtained by tapping, collecting, squeezing, or extraction with water, followed by evaporation of water by boiling in most cases; (ii) foodstuffs were characterized by the presence of high amounts of glucose and fructose and/or

Table 2. Quantities (Percent) of Glucose (Glc), Fructose (Fru), and Saccharose (Sac) and Content (Milligrams per Kilogram) of Hydroxymethylfurfural (HMF) in Foodstuffs[a]

	1	2	3	4	5	6	7	8	9	10	11	12	14	13	15	16
Glc	1.3	7.6	ni	1.0	ni	19.4	25.1	10.2	14.0	ni	24.5	14.1	15.1	16.3	19.9	3.6
Fru	1.1	6.7	ni	1.2	ni	24.3	14.4	39.5	34.4	ni	28.4	14.7	15.4	16.6	55.6	2.7
Sac	60.7	44.6	ni	57.1	ni	nd	nd	7.8	4.6	ni	0.2	32.0	26.5	28.6	nd	23.9
HMF	nd	ni	58	n.d.	123	121	317	16	338	ni	259	17	26	14533	7	ni

[a] Numbers correspond to products of **Table 1**. ni, not investigated; nd, not detected. Data are average of two replicates.

saccharose; (iii) the resulting concentrates represented sweet and syrupy or highly viscous liquids, which were light or dark brown (maple syrup, date syrup, juice syrups) or almost black (palm syrup, Pekmez, Arrope); and (iv) the concentrates contained HMF, serving as an indicator for heat treatment and progress of the Maillard reaction.

The sample of Arrope contained exceptionally high amounts of HMF (14.5 g/100 g of sample), indicating the most severe treatment, that is, concentration of grape must by boiling. This is of interest as Arrope is added as sweetener and colorant to wines of the Sherry type. Such fortified wines have been found to contain much higher amounts of D-amino acids in comparison to table wines (*19*). The browning of foods containing reducing sugars and amino acids is accompanied by the formation of flavor compounds and HMF, which are known as products of nonenzymatic browning or the Maillard reaction. That reaction starts with the reversible formation of Schiff bases (azomethines) from amino acids and reducing sugars such as glucose, followed by cyclization yielding *N*-glucosyl amino acids. These compounds rearrange in a reaction known as Amadori rearrangement to relatively stable fructose-amino acids (Amadori compounds). These compounds can undergo 1,2- or 2,3-enolization followed by release of amino acids and deoxyosones. The amino acids released can participate again at the reaction. At advanced stages of the Maillard reaction they are irreversibly converted into other compounds (Strecker aldehydes, heterocyclic compounds, and melanoidins) (*22*, *24*).

From the data it is deduced that the conversion of L-amino acids to D-amino acids proceeds in the Amadori compounds. Indeed, Amadori compounds (fructose-amino acids) have been isolated or determined in foods such as freeze-dried apricots (*37*), dried preparations of vegetables and fruits (*38*−*40*), fruit juice concentrates, soy sauce (*41*), dried milk products (*42*), and tobacco (*43*). This explains the occurrence and formation of D-amino acids in some of these or related food products (*19*, *44*, *45*) by the tentative racemization mechanism presented in **Figure 2**.

To test the hypothesis on the generation of D-amino acids from Amadori compounds, synthetic fructose-L-phenylalanine was synthesized. On acidic total hydrolysis only L-Phe was released. This demonstrates that the Amadori compound consisted of L-Phe almost exclusively. On brief heating of Fru-L-Phe, however, a mixture of L-Phe and D-Phe was released. With regard to mechanistic details of amino acid racemization, it is assumed that the enolization of the Amadori compound together with heating activates the C^{α}-hydrogen of the amino acid. On C^{α}-abstraction of this hydrogen a carbanion is formed. In addition, in the Amadori compound an intramolecular hydrogen bridge with the formation of a six-membered ring might favor an intramolecular prototropy accompanied with formation of an carbanion (cf. **Figure 2**). Reattachment of the proton is possible from both sides of the carbanion, resulting in partial racemization (epimerization) of the amino acid. Steric constraints and electronic features (mesomeric and inductive effects, acidity, basicity) of the amino acid side chain govern the extent of racemization. The sterically highly constrained side chain of valine explains the observed low extent of racemization of this amino acid (*21*). These features control also the kinetics of formation and decay as well as the stability of Amadori compounds. Among amino acids Ala represents a favorable case as it shows low steric hindrance but sufficient tendency to form a carbanion and moderate reactivity in subsequent decay processes (cf. **Table 1**). This explains the abundance of D-Ala in the foodstuffs analyzed. The proposed racemization mechanism is depicted in **Figure 2**.

The kinetics of the Maillard reaction in a food system are dependent on structures and concentrations of educts, reaction temperature and time, pH, water activity, the redox state of the system, and the presence of catalysts including metal ions (*29*, *38*, *46*−*48*). Consequently, the racemization of amino acids in the course of this reaction is also governed by these parameters. This explains the high racemization rate of amino acids in plant juices concentrated by boiling over longer periods of time. The water activity of food is also lowered by high concentrations of sugars or other water-binding or -immobilizing components. Notably, reducing sugars, which are required for the Maillard reaction, are also easily formed from nonreducing oligo- or polysaccharides under moderately acidic conditions as occur in plant juices (*49*) or on hydrothermolysis of saccharides (*50*).

The proposed racemization mechanism demonstrates a new route for the generation of D-amino acids from L-amino acids (and vice versa) as a result of the Maillard reaction. The mechanism is primarily not dependent on established chemical pathways such as base- or acid-catalyzed racemization or mechanisms requiring plant transaminases or microbial racemases or epimerases. The findings should also be taken into account when D-amino acids are used as chemical markers for age dating (maturation) of food, microbial spoilage, or definite

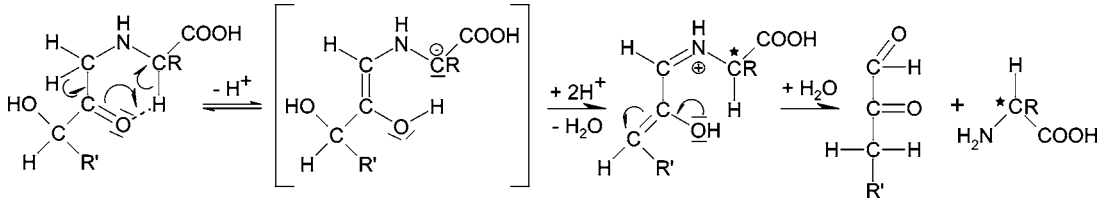

Figure 2. Tentative racemization mechanism of an amino acid in the Amadori compound (fructose-L-amino acid) via intermediate formation of a carbanion followed by reprotonation and decay with release of racemized amino acid; asterisk refers to racemized amino acid.

proof of suspected adulteration of fruit juices by the addition of racemic amino acid.

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