Amino Acids (2010) 38:951–958
DOI 10.1007/s00726-009-0304-1

ORIGINAL ARTICLE

Gas chromatographic determination of amino acid enantiomers in bottled and aged wines

Hatem Salama Mohammed Ali · Ralf Pätzold ·
Hans Brückner

Received: 13 February 2009 / Accepted: 6 May 2009 / Published online: 7 June 2009

Abstract Free L- and D-amino acids were determined by chiral GC-MS in 26 wines, comprising white wines, red wines, ice wines and sparkling wines. The aim of the work was to investigate whether quantities and pattern of D-amino acids, in particular D-proline, correlate with the storage time of bottled wines. The relative quantities with respect to the corresponding L-enantiomer ranged in white wines from 0.4 to 3.9% D-Ala, 0.9–8.3% D-Asx, and 0.5–8.9% D-Glx, in red wines from 2.9 to 10.6% D-Ala, 2.2–10.9% D-Asx, and 3.9–7.4% D-Glx, and in sparkling wines from 2.2 to 9.8% D-Ala, 2.1–4.4% D-Asx and 1.3–6.1% D-Glx. Low relative quantities of 0.3–0.7% D-Pro were detected in three white wines stored for more than 20 years and did not exceed 0.2% D-Pro in two red wines stored for 10 and 20 years, respectively. An ice wine stored for 24 years contained 0.9% D-Pro, 6.4% D-Glx, 3.0% D-Asp and 1.5% D-Ala. The data confirm the presence of D-amino acids in wines. They do not provide evidence for a correlation between the storage time of bottled wines and quantities of D-amino acids.

Keywords Wine amino acids · D-Amino acids · D-Pro · Age dating · Gas chromatography-mass spectrometry · Chirasil®-L-Val

---

H. S. M. Ali · R. Pätzold · H. Brückner (✉)
Department of Food Sciences, Interdisciplinary Research Centre for BioSystems, Land Use and Nutrition (IFZ),
Institute of Nutritional Science, University of Giessen,
Heinrich-Buff-Ring 26-32, 35392 Giessen, Germany
e-mail: hans.brueckner@ernaehrung.uni-giessen.de

Present Address:
H. S. M. Ali
Department of Food Science and Technology,
National Research Centre, El-Tahrir Street,
Giza, Dokki 12311, Egypt

Introduction

Wines are defined as the alcoholic fermented juices or musts of cultivars and crosses of the genus *Vitis* in the botanical family Vitaceae. Grape juice is rich in glucose and fructose as well as amino acids. Alcoholic fermentation is performed traditionally by mixtures of yeasts growing naturally on grapes or, nowadays preferably, by adding selected strains of *Saccharomyces cerevisiae* to the grape must after pasteurization. For their growth, i.e. formation of biomass, the yeasts use amino acids as nitrogen sources and convert the sugars into ethyl alcohol and carbon dioxide. Amino acids are also important precursors of flavor compounds and biogenic amines. Owing to their abundance and importance, many attempts have been undertaken to use the pattern of amino acids for identification, classification and proof of authenticity of grape juices and wines. Consequently, the majority of reports on amino acids in wines deal with methods for their quantitative analysis (Herbert et al. 2000; Hernández-Orte et al. 2003; Moreno-Arribas et al. 1998).

Considerably few reports deal with the stereochemistry of wine amino acids, i.e. the possible occurrence of the mirror images of the common L-amino acids, namely D-amino acids or D-enantiomers (Brückner and Hausch 1989; Chaves das Neves and Noronha 1995; Calabrese et al. 1995; Kato et al. 1995; Brückner et al. 1995; Calabrese and Stancher 1999; Jin et al. 1999; Tsunoda et al. 1999; Pätzold et al. 2003).

The finding that L-amino acids are subjects of a time-dependent conversion into D-amino acids, a process called enantiomerization, isomerization or racemization, attracted much attention. Since racemization of bonded or free amino acids under constant conditions is treated as first-order kinetics, bonded D-amino acids released on acidic hydrolysis are used for the age dating of fossilized and recent organisms. This method is also referred to as amino acid age dating. Amino acid racemization, however, is very much dependent on temperature, pH and presence of catalysts (Bada 1972; Bada and Schroder 1975).

Nevertheless, it was promising to correlate the kinds and quantities of D-amino acids in foodstuffs with their age, in particular when commercial value and sensory qualities are correlated with age. This is the case, for example, for various types of wine matured or stored for long periods of time, traditional balsamic vinegar that is aged for decades or certain hard cheeses ripened for several years (Brückner and Hausch 1989, 1990; Palla et al. 1989; Marchelli et al. 1996). Consequently, the use of D-amino acids for assessing the quality and genuineness of food and drinks together with their nutritional and physiological relevance is a matter of ongoing research (see the monograph of Konno et al. 2007).

The chemistry of ripening and formation of D-amino acids in foodstuffs, however, is complex. In particular, it had been realized that microbial fermented foods are rich in D-amino acids owing to the presence of bacteria and their racemases (Brückner et al. 1993; Friedman 1999). Furthermore, it has been recognized that D-amino acids are formed in the course of the Maillard reaction, i.e. reaction of reducing sugars and amino compounds such as amino acids (Erbe and Brückner 1998; Brückner et al. 2001; Pätzold and Brückner 2006a, b, 2007; Kim and Lee 2008; 2009).

It had been proposed that quantities of D-Pro in typical Italian table wines stored for extended periods of time are suitable markers for age dating (Calabrese and Stancher 1999). We had analyzed a large number of fortified (dessert) wines and found an abundance of D-amino acids, but no or less than 1% of D-Pro (Pätzold et al. 2003). To clear this discrepancy, we investigated bottled table wines stored for up to 36 years. The main focus was laid on D-amino acids in German white wines, but several red wines from Spain and France and sparkling wines were also analyzed.

Materials and methods

For enantioselective separations and quantification of amino acids, a fused silica capillary column Chirasil®-L-Val together with a GC-MS instrument as described previously were used (Ali et al. 2006; Pätzold and Brückner 2006a, b).

Relative amounts of D-amino acids were calculated according to the Eq. 1

$$\%\text{D} = 100A_{\text{D}}/(A_{\text{D}} + A_{\text{L}}) \quad (1)$$

where %D is the relative amount of the D-amino acid to be determined, and A_{D} and A_{L} are the peak areas (ion abundances) of the D- or L-enantiomer, respectively, determined by GC-SIM-MS. For quantification of amino acids, response factors in a standard mixture were determined in relation to the internal standard (IS) L-Nle (norleucine) (Erbe and Brückner 1998). Equimolar amounts of amino acids of the standard mixture, including L-Nle, were injected into the GC-MS system. Response factors were calculated according to Eq. 2

$$f_R = A_{\text{LAA}}/A_{\text{IS}} \quad (2)$$

where f_R is the response factor of the amino acid to be determined, A_{LAA} the peak area of amino acid to be determined, and A_{IS} the peak area of the IS obtained from the standard amino acid mixture. Amino acids in samples were quantified according to the Eq. 3

$$c_{\text{LAA}} = 1/f_R x (A_{\text{LAA}}/A_{\text{IS}}) x\, c_{\text{IS}}, \quad (3)$$

which is equal to

$$c_{\text{LAA}} = (c_{\text{IS}}/f_R) x (A_{\text{LAA}}/A_{\text{IS}})$$

where c_{LAA} is the amount of L-amino acid, f_R the response factor, A_{LAA} the peak area of the L-amino acid in the sample, A_{IS} the peak area of the IS added to the sample, and c_{IS} the amount of the IS added to the sample. Note that the parantheses in Eq. 3 in previous reports have not been placed correctly (Ali et al. 2006; Pätzold and Brückner 2005a, 2006a) but computations were performed according to Eq. 3 above.

If required, from the relative quantities of D-amino acids (%D) and the quantities of L-amino acids c_{LAA} presented in Tables 2, 3 and 4, the absolute quantities of D-amino acid c_{DAA} can be calculated according to Eq. 4.

$$c_{\text{DAA}} = c_{\text{LAA}}(\%\text{D})/(100 - \%\text{D}) \quad (4)$$

Treatment of wine samples

To aliquots of wines (5 mL) the internal standard L-Nle (100 μL of a 10 mM solution in 0.01 M HCl) was added. Samples were adjusted to pH 2.3 by the addition of 0.1 M HCl and slowly passed through Pasteur pipettes equipped with plugs of glass wool and filled with Dowex 50 W X 8 ion exchanger (H^+-form; bed volume 0.5 × 3.5 cm). AAs were eluted with 4 M aqueous NH_3 and converted into *N* (*O*)-pentafluoropropionyl 2-propyl esters as described previously (Ali et al. 2006; Pätzold and Brückner 2005a, b, 2006a, b).

Sources and storage of wine

Bottled wines were commercial products; names, vintages and analytical data of the wines are compiled in Table 1. Wines were analyzed in 2003. Bottles were corked and stored horizontally in the cellar of the senior author at an average temperature of about 15°C. Even the old wines did not show any turbidity and were still palatable. Contents of alcohol (% by volume) are given as indicated on bottle labels. The pH was measured using a calibrated glass electrode and quantities of glucose (Glc) and fructose (Fru) were determined photometrically using an enzyme assay (catalog no.0716260; R-Biopharm, Darmstadt, Germany).

Results

The wines compiled in Table 1 are characterized with regard to type, geographic and regional origin, vintage, contents of ethyl alcohol (EtOH), measured pH and contents of glucose and fructose. Contents of EtOH ranged from 7.5 to 14.0% by volume and the pH ranged from 2.64 to 4.10. The quantities of reducing sugars in wines aged up to 10 years ranged from 0.8 to 10.4 g L^{-1} Glc and 1.0–13.0 g L^{-1} Fru. Quantities of Glc and Fru were larger in sparkling wine, approaching 15.4 g L^{-1} Glc and 17.7 g L^{-1} Fru in French sparkling wine no. 23 (see "Discussion"). Total quantities of L-amino acids (including non-chiral Gly and the typical plant amino acid GABA) and relative quantities of the corresponding D-amino acids determined by GC-SIM-MS are presented in Tables 2, 3 and 4. Representative chromatograms of wines aged for 19 years (no. 20) and 36 years (no. 16) are shown in Fig. 1.

Among L-amino acids, Pro is the most abundant in wines (0.49–2.00 g L^{-1}) followed by L-Ala (0.01–0.39 g L^{-1}), Glx (0.05–0.48 g L^{-1}) and Asx (0.01–0.28 g L^{-1}). Lower

Table 1 Characterization of wines

No[a]		Vintage	Alcohol (% vol)	pH	Glc g L^{-1}	Fru g L^{-1}
1	Spätlese[b], Rheinhessen, 'Erben', Germany	2003	10.0	3.10	9.1	12.5
2	Riesling, Qualitätswein, Mosel-Saar-Ruwer, 'Erben', Germany	2003	11.0	2.80	8.6	11.2
3	Riesling, Qualitätswein, 'Grünes Land', Germany	2003	11.5	2.70	7.3	11.2
4	Riesling, Kabinett, Mosel-Saar-Ruwer, 'Klüsserather Bruderschaft', Germany	2003	10.5	2.83	5.6	6.5
5	Riesling, Spätlese, Mosel-Saar-Ruwer, 'Bernkastler Kurfürstlay', Germany	2002	8.0	2.74	7.7	8.3
6	Riesling, Qualitätswein, Mosel-Saar-Ruwer, 'Bernkastler Kurfürstlay', Germany	2001	9.0	3.10	3.0	3.7
7	Müller-Thurgau, Qualitätswein, Mosel-Saar-Ruwer, 'Moselland Akzente', Germany	2001	9.5	3.17	2.6	5.1
8	Riesling, Spätlese, 'Erdener Treppchen', Germany	2000	7.5	3.16	2.6	2.8
9	Riesling, Kabinett, 'Zeltinger Himmelreich', Germany	1999	8.5	2.64	0.8	1.0
10	Riesling, Spätlese, 'Reiler Goldlay', Germany	1997	11.0	3.10	0.9	2.8
11	Riesling, Kabinett, 'Bopparder Hamm-Ohlenberg', Germany	1986	12.0	3.31	0.8	1.0
12	Riesling, Spätlese, 'Reiler vom heißen Stein', Germany	1983	9.0	3.33	1.1	1.9
13	Kerner, Spätlese, Rheinhessen, 'Sprendlinger Hölle', Germany	1981	8.5	3.47	0.4	0.5
14	Silvaner, Kabinett, 'Birkweiler Kastanienbusch', Germany	1981	9.5	3.41	1.0	0.6
15	Ruländer, Baden, 'Leiselheimer Vulkanfelsen', Germany	1979	9.0	4.10	0.5	0.1
16	Beerenauslese, 'Erdener Treppchen', Germany	1967	8.5	3.70	0.2	0.3
17	Tempranillo, Cabernet Sauvignon, 'Osborne Solaz', Spain	2001	13.0	3.35	10.4	13.0
18	'Chateau Smith Haut Lafitte', France	1994	–	3.60	1.8	2.3
19	Tempranillo, Cabernet Sauvignon, Grand Reserva 'Tarragona', Spain	1994	14.0	3.63	0.4	1.0
20	'Caves de Sarragan', AOC, France	1984	13.0	3.80	0.2	0.3
21	Eiswein, Silvaner, Riesling, Rheinhessen, 'Mainzer Domherr', Germany	2001	7.5	3.44	9.9	10.2
22	Eiswein, Riesling, Württemberg, 'Reiner Altenberg', Germany	1979	–	3.50	2.6	2.8
23	'Napoleon, blanc de blanc', France	2002	10.5	3.00	15.4	17.7
24	'Napoleon, muscat rosé', France	2001	11.0	3.20	12.9	14.4
25	'Kupferberg Gold', Germany	2002	11.0	2.71	12.5	15.3
26	'Henkel Trocken', Germany	2002	11.5	2.67	11.7	13.0

[a] Wines were analyzed in 2003; nos. 1–16 represent white wines; nos. 17–20 red wines; nos. 21 and 22 ice wines and nos. 23–26; sparkling wines;– not given

[b] German quality wines are classified as table wines (Tafelwein), quality wines with vintage (with all the required characteristics of the growing region), and special high-quality wines, denoted according to increasing quality as Kabinett, Spätlese, Auslese, Beerenauslese and for the top quality as Trockenbeerenauslese. Eisweine (ice wines) are prepared from frozen grapes harvested at a temperature below −7°C. The sparkling wines are produced by the tank fermentation process, where a second fermentation of base wines is carried out with the addition of sugar and yeast at a pressure of about 7 bars over a 3–4-week period

Table 2 Quantities of amino acids (L) (mg L^{-1}) and relative amounts (D) (%) in white wines (nos. 1–16)

	1		2		3		4		5		6		7		8	
	L	% D	L	% D	L	% D	L	% D	L	% D	L	% D	L	% D	L	% D
Ala[a]	134	2.9	30.4	1.6	88.0	1.9	94.1	0.5	132	0.4	39.1	0.5	142	0.7	72.2	2.4
Val	38.1	0.0	2.4	0.0	27.2	0.0	19.5	0.0	59.0	0.0	14.1	0.0	32.8	0.0	27.8	0.0
Thr	72.6	0.0	4.7	0.0	40.1	0.0	17.0	0.0	74.3	0.0	31.6	0.0	41.0	0.0	28.1	0.0
Gly[b]	53.3	–	9.3	–	50.7	–	27.5	–	59.1	–	35.2	–	24.6	–	85.6	–
Ile	1.0	0.0	1.1	0.0	22.4	0.0	15.8	0.0	39.8	0.0	27.5	0.0	22.6	0.0	17.8	0.0
Pro	890	0.0	489	0.0	904	0.0	914	0.0	701	0.0	1,269	0.0	631	0.0	967	0.2
Leu	9.6	0.0	1.0	0.0	14.4	0.0	9.1	0.0	16.7	0.0	14.2	0.0	8.9	0.0	9.3	0.0
Ser	22.3	0.0	2.4	0.0	18.6	0.0	4.4	0.0	23.1	0.0	15.6	0.0	6.3	0.0	11.0	0.0
GABA[b]	232	–	2.7	–	65.4	–	17.0	–	92.3	–	170.1	–	19.4	–	10.1	–
Asx	120	1.0	12.0	5.8	122	1.4	64.6	1.9	109	0.9	239	1.2	28.8	1.1	87.5	2.2
Met	12.0	0.0	2.0	0.0	15.2	0.0	7.0	0.0	12.8	0.0	26.1	0.0	3.0	0.0	6.2	0.0
Phe	53.7	0.0	6.2	0.0	57.1	0.0	33.6	0.0	83.4	0.0	129	0.3	21.1	0.0	46.2	0.0
Glx	166	1.5	25.2	3.1	142	8.9	68.5	4.3	134	0.5	304	1.2	24.5	1.6	114	1.6
Tyr	71.1	0.0	7.1	0.0	51.4	0.0	19.7	0.0	65.0	0.0	67.3	0.0	5.4	0.0	36.1	0.0
Orn	97.3	0.0	2.4	0.0	44.7	0.0	3.5	0.0	34.0	0.0	33.0	0.3	2.8	0.0	4.2	0.0
Lys	75.2	0.0	4.0	0.0	91.8	0.0	27.2	0.0	63.8	0.0	112	0.9	3.5	0.0	118	0.0

	9		10		11		12		13		14		15		16	
	L	% D	L	% D	L	% D	L	% D	L	% D	L	% D	L	% D	L	% D
Ala[a]	11.7	3.3	46.2	3.9	337	0.6	87.0	0.5	318	0.7	119	1.0	232	3.9	61.3	2.1
Val	3.1	0.0	13.6	0.0	54.7	0.0	24.2	0.0	143	0.0	38.0	0.0	34.3	0.0	33.6	0.0
Thr	2.2	0.0	17.4	0.0	199	0.0	60.0	0.0	256	0.0	54.5	0.0	48.1	0.0	40.5	0.0
Gly[b]	17.3	–	35.8	–	129	–	43.4	–	14.0	–	67.2	–	74.1	–	64.2	–
Ile	2.8	0.0	15.7	0.0	34.7	0.0	25.1	0.0	109	0.0	26.3	0.0	24.6	0.0	27.0	0.0
Pro	1,237	0.2	1,017	0.2	928	0.1	1,595	0.2	1,732	0.2	1,019	0.3	1,745	0.3	1,308	0.7
Leu	1.7	0.0	10.0	0.0	20.1	0.0	5.5	0.0	0.0	0.0	65.0	0.0	14.2	0.0	59.0	0.0
Ser	6.1	0.0	8.1	0.0	39.3	0.0	140	0.7	364	0.0	19.1	11.5	82.8	0.0	8.4	0.0
GABA[b]	2.5	–	62.6	–	0.0	–	169	–	0.0	–	77.6	–	0.0	–	64.2	–
Asx	69.3	8.3	120	3.3	72.5	3.9	191	2.7	633	2.1	127	2.5	262	3.8	94.0	2.1
Met	6.6	0.0	19.2	0.0	26.3	0.0	22.8	0.0	61.5	0.0	20.6	0.0	12.9	0.0	16.1	0.0
Phe	39.0	4.3	101	2.0	88.7	1.4	105	0.2	343	1.0	58.0	0.2	45.8	0.6	56.4	0.5
Glx	113	3.8	123	4.3	419	1.1	200	0.7	573	0.9	123	0.8	73.2	3.1	94.0	2.1
Tyr	20.4	0.0	57.0	0.0	139	0.0	96.1	0.0	273	0.0	60.7	0.0	39.0	0.0	88.0	0.0
Orn	4.3	0.0	21.5	0.0	66.6	3.6	28.0	1.8	154	0.7	30.0	1.3	199	1.8	8.4	0.0
Lys	28.2	0.0	80.6	2.1	117	0.0	146	0.7	368	0.3	92.9	0.3	27.5	1.9	68.0	0.3

[a] Amino acids in Tables 2, 3 and 4 are listed according to their order of elution from the GC column

[b] Non-chiral amino acids: data presented in the tables are the average of two sample workups and the two injections. Detection limit about 0.05–0.1% D-enantiomer

quantities of other L-amino acids are also detected (Tables 2, 3, 4). The data on quantities of amino acids in wines agree with those reported in the literature, with the restriction that Arg, representing together with Pro a major amino acid, could not be determined in this study. With regard to the aim of the work, emphasis in the following was made on the relative quantities of D-amino acids in relation to bottling (storage) age and type of the wines. Analyses of white wines (nos. 1–16) are presented in Table 2. Despite the fact that L-Pro is the most abundant amino acid in wines, no D-Pro was detected in white wines nos. 1–7. In wines nos. 8–15, relative quantities did not exceed 0.3%, and in wine no. 16 quantities of just 0.7% D-Pro could be detected. The GC-SIM-MS of this wine (no. 16) stored for 36 years is presented in Fig. 1a. The insert shows the section of a chromatogram resulting from

Table 3 Quantities of amino acids (L; mg L^{-1}) and relative amounts (D; %) in red wines (nos. 17–20) and ice wines (nos. 21–22)

	17		18		19		20		21		22	
	L	% D	L	% D	L	% D	L	% D	L	% D	L	% D
Ala[a]	150	2.9	213	5.5	185	5.4	161	10.6	369	1.1	390	1.5
Val	9.5	0.0	28.1	0.0	34.0	0.0	50.0	0.0	156	0.0	190	0.0
Thr	23.2	0.0	48.3	0.0	20.0	0.0	48.1	0.0	139	0.0	180	0.0
Gly[b]	61.1	–	106	–	90.7	–	140	–	98.3	–	144	–
Ile	5.4	0.0	24.6	0.0	27.0	0.0	28.4	0.0	88.1	0.0	134	0.0
Pro	1,605	0.1	1,515	0.1	1,689	0.2	1,533	0.2	680	0.1	1,084	0.9
Leu	6.2	0.0	49.1	0.0	91.6	0.0	54.1	0.0	28.5	0.0	66.1	0.0
Ser	8.1	0.0	17.7	0.0	0.0	0.0	22.8	0.0	28.4	0.0	121	0.0
GABA[b]	166	–	70.0	–	101	–	72.3	–	154	–	356	–
Asx	89.0	2.6	84.2	7.7	276	2.2	110	10.9	78.3	0.9	190	3.0
Met	12.0	0.0	9.0	0.0	21.5	0.0	10.3	0.0	14.0	0.0	33.0	0.0
Phe	32.2	0.9	42.4	0.2	128	0.5	36.7	1.0	69.1	0.0	182	1.3
Glx	135	3.9	130	4.6	475	5.0	135	7.4	84.0	1.4	199	6.4
Tyr	15.5	0.0	61.2	0.0	51.5	0.0	46.4	0.0	21.7	0.0	137	0.0
Orn	45.1	1.4	68.7	1.5	95.2	1.8	4.2	7.1	12.9	0.0	70.5	2.8
Lys	38.9	0.0	87.4	0.0	319	0.6	60.0	0.5	6.5	0.0	72.0	0.6

Table 4 Quantities of amino acids (L) (mg L^{-1}) and relative amounts (D) (%) in sparkling wines (nos. 23–26)

	23		24		25		26	
	L	% D	L	% D	L	% D	L	% D
Ala[a]	10.2	9.8	44.5	9.0	107	5.4	64.8	2.2
Val	2.3	0.0	6.3	0.0	39.2	0.0	19.0	0.0
Thr	5.0	0.0	7.2	0.0	31.9	0.0	23.4	0.0
Gly[b]	25.1	–	40.4	–	80.3	–	84.5	–
Ile	4.9	0.0	3.3	0.0	11.8	0.0	9.0	0.0
Pro	1,203	0.0	2,000	0.1	1,906	0.1	1,245	0.1
Leu	3.2	0.0	1.3	0.0	15.3	0.0	4.7	0.0
Ser	0.0	0.0	3.5	0.0	0.0	0.0	12.6	0.0
GABA[b]	46.2	–	42.4	–	87.4	–	87.4	–
Asx	91.4	4.4	27.5	4.4	96.4	2.3	72.3	2.1
Met	5.1	0.0	1.5	0.0	8.4	0.0	7.5	0.0
Phe	34.3	0.3	7.0	1.4	51.6	0.0	31.5	0.0
Glx	108	6.1	49.4	6.1	93.1	2.2	176	1.3
Tyr	20.3	0.0	23.1	0.0	68.1	0.0	65.5	0.0
Orn	55.0	1.8	68.4	1.5	44.1	2.5	38.4	0.0
Lys	83.4	1.6	26.6	1.1	89.1	0.0	80.2	0.5

an about 30-fold dilution of derivatized analytes in order to quantify Pro enantiomers.

Analyses of red wines and ice wines are presented in Table 3. In red wines, relative quantities of D-Pro (0.1–0.2%), D-Ala (2.9–10.6%), D-Asx (2.0–10.9%), and D-Glx (3.9–7.4%) could be determined. The GC-SIM-MS of the French red wine no. 20, stored for 19 years, is shown in Fig. 1b. The insert shows the section of a chromatogram resulting from the analysis of a sample diluted about 30-fold in order to calculate the relative quantities of D-Pro.

Among the two ice wines investigated, the wine stored for 24 years (no. 22) contained relative quantities of 0.9% D-Pro, 1.5% D-Ala, 3.0% D-Asx and 6.4% D-Glx together with a few other D-amino acids. Just 0.1% D-Pro was detected in the ice wine no. 21 stored for 2 years, together with a few other D-amino acids in the low percentage range. Data of sparkling wines (nos. 24–26) are compiled in Table 4. No or 0.1% D-Pro was detected together with D-Ala (2.2–9.8%), D-Asx (2.1–4.4%), and D-Glx (1.3–6.1%) and low amounts of a few other D-amino acids. Quantities of L-amino acids were much lower in sparkling wines in comparison to white and red table wines.

Discussion

Chiral analyses of white and red wines, special ice wines and sparkling wines were performed with the aim of determining whether or not there was a correlation between the type of wine and storage time following the bottling of wine. The period prior to bottling of wine is named maturation and the storage time following bottling is called aging. Comparatively few reports deal with the enantioselective analysis of wine amino acids. Chaves das Neves and Noronha (1995) investigated elementary (base) wines made from the Portuguese grape cultivar 'Roupeiro' under controlled conditions. The authors determined the ratio of amino acid enantiomers using GC on Chirasil®-L-Val.

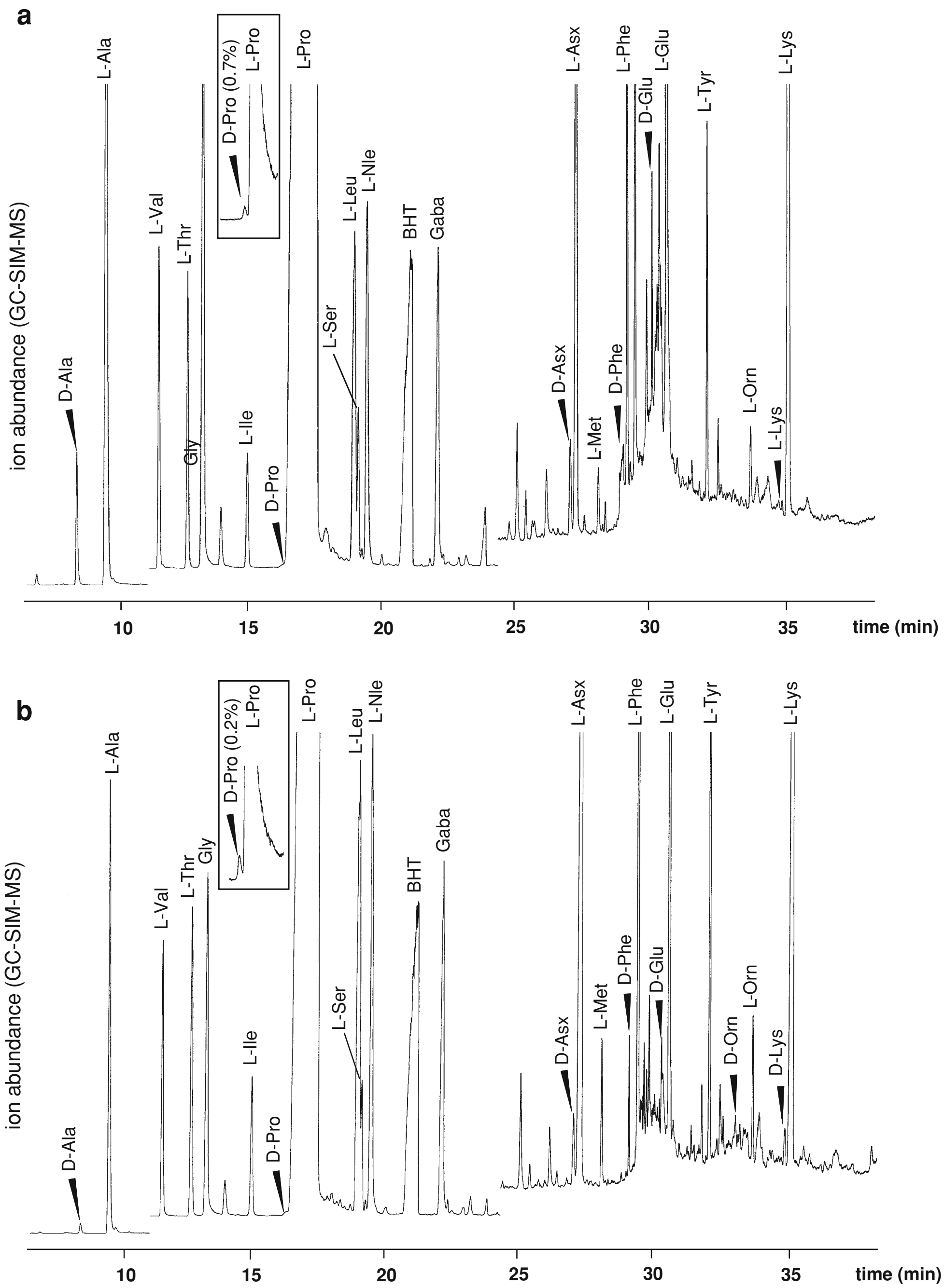

Fig. 1 GC-SIM-MS of *N*(*O*)-pentafluoropropionyl 2-propyl esters of amino acids of (**a**) German white wine no. 16 (‘Erdener Treppchen’, vintage 1967) stored for 36 years, and (**b**) of French red wine no. 20 (‘Caves de Sarragan’, vintage 1984) stored for 19 years (analyses were performed in 2003). Inserts represent sections of chromatograms of analytes diluted to about 1:30 in order to resolve the proline enantiomers. BHT refers to 3,5-di-*tert*-butyl-4-hydroxytoluene (2,6-di-*tert*-butyl-*p*-cresol) added as antioxidant before the derivatization procedures (Erbe and Brückner 1998; Pätzold and Brückner 2005a)

After 1 year of maturation in large storage containers made of concrete, the wines were bottled and analyzed regularly for an aging period of 11 years. Amounts of ≥60% D-Ala were already detected in wines aged only a few years, together with ≥20% of the D-enantiomers of Val, Leu, Ser, Asx, Met, Phe and Glx. Notably, no D-Pro was detected. Those authors state that it was not possible to correlate bottling age and quantities of D-amino acids. Calabrese et al. (1995) determined the quantities of L- and D-Pro in 23 well-characterized Italian red and 7 white wines using HPLC following pre-column derivatization with chiral Marfey’s reagent (for a review of the popular methods see Bhushan and Brückner 2004). The authors reported that young wines up to 2 years did not contain detectable amounts of D-Pro, but wines of 2–5 years of age had about 0.2% D-Pro. Quantities of D-Pro in older wines increased and amounted to about 15% D-Pro in a red wine aged for 15 years. Kato et al. (1995) investigated several white, red and rosé wines for the presence of D-amino acids by chiral phase HPLC using pre-column derivatization with 4-fluoro-7-nitro-2,1,3-benzoxadiazole. The authors detected the presence of D-Ala in all wines and of D-Leu and D-Ile in certain wines amounting to 25.5, 7.5 and 5.0%, respectively. They did not report on the detection of D-Pro. Using capillary electrophoresis together with chiral cyclodextrin additives, Tsunoda et al. (1999) determined 2.19% D-Asp in a red wine and 3.21% D-Asp in a white wine. Using a fluorescent chiral derivatizing reagent and HPLC, Jin et al. (1999) determined 4.0% D-Ala and 10.1% D-Glu in a wine sample, but no D-Pro was detected. The latter authors focused on the analytical methodology of amino acid enantioseparation. Consequently, the wines were not further characterized.

In a continuing work Calabrese and Stancher (1999) studied again the isomerization of Pro in Italian 'Merlot' and 'Cabernet' wines using Marfey's reagent and found an increase of the D/L ratio of Pro with bottling age. From the data, they deduced a first-order kinetic process considering an aging period of about 10 years and postulated an increasingly overlaying microbial (enzymatic) activity in bottled wines aged for more than 10 years.

In a previous work, we had found that in particular fortified wines (desert or liquor wines) such as sherry, port, Madeira or marsala contain high relative amounts of D-amino acids (Pätzold et al. 2003). However, no D-Pro or quantities exceeding 0.8% could be detected in 23 sherry wines. In the work presented here, white and red table wines of known bottling age were analyzed. For comparison two special ice wines and four sparkling wines were also analyzed. In French sparkling wines, D-Ala approached 9.0 and 10.2%, respectively. Low amounts of other D-amino acids were also detected but quantities of D-Pro did not exceed 0.1%. In the ice wine stored for 24 years 0.9% D-Pro was detected and in the ice wine stored for 3 years, only 0.1% D-Pro. No or low amounts of other D-amino acids were found in these wines.

In summary, from the data of wines compiled in Table 1, no correlation between storage time of bottled wines and relative quantities of D-amino acids presented in Tables 2, 3 and 4 could be deduced. In particular, the quantities of D-Pro do not correlate with the age of the wines. Thus, it appears that the origin and composition of grapes, specific vinification procedures and individual treatments and processing of grape musts are responsible for the D-amino acids detectable in the resulting wines. Bacteria are commonly present in musts and consequently are integrated in the winemaking process. Therefore, D-amino acids will be formed inevitably. This has been shown for e.g., lactobacilli and for the malolactic fermenting *Leuconostoc oenos* in wine (Brückner et al. 1993). Further, it should be stressed that amino acids are important nitrogen sources for the growth of yeasts that are responsible for the alcoholic fermentation of grape must. Consequently, the quantities of amino acids decrease on fermentation. It was reported that strains of yeasts involved in winemaking such as *S. cerevisiae* and *S. uvarum* or *Metschnikowia kamienski* metabolize L-amino acids, but not D-amino acids such as D-Ala, D-Asp, D-Glu and D-Pro (LaRue and Spencer 1967). In contrast, *Hansenula anomala* metabolizes D-Ala and D-Glu, but not D-Asp and D-Pro. Notably, species of yeasts and their respective frequency change in the course of the winemaking and maturation process. Furthermore, some yeasts contain D-amino acid oxidases or D-amino acid acetylases, the action of which will affect the ratio of free D- and L-amino acids. The relative high abundance of D-Ala, D-Asp and D-Glu in sparkling wines (Table 4) might be the result of a second fermentation of the base wines with strains of special yeasts such as *S. bayanus*. This process is commonly carried out with the addition of invert sugar, a mixture of glucose and fructose (see legend of Table 1).

It had also been demonstrated that intermediates of the Maillard reaction, in particular the fructose amino acids resulting from the AMADORI rearrangement of *N*-glycosyl amino acids, are potential intermediates of amino acid racemization (Brückner et al. 2001; Ali et al. 2006; Pätzold and Brückner 2006a, b; Kim and Lee 2008, 2009). The Maillard reaction proceeds also in the course of maturation and aging of wines (Jackson 2000) and might contribute to the formation of D-amino acids in wines. In spite of that, no correlation could be deduced from the contents of glucose and fructose in table wines (see Table 1) and the D-amino acids determined therein.

These facts outlined above make the reliable interpretation of data resulting from the enantioanalysis of amino acids in wines of unknown vinification and manufacturing history extremely difficult. In conclusion, quantities and kinds of D-amino acids are not considered suitable markers for the age dating of bottled wines.

Acknowledgment H·S.M.A. thanks the Arab Republic of Egypt for a scholarship.

References

Ali H, Pätzold R, Brückner H (2006) Determination of L- and D-amino acids in smokeless tobacco products and tobacco. Food Chem 99:803–812. doi:10.1016/j.foodchem.2005.08.056

Bada JL (1972) Kinetics of racemization of amino acids as a function of pH. J Am Chem Soc 94:1371–1373. doi:10.1021/ja00759a064

Bada JL, Schroder RA (1975) Amino acid racemization reactions and their geochemical implications. Naturwissenschaften 62:71–79. doi:10.1007/BF00592179

Bhushan R, Brückner H (2004) Marfey's reagent for chiral amino acid analysis: a review. Amino Acids 27:231–247. doi:10.1007/s00726-004-0118-0

Brückner H, Hausch M (1989) Detection of free D-amino acids in food by chiral phase capillary gas chromatography. J High Resolut Chromatogr 12:680–684. doi:10.1002/jhrc.1240121012

Brückner H, Hausch M (1990) D-amino acids in dairy products: detection, origin and nutritional aspects 2. Ripened cheeses. Milchwissenschaft 45:421–425

Brückner H, Becker D, Lüpke M (1993) Chirality of amino acids of microorganism used in food technology. Chirality 5:385–392. doi:10.1002/chir.530050521

Brückner H, Justus J, Kirschbaum J (2001) Saccharide-induced racemization of amino acids in the course of the Maillard reaction. Amino Acids 21:429–433. doi:10.1007/s007260170007

Calabrese M, Stancher B (1999) A study of the proline isomerisation in typical Italian wines. J Sci Food Agric 79:1357–1360. doi:10.1002/(SICI)1097-0010(199908)79:11<1357::AID-JSFA371>3.0.CO;2-3

Calabrese M, Stancher B, Riccobon P (1995) High performance liquid chromatography determination of proline isomers in Italian wines. J Sci Food Agric 69:361–366. doi:10.1002/jsfa.2740690313

Chaves das Neves HJ, Noronha JP (1995) Recognition of vinification technology through gas chromatographic data on enantiomeric purity of free amino acids. Fresenius J Anal Chem 352:783–787. doi:10.1007/BF00323064

Erbe T, Brückner H (1998) Chiral amino acid analysis of vinegars using gas chromatography—selected ion monitoring mass spectrometry. Z Lebensm Unters Forsch A 207:400–409. doi:10.1007/s002170050352

Friedman M (1999) Chemistry, nutrition, and microbiology of D-amino acids. J Agric Food Chem 47:3457–3479. doi:10.1021/jf990080u

Herbert P, Barros P, Ratola N, Alves A (2000) HPLC determination of amino acids in musts and port wine using OPA FMOC derivatives. J Food Sci 65:1130–1133. doi:10.1111/j.1365-2621.2000.tb10251.x

Hernández-Orte P, Ibarz MJ, Cacho J, Ferreira V (2003) Amino acid determination in grape juices and wines by HPLC using a modification of the 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC) method. Chromatographia 58:29–35

Jackson RS (2000) Wine science. Chemical constituents of grapes and wine, Academic Press, San Diego, pp 232–280

Jin D, Miyahara T, Oe T, Toyo'oka T (1999) Determination of D-amino acids labeled with fluorescent chiral reagents, R(−)- and S(+)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(*N*, *N*-dimethylaminosulfonyl)-2,1,3-benzoxadiazoles, in biological and food samples by liquid chromatography. Anal Biochem 269:124–132. doi:10.1006/abio.1998.3090

Kato M, Fukushima T, Santa T, Homma H, Imai K (1995) Determination of D-amino acids, derivatized with 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F), in wine samples by high-performance liquid chromatography. Biomed Chromatogr 9:193–194. doi:10.1002/bmc.1130090409

Kim J-S, Lee Y-S (2008) Effect of reaction pH on enolization and racemization reactions of glucose and fructose on heating with amino acid enantiomers and formation of melanoidins as a result of the Maillard reaction. Food Chem 108:582–592. doi:10.1016/j.foodchem.2007.11.014

Kim J-S, Lee Y-S (2009) Enolization and racemization reactions of glucose and fructose on heating with amino-acid enantiomers and the formation of melanoidins as a result of the Maillard reaction. Amino Acids 36:465–474. doi:10.1007/s00726-008-0104-z

Konno R, Brückner H, D'Aniello A, Fisher G, Fujii N, Homma H (eds.) (2007) D-Amino acids—a new frontier in amino acids and protein research. Practical methods and protocols. Nova Science, New York

LaRue TA, Spencer JFT (1967) The utilization of D-amino acids by yeasts. Can J Microbiol 13:777–788

Marchelli R, Dossena A, Palla G (1996) The potential of enantioselective analysis as a quality control tool. Trends Food Sci Technol 7:112–119. doi:10.1016/0924-2244(96)10011-X

Moreno-Arribas V, Pueyo E, Polo MC, Martin-Alvarez P (1998) Changes in the amino acid composition of the different nitrogenous fractions during the aging of wine with yeasts. J Agric Food Chem 46:4042–4051

Palla G, Marchelli R, Dossena A, Casnati G (1989) Occurrence of D-amino acids in food. J Chromatogr 475:45–53

Pätzold R, Brückner H (2005a) Chiral separation of amino acids by gas chromatography. In: Molnár-Perl I (ed) Quantitation of amino acids and amines by chromatography. Journal of Chromatography Library, vol 70. Elsevier, Amsterdam, pp 98–118

Pätzold R, Brückner H (2005b) Mass spectrometric detection and formation of D-amino acids in processed plant saps, syrups, and fruit juice concentrates. J Agric Food Chem 53:9722–9729

Pätzold R, Brückner H (2006a) Gas chromatographic determination and mechanism of formation of D-amino acids occurring in fermented and roasted cocoa beans, cocoa powder, chocolate and cocoa shell. Amino Acids 31:63–72

Pätzold R, Brückner H (2006b) Gas chromatographic detection of D-amino acids in natural and thermally treated bee honeys and studies on the mechanism of their formation as a result of the Maillard reaction. Eur Food Res Technol 223:347–354

Pätzold R, Nieto-Rodriguez A, Brückner H (2003) Chiral gas chromatography analysis of amino acids in fortified wines. Chromatographia Suppl 57:S207–S211

Tsunoda M, Kato M, Fukushima T, Santa T, Homma H, Yanai H, Soga T, Imai K (1999) Determination of aspartic acid enantiomers in bio-samples by capillary electrophoresis. Biomed Chromatogr 13:335–339