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Vitamin Content and Amino Acid Composition of Pickled Garlic Processed with and without Fermentation

ALFREDO MONTAÑO,* FRANCISCO JAVIER CASADO, ANTONIO DE CASTRO, ANTONIO HIGINIO SÁNCHEZ, AND LUIS REJANO

Instituto de la Grasa (C.S.I.C.), Apartado 1078, 41012 Seville, Spain

The effect of processing, with and without fermentation, upon the nutritional composition of pickled garlic was evaluated. On a dry basis, the fermented product had a higher content of riboflavin, α-tocopherol, and most individual amino acids but a lower thiamin level than the unfermented product. Ascorbic acid was totally lost during processing. The chemical scores for the unfermented and fermented product were 88 and 108%, respectively, with the limiting amino acid being leucine. Water blanching (90 °C for 4 min) affected only the ascorbic acid content, whereas fermentation significantly affected the contents of thiamin, ascorbic acid, and α-tocopherol, as well as glutamic acid and arginine. For each processing type, the effect of the preservation method and storage time on vitamins and amino acid composition was also analyzed. In the case of the fermented product, usage of the corresponding fermentation brine plus refrigerated storage was also assayed as the packing/preservation method and was found to give the best result from a nutritional standpoint.

KEYWORDS: Garlic; *Allium sativum* L.; pickles; fermentation; nutritional composition; amino acids; vitamins; processing; blanching; preservation; storage

INTRODUCTION

Garlic (*Allium sativum* L.) is used in all parts of the world as a spice and food. Among the different garlic-based products on the market, pickled garlic is becoming increasingly accepted by consumers, particularly in Spain. This could be attributable, at least partially, to some reported beneficial effects of garlic on health (*1*). Although different methods for pickling garlic with and without a fermentation step have been published (*2*, *3*), data on the nutritional composition of this product have not been reported. Commonly used food composition tables (*4*−*6*) include data only for raw garlic. The composition of pickled garlic can be expected to differ from that of raw garlic, because the different processing steps (blanching and fermentation), as well as the preservation/storage method, are known to affect the nutritional composition of vegetable products (*7*−*9*). Irrespective of the processing method selected, blanching to eliminate the typical garlic pungency is mandatory (*2*). Optimization of this step has recently been studied (*10*). However, the effect of blanching or that of the fermentation step in the case of the fermented product on garlic nutrients has not been investigated. Vitamins of the B group (mainly thiamin) as well as vitamin C (ascorbic acid) appear to be the most susceptible to loss during processing of vegetable products. In fact, thiamin and ascorbic acid are frequently used as research indicators of the severity of food processing (the assumption being that, if these nutrients are well-retained in food, all other nutrients will be also, with percentage retentions as high or higher) (*11*). In contrast, liposoluble vitamins (β-carotene and α-tocopherol) are hardly affected during processing of vegetables (*12*, *13*). Although protein content is low in vegetables, a knowledge of their amino acid composition is important, particularly in the dietary treatment of patients with disorders of amino acid and protein metabolism (*14*).

Generally, packing of processed vegetables is done using an acidified brine as cover liquor, followed by an appropriate preservation method (pasteurization and addition of preservatives). When preservatives are added, the product is generally held under refrigeration at 5 °C (*15*). In the case of fermented vegetables, the fermentation brines can be used as packing solutions, thereby significantly diminishing the pollution levels of the industrial wastewaters (*16*, *17*). This packing procedure has not previously been studied in the case of fermented garlic.

The objectives of this paper were to study the amino acid composition and levels of selected vitamins in pickled garlic processed with and without fermentation and to know the effect of different packing methods and storage time on the mentioned nutrients.

MATERIALS AND METHODS

Preparation of Pickled Garlic. Two different processes for preparing pickled garlic were studied: (1) packing directly with an acidified brine after blanching and (2) packing after the blanching and fermentation steps (**Figure 1**). Each preparation was carried out with a separate batch of garlic bulbs. Both batches were of Spanish "purple garlic" type, purchased in the same local store. The bulbs were cracked to

* To whom correspondence should be addressed. Telephone: +34-95-4691054. Fax: +34-95-4691262. E-mail: amontano@cica.es.

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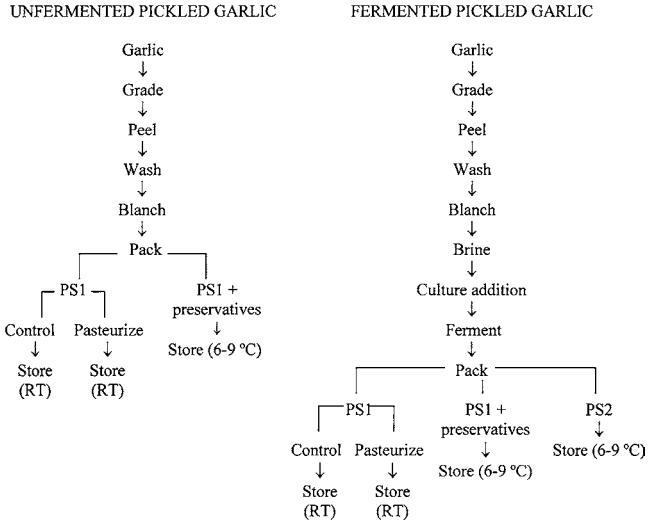

Figure 1. Flow diagrams for the two studied methods of pickled garlic processing. PS1 = packing solution 1 = acidified fresh brine. PS2 = packing solution 2 = fermenting olive brine. RT = room temperature.

separate the cloves. The defective and smallest ones were discarded, and the rest were peeled. In the first preparation type, the garlic cloves were blanched in water at 90 °C for 4 min and immediately packed. A portion of blanched garlic cloves was packed into "8 Par" glass bottles (125 g of garlic cloves and 125 mL of cover liquor capacity) using, as cover liquor, a brine acidified with a lactic−acetic acid mixture (packing solution 1, PS1). Concentrations of acid and NaCl in this brine were calculated to give equilibrium values of 1.5% acidity (as lactic acid) and 3.0% salt. One-half of the packed product was pasteurized for 5.5 min in a water bath at 90 °C (cover brine was added at approximately 70 °C), and the remainder was left as a nonpasteurized control. Both packed products were stored at room temperature. The remaining portion of blanched garlic cloves was also packed into 8 Par bottles with the same cover brine, except that it was fortified with 0.27% potassium sorbate and 0.24% sodium benzoate and stored under refrigeration (6−9 °C). In the second preparation type, garlic cloves were blanched in the same way as the first preparation and then subjected to lactic acid fermentation. For this, blanched garlic cloves (14.5 kg) were placed in an appropriate fermentation vessel, covered with brine (10 L of 9% NaCl, w/v) and inoculated (2 h after brining) with a starter culture of *Lactobacillus pentosus*. The initial population in brine after inoculation was 2.7×10^7 CFU/mL. Fermentation was carried out in a room maintained at 30 °C for 7 days. Then, the garlic cloves were divided into three portions. Two portions were packed and preserved in the same way as the first preparation type (i.e., packing/preservation methods: untreated control, pasteurized, and preservatives and refrigeration), while the third portion was packed with its own fermenting brine (packing solution 2, PS2). This brine was centrifuged (16000*g* for 20 min) before using, to remove particles in suspension. The packed product was kept refrigerated during storage.

Brine Analyses. The pH of brine during the fermentation step was measured using a Metrohm 670 Titroprocessor (Herisau, Switzerland). Microbiological analysis comprised aerobic plate count (PCA), *Enterobacteriaceae* (VRBD), lactobacilli (MRS ± 0.02% w/v sodium azide), enterococci (Slanetz and Bartley), molds and yeasts (OGYE), and sulfite-reducing clostridia (DRCM). Media were from Oxoid Ltd., Basingstoke, U.K. (PCA, MRS, Slanetz and Bartley, and OGYE) and Merck, Darmstadt, Germany (VRBD and DRCM).

Water and Nitrogen Contents of Garlic. Water content was estimated by weighing aliquots of freeze-dried homogenized samples before and after freeze drying. Nitrogen contents were determined by the Kjeldahl method, using a Büchi digestion unit (Büchi Labortechnik AG, Flawil, Switzerland) and a Kjeldahl distillation unit (Büchi).

Amino Acid Analysis. For the determination of the amino acids, except tryptophan, cyst(e)ine, and methionine, freeze-dried samples of garlic (150 mg) were subjected to acid hydrolysis with 5 mL of 6 M HCl under nitrogen atmosphere for 24 h at 110 °C. Each hydrolyzate was washed into a 50 mL volumetric flask and made up to the mark with Milli-Q water. An aliquot was spiked with DL-norleucine (Sigma, St Louis, MO) as an internal standard and dried under vacuum on a rotary evaporator. The dried mass was washed with Milli-Q water and evaporated to dryness, and 1 mL of 20 mM HCl was added to the residue. The amino acids were subjected to HPLC analysis after derivatization with 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate (AQC). AQC was purchased as part of an AccQ Fluor Reagent Kit from Waters. For derivatization, 10 μL of sample or amino acid standard mixture was combined with 70 μL of 200 mM borate buffer (Waters AccQ.Fluor Borate Buffer). The samples were mixed, and 20 μL of AccQ.Fluor reagent (AQC dissolved in acetonitrile) was added. After an immediate mixing, the samples were heated for 10 min at 55 °C to degrade a tyrosine byproduct. For analysis, samples of 5 μL were injected into the HPLC system. This consisted of a Waters 2690 Separations Module (Waters Assoc., Milford, MA), a Jasco FP-920 fluorescence detector (Jasco Corp., Tokyo, Japan) (an excitation wavelength at 250 nm, an emission wavelength at 395 nm, and a flow cell of 5 μL), and a computer with Waters Millenium 32 Chromatography Manager, version 3.00. The separation of the AQC derivatives was carried out using a 3.9 × 150 mm AccQ.Tag column (Waters, WAT052885), held at 37 °C and gradient elution. Three eluents were used. Eluent A consisted of 140 mM sodium acetate with 17 mM triethylamine, titrated to pH 5.05 with phosphoric acid. A total of 1 mg of disodium EDTA L^{-1} and 0.01% sodium azide were added. Eluents B and C were acetonitrile and Milli-Q water, respectively. The flow rate was 1.0 mL/min. The gradient profile is given in **Table 1**. Variation coefficients of the amino acid determination, including hydrolysis, were from 3.2 to 6.2%. Because of the partial conversion of asparagine and glutamine into aspartic acid and glutamic acid, respectively, during hydrolysis, the data for asparagine plus aspartic acid and glutamine plus glutamic acid are reported as Asx and Glx, respectively.

Table 1. Gradient Profile Used for Separation of Amino Acids as AQC Derivatives

time (min)	A (%)	B (%)	C (%)	curve number (Waters 2690 pump)
initial	100	0	0	
0.5	99	1	0	11[a]
18.0	95	5	0	6[b]
19.0	91	9	0	6
29.5	83	17	0	6
33.0	0	60	40	11
36.0	100	0	0	11

[a] Maintains start condition until next step. [b] Linear.

Cyst(e)ine and methionine were analyzed as cysteic acid or methionine sulfone after oxidation of the sample with performic acid and hydrolysis with 6 M HCl, according to the method described by Moore (*18*). Cysteic acid and methionine sulfone were derivatized with AQC and separated as described above. Method precision for Cys and Met was 0.1 and 4.7%, respectively.

Tryptophan was analyzed after alkaline hydrolysis with 4.2 M NaOH, according to the method described by Nielsen and Hurrell (*19*). Chromatographic conditions were similar to those used by Delhaye and Landry (*20*), except that a μ-Bondapak C_{18} (10 μm, 3.9 × 300 mm) column (Waters) maintained at 40 °C and fluorimetric detection (excitation at 285 nm and emission at 345 nm) were used. Method precision was 0.8%. All amino acids were estimated in duplicate. The chemical score of the limiting amino acid was determined (*21*) with respect to the FAO/WHO/UNU (*22*) and FAO/WHO (*23*) recommendations.

Vitamin Analyses. For the determination of thiamin and riboflavin, freeze-dried samples of garlic (5−10 g) were placed in volumetric flasks (100 mL), mixed with 75 mL of 0.1 N HCl, and the flasks were placed in a water bath at 95 °C for 30 min. After cooling to room temperature, 5 mL of 6% taka-diastase (Fluka) in 2.5 M sodium acetate was added, and the samples were incubated for 3 h at 48 °C. After cooling, the samples were brought to volume with distilled water and then filtered

through Whatman no. 41 filter paper. An aliquot of the filtrate was filtered through a 0.45 μm membrane filter and then used for the chromatographic determination of riboflavin. Another aliquot (0.5 mL) of the first filtrate was treated with 0.5 mL of 1% potassium ferricyanide in 15% aqueous NaOH to form the fluorescent derivative (thiochrome) from thiamin. After 1 min, 0.2 mL of glacial acetic acid was added, and the mixture was filtered through a 0.45 μm membrane filter prior to HPLC analysis. Separate chromatographic runs were carried out for the determination of each vitamin, using chromatographic conditions identical to those of Arella et al. (*24*), except that a μ-Bondapak C_{18} (10 μm, 3.9 × 300 mm) column (Waters) and a flow rate of 1.5 mL/min were used.

Ascorbic acid was extracted and analyzed by HPLC as described by Casado et al. (*25*). The sample (40 g of garlic cloves) was blended with an equal weight of 6% HPO_3 containing 1 mM EDTA. An aliquot (40 g of portion) was diluted with 3% HPO_3 containing 1 mM EDTA in a volumetric flask (100 mL). After filtration through Whatman no. 41 filter paper, an aliquot (1.0 mL) of the filtrate was mixed with 0.2 mL of 0.2% dithiothreitol solution (prepared in 0.2 M sodium phosphate buffer at pH 7) and 0.1 mL 45% (w/v) dipotassium hydrogen phosphate with a vortex mixer. After 10 min in darkness at room temperature, reduction was stopped by addition of 0.2 mL of 2 M phosphoric acid. Before injection, samples were clarified by filtration through a 0.45 μm filter. Separation was performed on a Luna 5μ C18(2) (250 × 4.6 mm inside diameter) column (Phenomenex, Torrance, CA) with a Tracer C18 guard column (Teknokroma, Barcelona, Spain), using deionized water (adjusted to pH 2.3 with orthophosphoric acid) as the mobile phase at a flow rate of 1.0 mL/min at ambient temperature. Ascorbic acid was monitored at 245 nm.

For the analysis of α-tocopherol, the extraction procedure of Malik et al. (*26*) was used. Briefly, garlic (10 g) was homogenized in 20 mL of phosphate-buffered saline (10 mM potassium phosphate and 150 mM sodium chloride at pH 7.4). After addition of 30 mL of 10 mM lithium dodecyl sulfate and 60 mL of ethanol, the mixture was extracted twice with portions (each of 120 mL) of heptane (containing 0.05% BHT as an antioxidant). The pooled heptane extracts were evaporated under reduced pressure at room temperature. The residue was dissolved in 10 mL of hexane, and filtered through a 0.45 μm membrane filter prior to HPLC analysis. Chromatographic conditions used were the same as those described for vegetable oils (*27*). Filtered extract (10 μL) was injected onto a Spherisorb W silica column (250 × 4.6 mm inside diameter, 5 μm, Teknokroma), using propan-2-ol in hexane (0.5/99.5, v/v) as the mobile phase at a flow rate of 1 mL/min. Elution was monitored by fluorescence (excitation wavelength at 290 nm and emission wavelength at 330 nm).

Statistical Analyses. The data were subjected to analysis of variance using the STATISTICA software, version 5.5 (*28*). Duncan's multiple range test was used for mean comparisons. Significant differences were determined at the $p < 0.05$ level.

RESULTS AND DISCUSSION

Changes in Nutrients during the Main Steps of Processing. The mean content of α-tocopherol in raw garlic (0.5 mg/100 g of wet weight) was slightly lower than some other authors have reported (*26*, *29*) but around 50-fold higher than that of commonly used food tables (*4*, *5*). Contents of ascorbic acid, thiamin, and riboflavin (9.20, 0.08, and 0.01 mg/100 g of wet weight, respectively) were lower than those reported by USDA (31.20, 0.20, and 0.11 mg/100 g of wet weight, respectively), but the total amino acid contents (4.5 g/100 g of wet weight versus 4.3 g/100 g of wet weight) or even the amino acid profile were quite similar. Discrepancies between the vitamin contents reported in this study and values published elsewhere may be due to differences in variety and growing conditions. Climatic and environmental factors such as light, temperature, rainfall, season, location, altitude, soil fertility, irrigation, and plant protection measures are known to affect the nutritional status of vegetable crops (*30*).

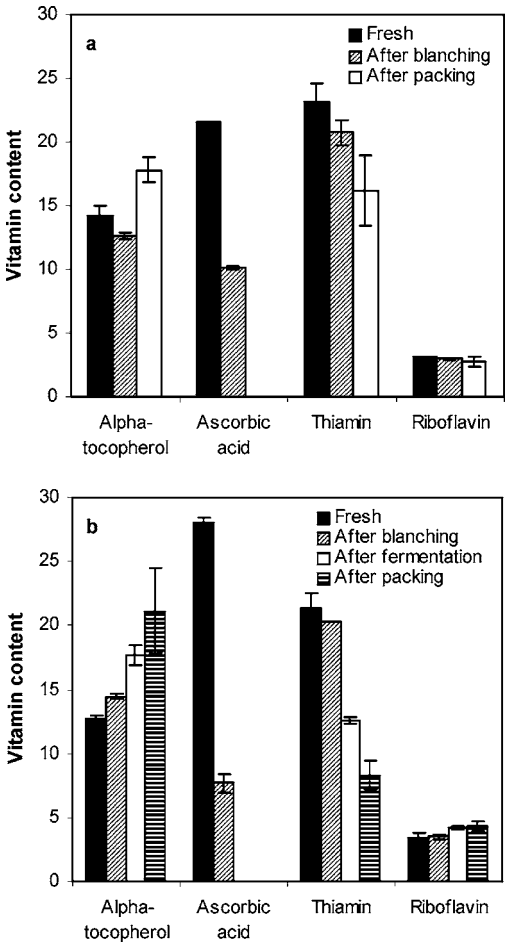

Figure 2. Changes in vitamins during processing in pickled garlic processed (a) without and (b) with the fermentation step. Values are the means of duplicate determinations in fresh and blanched garlic. In packed garlic, values are the means of three different preservation methods, each analyzed twice during storage, with analyses in duplicate ($n = 12$). Error bars represent 1 standard deviation. α-Tocopherol and ascorbic acid contents are expressed as mg/kg of dry weight and mg/100 g of dry weight, respectively. Thiamin and riboflavin contents are expressed as μg/10 g of dry weight.

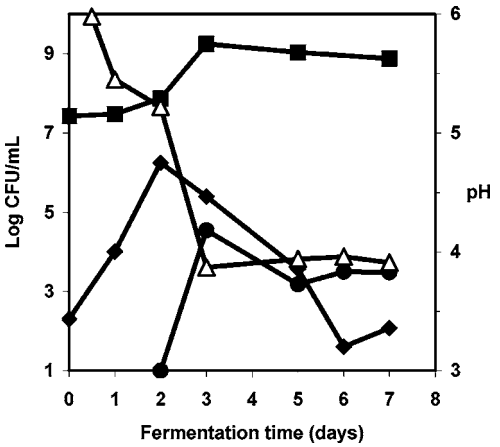

Figure 3. Populations of lactobacilli (■), *Enterobacteriaceae* (◆), and enterococci (●) and evolution of pH (△), during the fermentation step of blanched garlic in brine.

The water content of garlic significantly ($p < 0.05$) increased in each step of processing. Thus, the values for this nutrient (expressed as g/100 g) were the following: 62.9 (fresh garlic),

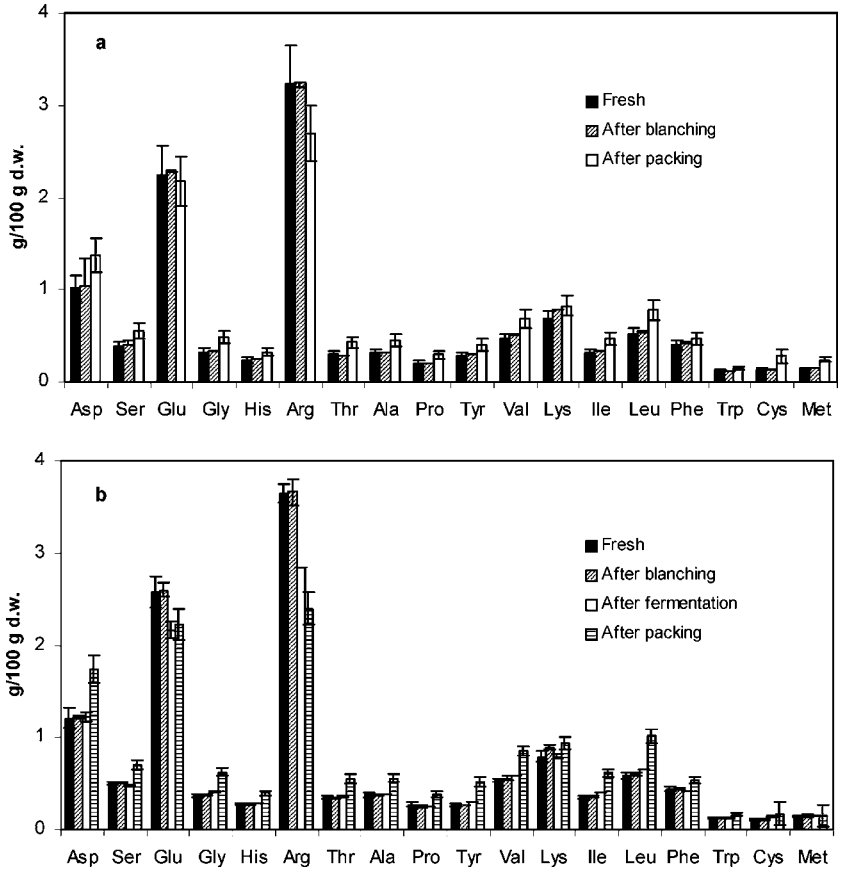

Figure 4. Changes in individual amino acids during processing in pickled garlic processed (a) without and (b) with the fermentation step. Values are the means of duplicate determinations in fresh and blanched garlic. In packed garlic, values are the means of three different preservation methods, each analyzed twice during storage, with analyses in duplicate ($n = 12$). Error bars represent 1 standard deviation.

64.4 (blanched garlic), and 76.3 (packed garlic) in the processing without fermentation; 62.9 (fresh garlic), 65.1 (blanched garlic), 71.9 (fermented garlic), and 82.6 (packed garlic) in the processing with fermentation. The changes in vitamin contents, expressed on a dry basis, during processing of pickled garlic, both with and without fermentation step, are shown in **Figure 2**. Blanching in hot water (90 °C for 4 min) significantly ($p < 0.05$) affected the ascorbic acid content of garlic. Considering both types of processing, the mean loss of this vitamin by blanching was 63%, but the other vitamins studied did not significantly change. After the fermentation step, garlic had a higher content of α-tocopherol but lower contents of thiamin and ascorbic acid (the latter was not detected) compared with garlic after blanching (**Figure 2b**). The increase in the content of α-tocopherol could be due to the percentage increase in the lipid content of garlic, which in turn, would be a result of the loss of water-soluble components. Mass balances with respect to thiamin contents before and after the fermentation step indicated that most losses were caused by leaching of the thiamin into the fermentation brine, and the same was found in the packing step. Thus, leaching appeared to be the main mechanism of thiamin loss during pickled garlic processing. The riboflavin content did not significantly change during processing either with or without the fermentation step. A feasible explanation for this could be that this vitamin, predominantly as two coenzyme forms (FMN and FAD), is usually bound to proteins in foods (*31*), thereby preventing loss by leaching of the riboflavin into the different aqueous solutions (blanching water, fermentation brine, and packing solution). The small increase in riboflavin content as a result of fermentation (**Figure 2b**) could be attributed to the loss of other components during this step. However, the increase was not statistically significant. Production of this vitamin by microorganisms growing in the brine during the fermentation step (**Figure 3**) does not seem likely. Moreover, molds and yeasts, microorganisms known to produce B-group vitamins in vegetable fermentations (*8*, *32*), were not detected.

Figure 4 shows changes in individual amino acids (expressed on a dry basis) during processing of each product. Blanching did not significantly ($p < 0.05$) affect the individual amino acid content in any case. However, during the fermentation step, the major amino acids in garlic, namely, glutamic acid and arginine, significantly decreased in concentration (**Figure 4b**). In the packing step, irrespective of the processing type, most individual amino acids increased in concentration (calculated on a dry basis), again with the exceptions of glutamic acid and arginine. The increases in concentration could be a consequence of the decrease in water-soluble components by leaching into the packing solutions. The changes in arginine and glutamic acid appear to indicate that these compounds existed as free amino acids in a significant proportion. As a result of all of these individual changes, the total amino acid content remained practically unchanged during all of the steps of pickled garlic processing (values not shown).

Effects of Packing/Preservation Method and Storage Time on Nutrients. Separate analyses of variance were carried out for unfermented and fermented packed pickled garlic to know the effect of both packing/preservation method (PM) and storage time (T) on the studied nutrients. In the unfermented product, where microorganisms were not detected (<1 CFU/mL) in any case (control, C; pasteurized, P; and refrigerated, R samples), significant ($p < 0.05$) differences because of one or both of the

Table 2. Effects of Preservation Method and Storage Time on Vitamins and Amino Acids of Unfermented Pickled Garlic

	factor effect[a]		factor				
			preservation method (PM)[b]			storage time (T)[c]	
nutrients	PM	T	C	R	P	1 month	11 months
vitamins[d]							
α-tocopherol	ns	ns	18.51	17.01	17.89	17.92	17.68
thiamin	*	ns	1.82a	1.48b	1.66ab	1.67	1.55
riboflavin	ns	ns	0.27	0.27	0.28	0.29	0.26
amino acids[e]							
aspartic acid	ns	ns	1.46	1.31	1.39	1.30	1.47
serine	ns	ns	0.56	0.54	0.57	0.52	0.59
glutamic acid	ns	ns	2.27	2.09	2.19	2.10	2.26
glycine	ns	ns	0.49	0.48	0.49	0.45	0.52
histidine	ns	ns	0.34	0.32	0.33	0.30	0.36
arginine	ns	ns	2.83	2.66	2.66	2.63	2.80
threonine	ns	ns	0.43	0.43	0.44	0.40	0.46
alanine	ns	ns	0.47	0.44	0.46	0.42	0.49
proline	ns	ns	0.30	0.30	0.30	0.27	0.33
tyrosine	ns	ns	0.45	0.37	0.42	0.37	0.45
valine	ns	ns	0.72	0.68	0.69	0.64	0.75
lysine	ns	ns	0.86	0.82	0.82	0.77	0.89
isoleucine	ns	ns	0.49	0.46	0.47	0.43	0.51
leucine	ns	ns	0.81	0.77	0.78	0.72	0.85
phenylalanine	ns	ns	0.49	0.46	0.46	0.44	0.51
tryptophan	ns	ns	0.13	0.17	0.14	0.15	0.15
cyst(e)ine	*	*	0.28b	0.23a	0.34c	0.23a	0.34b
methionine	ns	ns	0.22	0.23	0.26	0.25	0.23
total amino acid	ns	ns	13.60	12.73	13.19	12.41	13.94

[a] Results of analysis of variance when the interaction and the within error terms were pooled. *, significant differences ($p < 0.05$); ns, no significant differences. When significant differences exist, mean values with the same letter indicate that there are no significant differences between them. [b] Values are means of two samplings, with analyses in duplicate ($n = 4$); C, garlic packed with acidified brine, left at room temperature; R, garlic packed with acidified brine, left refrigerated (6–9 °C); P, garlic packed with acidified brine and then pasteurized (90 °C, 5.5 min), left at room temperature. [c] Values are means of the three preservation methods, with analyses in duplicate ($n = 6$). [d] mg/kg of dry weight. [e] g/100 g of dry weight.

Table 3. Effects of Preservation Method and Storage Time on Vitamins and Amino Acids of Fermented Pickled Garlic

	factor effect[a]		factor					
			preservation method (PM)[b]				storage time (T)[c]	
nutrients	PM	T	C	R	P	FB/R	1 month	11 months
vitamins[d]								
α-tocopherol	*	*	25.31a	18.97b	22.87c	19.89b	23.26a	20.26b
thiamin	*	ns	0.79a	0.97b	0.85a	1.04b	0.95	0.88
riboflavin	*	ns	0.39a	0.45b	0.43ab	0.56c	0.44	0.47
amino acids[e]								
aspartic acid	*	ns	1.65a	1.73a	1.82a	1.33b	1.63	1.63
serine	*	ns	0.70a	0.69a	0.71a	0.51b	0.66	0.64
glutamic acid	ns	ns	2.12	2.23	2.33	2.21	2.23	2.22
glycine	*	ns	0.62a	0.61a	0.63a	0.45b	0.59	0.57
histidine	*	ns	0.40a	0.39a	0.41a	0.32b	0.38	0.37
arginine	ns	ns	2.39	2.34	2.47	2.67	2.51	2.43
threonine	*	ns	0.54a	0.54a	0.57a	0.40b	0.53	0.50
alanine	*	ns	0.54a	0.56a	0.58a	0.42b	0.53	0.52
proline	*	ns	0.39a	0.39a	0.40a	0.30b	0.39	0.35
tyrosine	*	ns	0.56a	0.47b	0.55a	0.40c	0.49	0.50
valine	*	ns	0.85a	0.83a	0.89a	0.64b	0.82	0.79
lysine	ns	ns	0.90	0.94	0.98	0.84	0.93	0.90
isoleucine	*	ns	0.61a	0.60a	0.63a	0.44b	0.58	0.56
leucine	*	ns	1.01a	0.99a	1.05a	0.71b	0.95	0.93
phenylalanine	ns	ns	0.53	0.52	0.55	0.47	0.53	0.50
tryptophan	*	ns	0.16ab	0.18b	0.15a	0.14a	0.15	0.17
cyst(e)ine	*	*	0.14a	0.11b	0.27c	0.09b	0.22a	0.09b
methionine	*	*	0.13a	0.13a	0.21c	0.09b	0.22a	0.05b
total amino acid	*	ns	14.22a	14.24a	15.21a	12.4b	14.33	13.72

[a] Results of analysis of variance when the interaction and the within error terms were pooled. *, significant differences ($p < 0.05$); ns, no significant differences. When significant differences exist, mean values with the same letter indicate that there are no significant differences between them. [b] Values are means of two samplings, with analyses in duplicate ($n = 4$); C, R, and P as indicated in **Table 2**; FB/R, garlic packed with its corresponding fermentation brine, left refrigerated (6–9 °C). [c] Values are means of the four preservation methods, with analyses in duplicate ($n = 8$). [d] mg/kg of dry weight. [e] g/100 g of dry weight.

mentioned factors were found only for thiamin and the amino acid cyst(e)ine (**Table 2**). Besides the significant increase in cyst(e)ine, most amino acids showed a tendency to increase in concentration during storage time, which could be attributable to the decrease of water-soluble components by leaching into the packing solutions.

In the fermented product, there was a significant effect of the packing method on all nutrients, except for glutamic acid, arginine, lysine, and phenylalanine (**Table 3**). Garlic packed with fermentation brine, maintained under refrigeration, (FB/R) had a lower content of water compared with the other fermented packed products (76 versus 82%). As a result, when data were expressed on a wet basis, the highest content of nutrients was found in this product (data not shown). Storage time significantly affected only the contents of α-tocopherol, cyst(e)ine, and methionine, which decreased (**Table 3**). The interaction between PM and T was significant ($p < 0.05$) in the case of both cyst(e)ine and methionine, indicating that all samples did not present the same evolution during storage time. Thus, concentrations of both amino acids decreased drastically after 11 months of storage in samples C, R, and FB/R, while they hardly changed in sample P (data not shown). In contrast to the case of the unfermented packed product, lactobacilli were detected in the fermented packed product, although only in samples C and FB/R. After 3 months of storage, the sample C contained a variable population of lactobacilli, which were not detected after 10 months. In sample FB/R, lactobacilli were present with populations above 10^7 CFU/mL after 3 months and around 10^4 CFU/mL after 10 months of storage. Enterobacteria, clostridia, and fungi were not detected in any sample.

Comparison between Fermented and Nonfermented Products. Table 4 shows, for both garlic products, the composition data (mean values) corresponding to three packing systems (C, R, and P), each analyzed twice (after 1 and 11 months of storage) and in duplicate ($n = 12$). In comparison with unfermented pickled garlic, the fermented product had higher contents of water, α-tocopherol, riboflavin, aspartic acid, serine, glycine, histidine, threonine, alanine, proline, tyrosine, valine, lysine, isoleucine, leucine, phenylalanine, and total amino acids but lower contents of thiamin and arginine. The remaining amino acid concentrations, as well as the total nitrogen content, in fermented versus unfermented pickled garlic were not significantly different at the 0.05 probability level. However, the high variability in the case of cyst(e)ine and methionine in the fermented product is noteworthy, reflecting the above-mentioned differences between pasteurized and unpasteurized samples. The percent recovery of the amino acids (based on the total amount of nitrogen for each amino acid) was 73% for the unfermented garlic product and 76% for the fermented garlic product. Because of the higher water content in the fermented product, when data were expressed on a wet basis (edible portion), the differences in nutritional composition between the two products were small and slightly favorable to the unfermented product (data not shown).

Table 5 shows the essential amino acid pattern of pickled garlic as compared with that of the reference established by FAO/WHO/UNU (*22*) and FAO/WHO (*23*). In both the unfermented and fermented products, all of the essential amino

Table 4. Comparison of the Nutritional Composition of Pickled Garlic Processed with and without Fermentation

nutrient	pickled garlic[a] unfermented	pickled garlic[a] fermented	*F* value[b]
water (g/100 g of wet weight)	76.30 ± 0.21	82.57 ± 0.11	666.08***
vitamins (on dry weight)			
α-tocopherol (mg/kg)	17.80 ± 0.29	22.39 ± 0.97	17.96***
thiamin (mg/kg)	1.62 ± 0.09	0.87 ± 0.03	73.33***
giboflavin (mg/kg)	0.28 ± 0.01	0.42 ± 0.01	55.91***
amino acids (g/100 g of dry weight)			
aspartic acid	1.38 ± 0.05	1.73 ± 0.05	28.41***
serine	0.56 ± 0.02	0.70 ± 0.01	36.26***
glutamic acid	2.18 ± 0.06	2.23 ± 0.05	0.45ns
glycine	0.49 ± 0.02	0.62 ± 0.09	44.22***
histidine	0.33 ± 0.01	0.40 ± 0.005	21.22***
arginine	2.72 ± 0.08	2.40 ± 0.04	14.19**
threonine	0.43 ± 0.02	0.55 ± 0.01	29.58***
alanine	0.45 ± 0.02	0.56 ± 0.01	26.05***
proline	0.30 ± 0.01	0.39 ± 0.01	25.55***
tyrosine	0.41 ± 0.02	0.52 ± 0.02	14.84**
valine	0.70 ± 0.03	0.86 ± 0.02	24.50***
lysine	0.83 ± 0.03	0.94 ± 0.02	8.30*
isoleucine	0.47 ± 0.02	0.61 ± 0.01	36.84***
leucine	0.79 ± 0.03	1.02 ± 0.02	36.88***
phenylalanine	0.47 ± 0.02	0.53 ± 0.007	9.44*
tryptophan	0.15 ± 0.006	0.17 ± 0.007	3.61ns
cyst(e)ine	0.28 ± 0.03	0.17 ± 0.06	2.97ns
methionine	0.24 ± 0.01	0.16 ± 0.05	2.67ns
total amino acid	13.18 ± 0.41	14.56 ± 0.27	7.71*
total nitrogen (g/100 g of dry weight)	2.95 ± 0.03	3.02 ± 0.03	1.94ns

[a] Data are means of three different preservation systems, each analyzed twice during storage time, with analyses in duplicate ($n = 12$) ± standard error of the mean. [b] *F* value, assessment of overall product differences obtained from analysis of variance; ***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; ns, not significant.

Table 5. Comparison of the Essential Amino Acid (EAA) Composition of Nonfermented and Fermented Pickled Garlic with the Suggested EAA Pattern of Requirements for Humans

EAA	FAO pattern[b]	pickled garlic[a] unfermented	pickled garlic[a] fermented	*F* value[c]
histidine	1.9	2.4 (1.26)	2.8 (1.47)	11.98**
threonine	3.4	3.2 (0.94)	3.8 (1.12)	10.24**
valine	3.5	5.1 (1.46)	6.0 (1.71)	10.03*
lysine	5.8	6.1 (1.05)	6.6 (1.14)	12.69**
isoleucine	2.8	3.5 (1.25)	4.3 (1.54)	13.13**
leucine	6.6	5.8 (0.88)	7.1 (1.08)	11.46**
phenylalanine	6.3[d]	3.5 (1.03)[d]	3.7 (1.17)[d]	4.88ns
tyrosine		3.0	3.7	7.75*
tryptophan	1.1	1.1 (1.00)	1.2 (1.09)	0.22ns
cyst(e)ine		2.1	1.8	0.89ns
methionine	2.5[e]	1.8 (1.56)[e]	1.6 (1.36)[e]	0.00ns
total	33.9	37.7	42.6	9.10*

[a] Mean content (g/16 g of N, $n = 12$), corrected to 100% nitrogen recovery. Essential amino acid score in parentheses. [b] Requirements for preschool child (2–5 years) (FAO/WHO/UNU, 1985; FAO/WHO, 1990). [c] *F* value, assessment of overall product differences obtained from analysis of variance; **, $p < 0.01$; *, $p < 0.05$; ns, not significant. [d] Phenylalanine and tyrosine. [e] Methionine and cysteine.

acids had a high chemical score, implying that essential amino acids present in pickled garlic have a high biological protein value. The first limiting amino acid was leucine, with chemical scores of 88 and 108% for the unfermented and fermented product, respectively. The fermented pickled garlic was thus clearly better in protein quality.

In summary, it can be stated that processing did affect the nutritional composition of pickled garlic, but the results greatly depended on the basis (wet or dry) used to express data. The nutrient most affected was ascorbic acid, which was totally lost during processing. Results indicate that the protein of pickled garlic, particularly that in the fermented product, is a good source of all essential amino acids. Preservation method and storage time (up to 11 months) hardly affected the nutritional composition in the case of unfermented pickled garlic. However, in the fermented pickled garlic, there were considerable losses of the sulfur-containing amino acids during storage. Packing with the corresponding fermentation brine plus refrigerated storage gave good results as an alternative preservation method, with the product being nutritionally superior to that using the other packing/preservation methods studied.

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