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D-Amino Acid Formation in Sterilized Alkali-Treated Olives

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The occurrence of D-amino acids in commercial ripe olives, a well-known sterilized alkali-treated product, was investigated by high-performance liquid chromatography (HPLC) with precolumn automatic derivatization. Absolute amounts of D-amino acids were in total 18.6−38.2 mg/100 g edible portion. The major D-amino acids were D-aspartic acid, D-glutamic acid, D-serine, and D-leucine. Furthermore, to evaluate the effects of sterilization time and olive pH on amino acid racemization, a simulated processing of green ripe olives was carried out. Serine (both free and bound form) was the most-racemized amino acid after heat treatment. Sterilization (15−35 min at 121 °C) increased the racemization values of both free and protein-bound amino acids, although in case of protein-bound phenylalanine the increase was not statistically significant. With an increase of pH from 8 to 10 units, the racemization values of all amino acids increased significantly, except for free forms of aspartic and glutamic acids. In general, the effects of the sterilization time and olive pH on total concentration (L + D enantiomers) of each amino acid were also significant.

KEYWORDS: D-Amino acids; table olive; alkaline treatment; sterilization; racemization

INTRODUCTION

Alkali-treated olives are the most important class of table olive commercialized in the world (*1*). They include different and well-known processing types, mainly Spanish-style green olives and black (or green) ripe olives. One important difference between these two processing types is that ripe olives are a low-acid product; therefore, a sterilization treatment is mandatory for proper preservation. Briefly, in the case of black ripe olives, the olives are processed in a lye solution, which leaches out the bitterness. After that, a series of cold water rinses removes all traces of lye solution. During this process, which takes several days, a flow of air bubbling through the olives produces the natural rich dark color. A trace of organic iron salt (ferrous gluconate) is added to act as a color fixer. Finally, the ripe olives are canned in a mild salt brine and are heat sterilized. The process is the same for green ripe olives, except that they are not exposed to air (oxidized) during processing and so retain their green color. Apart from ripe olives, very few heated alkali-treated foods are known (an example is tortillas of maize).

Excepting the destruction of particular vitamins, the major chemical reactions that occur during food processing are those that proteins undergo (*2*). During heat treatment under alkaline conditions, the conversion of free or protein-bound L-amino acids into their mirror images (enantiomers), named D-amino acids, and other reactions (e.g., cross-linking in the protein, Maillard reaction) may occur (*3*), which tend to make the amino acids less available and, in general, the protein less digestible (*4*). The process that changes the chirality of amino acids is commonly referred to as racemization, although in the strict sense, racemized amino acids consist of exactly equal amounts of the D- and L-enantiomers. Although amino acid racemization in heated alkali-treated proteins has been extensively studied (*5*−*8*), racemization in whole food subjected to conditions similar to those that may occur in actual processing has scarcely been reported. Chang et al. (*9*) investigated amino acid racemization in alkali-treated duck eggs, a popular food in China. Although the total protein content of table olives is low (<2 g/100 g) (*10*), the content of D-amino acids in heated alkali-treated olives could be relatively high in comparison with other processed foods, especially at extreme process conditions (high olive pH or long heating times).

Available analytical methods for analysis of D-amino acids include gas chromatographic (GC) methods, high-performance liquid chromatographic (HPLC) methods, high-performance capillary electrophoretic (HPCE) methods, and enzymatic methods (*11*), although HPLC is predominant in foods. Among the various HPLC methods proposed for analysis of D-amino acids in food samples, that using *o*-phthaldialdehyde (OPA) in combination with a chiral thiol such as *N*-isobutyryl-L- or D-cysteine (IBLC or IBDC) has been tested thoroughly with respect to its robustness and reliability in various matrixes (*12*).

The objectives of the present work were to examine the relative and absolute amounts of D-amino acids, obtained using the above-mentioned HPLC method, in commercial black ripe olives, and to study the amino acid racemization during sterilization treatment of alkali-treated olives, evaluating the effects of time of heat treatment and pH of olives in a simulated process.

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Table 1. Chemical Characteristics (in Cover Liquor) of Commercial Presentations of Black Ripe Olives Analyzed in the Present Study

	black ripe olives[a]						
	A	B	C	D	E	F	G
presentation	plain	pitted	plain	plain	plain	plain	slices
pH	6.68	6.46	5.93	5.98	5.98	6.17	6.16
titratable acidity (% as lactic acid)	0.04	0.04	0.10	0.08	0.12	0.15	0.04
salt (% NaCl)	2.90	2.72	3.34	2.13	2.08	2.02	2.73

[a] Company codes are identified by letters A–G.

MATERIALS AND METHODS

Reagents. Deionized water was obtained from a Milli-Q system (Millipore, Bedford, MA). The D- and L-amino acids standards were of the highest available purity from Sigma (St. Louis, MO) and included aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), serine (Ser), glutamine (Gln), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), alanine (Ala), tyrosine (Tyr), valine (Val), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), and lysine (Lys). The internal standard D-alpha-amino-*n*-butyric acid (D-Abu) was obtained from Aldrich (Milwaukee, WI). *o*-Phthaldialdehyde (OPA) was supplied by Sigma. *N*-Isobutyryl-L-cysteine (IBLC) and *N*-isobutyryl-D-cysteine (IBDC), both with an optical purity ≥ 99.5%, were obtained from Fluka (Buchs, Switzerland). The amino acid L-*homo*-arginine (L-*homo*-Arg) was also from Fluka. All other chemicals and solvents were of analytical or chromatographic grade from several suppliers. The OPA/IBLC or OPA/IBDC reagent was dissolved in 1 M potassium borate buffer at pH 10.4 (170 mM OPA and 260 mM IBLC or IBDC). The reaction buffer was 0.4 M borate buffer, pH 10.4. The mobile phase for HPLC analysis of amino acids consisted of (A) 23 mM sodium acetate adjusted to pH 5.95 with 10% (v/v) acetic acid and (B) 600 mL methanol plus 50 mL acetonitrile.

Apparatus. The HPLC system consisted of a Jasco model PU-2089 pump (Jasco Corp., Tokyo, Japan), a Jasco model AS-2057 autosampler, a Jasco model FP-920 fluorescence detector (λex = 230 nm, λem = 445 nm), and a computer with Jasco-Borwin chromatography software version 1.5. A Luna 5μ C18(2) (250 × 4.6 mm) column (Phenomenex, Torrance, CA) with a C18 guard column (Phenomenex) was used for separation of D- and L-amino acids. The column was kept at constant temperature in a Gecko-2000 column heater (CIL Cluzeau Info Labo, Paris, France) at 30 °C.

Samples. Commercial packed black ripe olives (A−G) were purchased from local supermarkets. Their chemical characteristics and forms of presentation are shown in **Table 1**. For the experiments to assess the effects of pH of the cover liquor and sterilization time on the formation of D-amino acids, green olives of Hojiblanca cultivar were used. Olives were sorted, and about 5 kg was treated in an experimental fermenter with 4.1 L of lye (2.2% w/v NaOH) for 6 h at 22 °C. This solution was removed, and the fruits were washed with 4.1 L of tap water for 4 h. This washing stage was repeated twice more but with distinct duration (for 15 h and then for 7 h), and finally the olives were packed into glass bottles, using three different cover liquors (packing A−C). The cover liquor of packing A consisted of 0.1 M sodium phosphate buffer at pH 7.5. Liquors of packings B and C (both 0.1 M borate buffer) were adjusted to pH 9.5 and 12.0, respectively. Prior to heating, bottles were stored at 5 °C for 7−13 days to allow equilibration of the cover liquor with the olives. Olive juice at equilibrium had the following pH values: packing A, 8.3; packing B, 9.2; and packing C, 10.3. Duplicate bottles of each packing were heated at 121 °C in a computer-controlled retort (Steriflow SAS, Paris, France) for various lengths of time (15, 21, or 35 min). At the end of the treatment, the bottles were cooled for 1 h in an ice bath and were immediately opened and drained. The corresponding liquid fractions were frozen until analysis. The excess water on the surface of the fruits was removed with a tissue, and the olives were pitted. A portion of pitted olives from each bottle was homogenized and subjected to freeze drying for estimating water content, and the rest was homogenized with the same weight of 1 N HCl (to avoid unwanted reactions of amino acids at alkaline pH) and then was subjected to freeze drying.

Chemical Analyses. The pH, titratable acidity (as percentage of lactic acid), and salt (percentage of NaCl) of cover liquor were determined using the routine methods (*13*). Water content of olives was estimated by weighing aliquots of freeze-dried homogenized samples before and after freeze drying. Fat content was determined using the Soxhlet technique as described previously (*13*).

For determination of total (free + bound) amino acids in commercial ripe olives, 0.1 g of freeze-dried and defatted sample was hydrolyzed with 5 mL of 6 N HCl at 110 °C under nitrogen atmosphere for 24 h. Each hydrolyzate was washed into a 50 mL volumetric flask and was made up to the mark with water. An aliquot was spiked with D-Abu (internal standard) and was dried under vacuum on a rotary evaporator. The dried mass was washed with water and was evaporated to dryness, and 1 mL of 20 mM HCl was added to the residue. The obtained solution was subjected to HPLC analysis after derivatization with OPA together with IBLC or IBDC, according to the method of Brückner et al. (*14*). Twenty microliters of the reaction buffer, 4 μL of reagent, and 8 μL of sample or standard solution were drawn up by the syringe of the autosampler and were mixed in an internal loop of the HPLC system. After 1.5 min, 8 μL of the reaction mixture was automatically injected onto the HPLC column. A linear gradient was applied for 80 min at a flow rate of 1.0 mL/min from 0 to 57% B. The column was then flushed with 80% B for 10 min to remove excess sample and reagents, followed by equilibration in 100% A for 15 min prior to initiating the next injection sequence. Because of the conversion of Asn and Gln into Asp and Glu, respectively, during hydrolysis, the data for Asn plus Asp and Gln plus Glu are reported as Asx and Glx, respectively.

For determination of free amino acids in packed alkali-treated green olives, 0.5 g of freeze-dried and defatted sample was homogenized with 50 mL of 70% (v/v) ethanol and 1 mL of 500 μM D-Abu in an ultrasonic bath for 5 min. The homogenates were centrifuged at 11 900*g* for 15 min at 2 °C, and the supernatants were decanted. The extraction procedure was repeated twice more, using 25 mL of 70% ethanol in each extraction. The supernatants were combined, were dried under vacuum on a rotary evaporator at 45 °C, were resuspended in 10 mL of water, and were filtered through a 0.20 μm filter. The final solution was analyzed by HPLC as described above.

For determination of protein-bound amino acids, 0.5 g of freeze-dried and defatted sample was extracted with 70% ethanol as described above, and the residue without free amino acids was freeze-dried. A portion (0.1 g) was hydrolyzed as described for total amino acids, and the final solution was subjected to HPLC analysis.

All analyses were performed in duplicate.

Statistical Analyses. Computations for basic statistics and analysis of variance (ANOVA) were carried out using the STATISTICA software, version 6.0 (*15*). The Student−Newman−Keuls test was used for means comparisons. Significant differences were determined at the $p < 0.05$ level.

RESULTS AND DISCUSSION

HPLC Method. The coefficient of variation (CV) of the peak areas ($n = 4$) of a standard mixture of amino acids (50 μM of L-amino acids and 2.5 μM of D-amino acids) derivatized with OPA-IBLC was <10% in all cases. For the retention times, CV was <1% for all amino acids. Linearity was observed in all cases ($R > 0.999$) in the range 0.5−500 μM (1−1000 pmol of amino acid injected). Detection limits, calculated from a 1.0 pmol injection and defined as 3 times the signal-to-noise ratio, were between 106 and 514 fmol (data not shown). The identity of the D-amino acids was confirmed using OPA-IBDC instead of OPA-IBLC as derivatizing reagent, reversing the elution of the derivatives of D- and L-amino acids. Under the chromatographic conditions used, when amino acids were derivatized with OPA-IBLC, D-Thr coeluted with Gly and L-*homo*-Arg, a nonprotein amino acid used by others (*12*) as internal standard, coeluted with L-Ala. Therefore, for quantification, we used D-Abu as internal standard, which was well resolved from other amino acids.

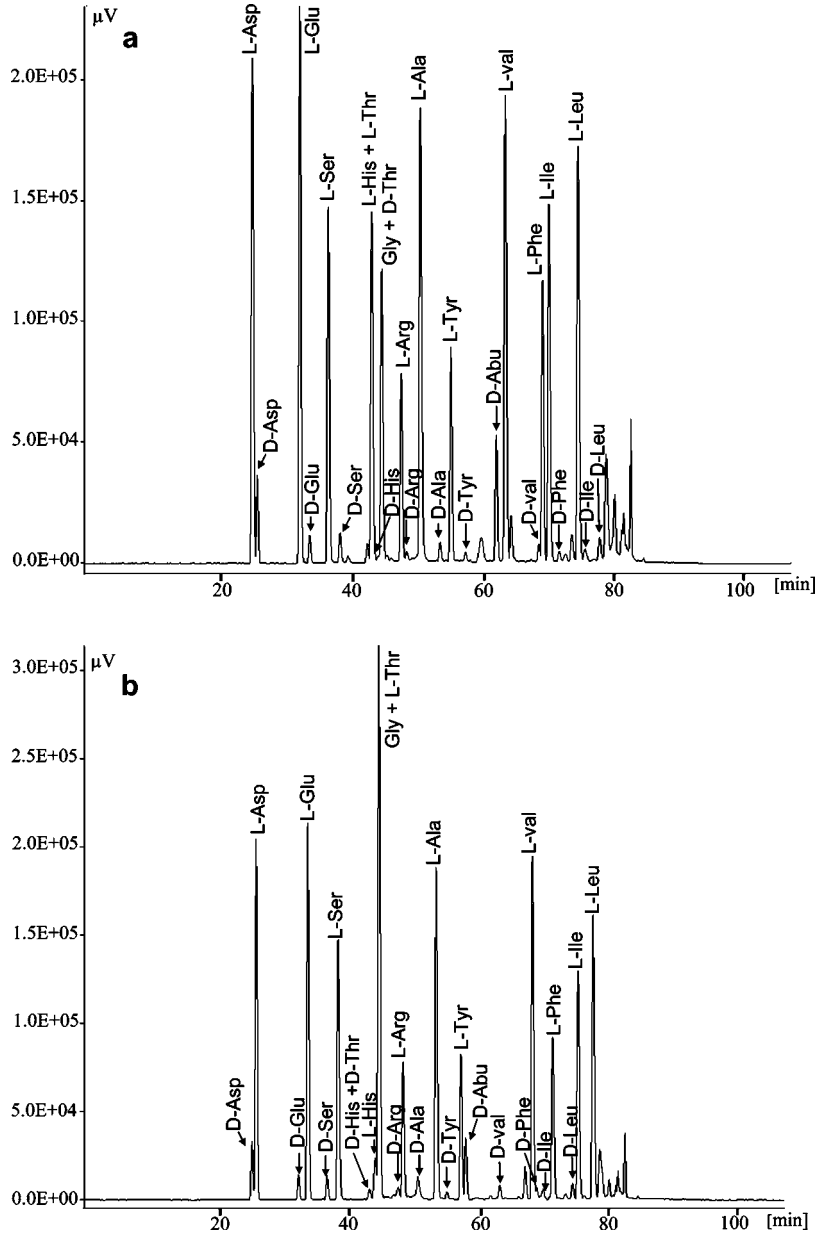

Figure 1. Elution profile of amino acids from a commercial sample of black ripe olives. The sample was totally hydrolyzed in 6 N HCl at 110 °C for 24 h, and amino acids were derivatized with (**a**) OPA-IBLC and (**b**) OPA-IBDC. Arrows mark positions of D-amino acids.

Total (Free + Bound) D-Amino Acids in Commercial Black Ripe Olives. The elution profile of amino acids derivatized with OPA-IBLC or OPA-IBDC from a selected sample of commercial black ripe olives is shown in **Figure 1**. Both the relative and the absolute amounts of D-amino acids in the samples analyzed are shown in **Table 2**. Significant amounts of D-Asx, D-Glx, D-Ser, D-His, D-Arg, D-Ala, D-Tyr, D-Val, D-Phe, D-Ile, and D-Leu were found. D-Lys was not detected. The most-abundant D-amino acids were D-Asx, D-Glx, D-Ser, and D-Leu. Total content of D-amino acids in samples averaged 26 mg/100 g edible portion. Total amino acids (L + D enantiomers) averaged 617 mg/100 g edible portion, which is in agreement with the amount (631 mg/100 g edible portion) previously found in black ripe olives (*10*). Data in the literature on total (free + bound) D-amino acids, or both free and bound D-amino acids, in foods are scarce. With the exception of a few cases such as coffee samples (*16*), most published data on D-amino acids in foods correspond to free amino acids only. The above-mentioned level of D-amino acids in black ripe olives is higher than that reported for free D-amino acids in fermented vegetables such as pickled cabbage juice (11.3 mg/100 mL), carrot juice (11.8 mg/ 100 mL), celery juice (3.28 mg/100 mL), or fermented alcoholic beverages (beer, 0.60 mg/100 mL; red wine, 0.34 mg/100 mL) but lower than that reported in Emmentaler cheese (83.2 mg/100 g) or fermented black beans (145 mg/100 g) (*17*, *18*). Taking into account that the serving size of table olives is around 15 g, and considering the above level of D-amino acids in black ripe olives, it can be calculated that about 4 mg of D-amino acids can be ingested per serving, which is lower than the amount of D-amino acids (10 mg) in a cup of "expresso" coffee (*16*).

Asx, Glx, and Leu were the major amino acids (data not shown), as previously reported in table olives (*10*). As demonstrated later, the relatively high levels of D-Ser can be attributed to the remarkable racemization rate of this amino acid during the sterilization treatment.

Formation of D-Amino Acids during Sterilization of Alkali-Treated Olives. Since the racemization rate for free amino acids has been observed to be different from that of amino acid residues in proteins (*8*), the present study was conducted

Table 2. Relative (%D) and Absolute Amounts of Total (Free + Bound) D-Amino Acids in Commercial Black Ripe Olives

	black ripe olives[a]															
	A		B		C		D		E		F		G		average[b]	
amino acid[c]	%D[d]	abs amt[e]	%D	abs amt	%D	abs amt	%D	abs amt	%D	abs amt	%D	abs amt	%D	abs amt	%D	abs amt
D-Asx	9.2	7.1	9.1	6.9	10.3	8.2	10.9	9.7	11.9	11.4	12.5	14.5	13.9	13.8	11.1 (16)	10.2 (30)
D-Glx	3.5	3.0	3.9	3.3	3.2	2.8	3.0	3.0	3.0	3.3	4.0	5.0	3.7	4.0	3.5 (12)	3.5 (23)
D-Ser	4.0	1.8	5.2	2.2	9.1	4.0	8.3	4.0	9.5	4.8	15.2	8.7	9.9	5.0	8.7 (42)	4.3 (52)
D-His	2.1	0.5	1.5	0.3	nd[f]	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.5 (174)	0.1 (177)
D-Arg	1.8	0.7	2.1	0.8	3.4	1.3	1.4	0.5	1.9	0.7	2.4	1.2	1.8	0.9	2.1 (31)	0.9 (32)
D-Ala	2.2	0.9	2.9	1.1	3.9	1.6	2.2	1.0	3.4	1.6	2.9	1.6	2.6	1.2	2.9 (22)	1.3 (25)
D-Tyr	2.1	0.8	2.6	1.0	3.7	1.5	1.6	0.7	2.7	1.2	2.3	1.5	2.5	1.3	2.5 (26)	1.1 (28)
D-Val	1.5	0.7	2.2	1.0	3.4	1.6	0.9	0.5	2.0	1.1	1.4	0.9	2.3	1.3	1.9 (42)	1.0 (38)
D-Phe	2.0	0.8	2.3	0.9	3.1	1.2	1.7	0.8	2.4	1.1	2.4	1.5	2.5	1.3	2.3 (19)	1.1 (26)
D-Ile	1.2	0.5	1.8	0.8	0.6	0.3	0.5	0.3	0.5	0.3	1.2	0.8	1.4	0.8	1.0 (49)	0.5 (50)
D-Leu	2.6	1.8	3.1	2.0	2.3	1.5	2.3	1.8	2.2	1.8	2.9	2.8	3.0	2.5	2.6 (14)	2.0 (23)
total D-amino acids[e]	18.6		20.1		23.9		22.1		27.2		38.2		32.1		26.0 (27)	
total (L + D) amino acids[e]	546		523		544		607		639		780		675		617 (15)	

[a] Company codes are identified by the letters A–G. Values are means of duplicate analyses. [b] Coefficient of variation (percentage) in parentheses. [c] Asx = Asp + Asn, calculated as Asp; Glx = Glu + Gln, calculated as Glu. [d] %D = 100D/(D + L), where D AND L are the amounts of D- AND L-amino acids, respectively. [e] Absolute amount (mg/100 g fresh weight). [f] nd, not detected.

for both free and protein-bound amino acids in olives. Before heat treatment, packed alkali-treated olives contained 574 mg/100 g fresh weight of total (free + protein-bound) amino acids, with 4.7% as free amino acids (data not shown). Arg, Glu, and Asn were the major free amino acids, which is in agreement with data reported in unfermented olive brine (*19*). The major protein-bound amino acids were Glu, Asp, and Leu.

In spite of buffer addition to maintain a constant pH, the pH of the cover liquor (the same as the pH of olive juice if equilibrium has been reached) decreased slightly during sterilization (data not shown). The maximum pH decrease was 1.1 (in packing C after 35 min heating). Heat-induced pH decrease also occurs in the real product (black ripe olives), as reported previously (*20*). This may be attributed to formation of organic acids from degradation of sugars (*21*) or saponification of the pectin ester groups (*22*). Studies to confirm these hypotheses are in progress in our laboratory.

(a) Free Amino Acids. The racemization study was focused on those free amino acids that showed notable racemization values during heat treatment, namely, Ser, Ala, Asp, Asn, and Glu (**Table 3**). Racemization of the other quantified free amino acids was null or negligible (data not shown). The presence of free D-amino acids in small amounts before heat treatment, particularly in the case of Ser (with about 5% of the D form), may be attributed to racemization occurring in the previous treatments of the olives (initial alkaline treatment with NaOH, washings, packing with hot buffer). Ser has been reported to be the amino acid with the highest racemization rate, after cysteine (Cys), in a free amino acid mixture under moderate alkaline treatment (*8*). Heat treatment of olives in alkaline cover liquors induced a significant ($p < 0.05$) increase in the extent of racemization of free amino acids, particularly in the case of Ser and Ala. After 35 min of heating, racemization values of about 20% and 11% were found for Ser and Ala, respectively. With increasing olive pH, the racemization values of Ser, Ala, and Asn increased, but the racemization value of Asp decreased slightly, and that of Glu did not significantly change.

Total amounts of each amino acid in olive pulp, with the exception of Asp, decreased during the first 15 min of heating (**Table 3**), which could be attributed to leaching of free amino acids into the cover liquor or losses by chemical reactions with reducing sugars (Maillard reaction) or degradation (e.g., formation of pyroglutamic acid from Glu) (*21*, *23*). Protein could also be partially hydrolyzed during heating, which would explain the observed increase in the total amount of Asp. With increasing olive pH, the total amount of each amino acid increased, which could be due to increased hydrolysis of protein at higher pH values.

Table 3. Effects of Heating Time (*t*) at 121 °C and Initial pH of Cover Liquor on the Racemization Values (%D) and Total Amounts (L + D, mg/100 g Fresh Weight) of Free Amino Acids in Packed Alkali-Treated Olives

		main effects						
		t (min)				pH		
amino acid[a]		0	15	21	35	8.3	9.2	10.3
Asp	%D[b]	0.47a	3.83b	3.16b	3.74b	3.56b	2.62a	2.21a
	L + D	1.53a	1.98b	1.67a	2.13b	1.07a	1.89b	2.46c
Glu	%D	0.48a	0.78a	1.44b	2.09c	1.10ab	1.01a	1.43b
	L + D	4.94b	2.89a	3.16a	3.16a	2.40a	3.81b	4.37c
Asn	%D	0.23a	1.62b	1.86b	3.33c	1.08a	2.23b	1.83b
	L + D	3.10b	1.10a	1.25a	1.15a	0.89a	2.25c	1.79b
Ser	%D	5.14a	11.95b	11.27b	19.84c	9.51a	11.86b	14.12c
	L + D	0.80b	0.52a	0.48a	0.52a	0.49a	0.58b	0.67c
Ala	%D	0.63a	5.84b	4.34b	11.09c	2.45a	5.15b	8.28c
	L + D	1.32d	0.86a	1.01b	1.22c	0.73a	1.08b	1.46c

[a] Mean values with different letters within a row for each effect are significantly different ($p < 0.05$). [b] %D = 100D/(L + D), where D and L are the amounts of D- and L-amino acids, respectively.

(b) Protein-Bound Amino Acids. Significant racemization values were found for more amino acids than for free amino acids (**Table 4**). Since the procedure used in this work does not allow the differentiation of D-amino acid isomers formed during acid hydrolysis, the racemization value in this case is the sum of three contributions: racemization occurring in the previous treatments of olives, racemization occurring during hydrolysis, and racemization occurring during sterilization. Both sterilization treatment and olive pH significantly ($p < 0.05$) affected (increased) the racemizacion values of protein-bound amino acids. The only exception was Phe: its racemization value was not significantly affected by sterilization time.

As in the case of free amino acids, Ser was again the most-racemized amino acid after heating. Beyond 15 min of heating, no further racemization of Ser was apparent. Similarly shaped

Table 4. Effects of Heating Time (t) at 121 °C and Initial pH of Cover Liquor on the Racemization Values (%D) and Total Amounts (L + D, mg/100 g Fresh Weight) of Protein-Bound Amino Acids in Packed Alkali-Treated Olives

		main effects						
		t (min)				pH		
amino acid[a]		0	15	21	35	8.3	9.2	10.3
Asx	%D[b]	7.15a	11.77c	10.48b	11.91c	9.27a	9.68a	12.03b
	L + D	76a	102c	102c	95b	99c	96b	87a
Glx	%D	2.30a	2.02a	2.72a	3.93b	1.90a	2.59b	3.73c
	L + D	80a	107b	112b	108b	106c	101b	97a
Ser	%D	6.66a	17.43c	14.49b	14.89b	8.34a	12.27b	19.49c
	L + D	40a	52b	51b	52b	51b	51b	45a
His	%D	4.50a	4.17a	6.39b	10.26c	4.73a	4.51a	9.40b
	L + D	21a	32b	35c	22a	25a	30b	28b
Arg	%D	2.74a	3.23a	3.73a	6.56b	2.99a	3.58a	5.62b
	L + D	42d	36b	39c	33a	49c	37b	28a
Ala	%D	2.19a	2.81a	3.04a	3.82b	2.00a	2.57a	4.32b
	L + D	37a	48b	50b	54c	49b	47b	46a
Tyr	%D	2.46a	2.50a	2.44a	4.09b	1.97a	2.85b	3.80c
	L + D	42a	54b	54b	50b	50a	48a	52a
Val	%D	0.97a	1.46ab	1.97b	3.74c	1.89a	1.40a	2.81b
	L + D	40a	58b	61b	59b	59b	55ab	50a
Phe	%D	2.98a	2.95a	2.99a	3.39a	2.15a	3.04b	4.04c
	L + D	43a	56b	54b	56b	55a	50a	51a
Leu	%D	2.46a	2.49a	3.51a	4.88b	2.94a	2.34a	4.73b
	L + D	66a	85b	90c	92c	85b	82ab	81a

[a] Mean values with different letters within a row for each effect are significantly different ($p < 0.05$). Asx = Asp + Asn, calculated as Asp; Glx = Glu + Gln, calculated as Glu. [b] %D = 100d/(L + D), where D and L are the amounts of D- and L-amino acids, respectively.

racemization curves for Ser (i.e., an increase of D enantiomer followed by leveling off or a slight decrease) have been reported in chicken muscle heated at 121 °C (*5*) and in soybean proteins treated in 0.1 N NaOH at 75 °C (*7*). This behavior can be explained by the effect of the protein structure and protein denaturation processes (*8*).

The increase in total (L + D) amounts of amino acids during the first 15 min of heating (**Table 4**) could be a consequence of the decrease in water-soluble components by leaching into the packing solution. Arg was the only exception. The total amount of this amino acid decreased during heating, and it was the most strongly affected by pH changes. This could be due to reaction with reducing sugars.

In summary, the content of D-amino acids in commercial samples of ripe olives has been measured. Of the total content of amino acids (≈0.6 g/100 g edible portion), some 4% was found as D-amino acids. The absolute amount found averaged 26 mg/100 g edible portion. The most abundant D-amino acids were D-Asx, D-Glx, D-Ser, and D-Leu. Since free amino acids represent only a low percentage (<5%) of total amino acids in ripe olives, D-amino acids are mainly from olive protein. Technological factors such as sterilization time and olive pH were both demonstrated to have a significant influence on racemization values and total amounts of amino acids in ripe olives.

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