Determination of ϵ-*N*-Pyrrolylnorleucine in Fresh Food Products

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ϵ-*N*-Pyrrolylnorleucine was determined in different fresh food products to study its presence as a normal component of food proteins. Twenty-two different products were screened: cod, cuttlefish, salmon, sardine, trout, beef, chicken, pork, broad bean, broccoli, chickpea, garlic, green pea, lentil, mushroom, soybean, spinach, sunflower, almond, hazelnut, peanut, and walnut. Foods were homogenized, their proteins were precipitated with trichloroacetic acid and hydrolyzed with 2 N NaOH for 20 h, and the ϵ-*N*-pyrrolylnorleucine content was determined by capillary electrophoresis. The ϵ-*N*-pyrrolylnorleucine, which was identified by HPLC/MS in sardine muscle hydrolysate, ranged in the 22 foods analyzed from 0.24 to 6.36 μmol/g. This concentration was correlated with the protein content of the food ($r = 0.687$, $p = 0.00041$). In addition, the ϵ-*N*-pyrrolylnorleucine/lysine ratio was found to be a function of the lipid, iron, and protein contents of the food ($r = 0.881$, $p < 0.0001$) and was directly correlated with lipid and iron contents and inversely correlated with the protein content. These results are in agreement with the oxidative stress origin proposed for ϵ-*N*-pyrrolylnorleucine and suggest that the ϵ-*N*-pyrrolylnorleucine/lysine ratio is a characteristic of each food. In addition, ϵ-*N*-pyrrolylnorleucine seemed to be a normal component of many fresh food products, in which it may be acting as a natural antioxidant.

Keywords: *ϵ-N-Pyrrolylnorleucine; fishes; meats; vegetables; nuts; capillary electrophoresis; natural antioxidants; amino−carbonyl reactions; oxidative stress*

INTRODUCTION

Lipid-derived aldehydes and other carbonyl compounds have in the past mainly attracted the interest of food chemists because these secondary autoxidation products are among the most important substances responsible for the flavor and flavor deffects of many foodstuffs and beverages (Esterbauer, 1982; Frankel, 1983; Grosh, 1987). However, more recent research has shown that these products are also produced in vivo as a consequence of lipid peroxidation (Dillard and Tappel, 1988; Esterbauer et al., 1991; Rice-Evans and Burdon, 1993). These carbonyl compounds are strong electrophiles and, therefore, are capable of reacting rapidly with nucleophiles, including amino acids and proteins. The actual carbonyl compounds involved in such reactions with cellular nucleophiles are not yet well-known (Ebeler et al., 1994), but different products of reaction between carbonyl compounds and reactive residues of biological macromolecules have been described (Chio and Tappel, 1969; Uchida and Stadtman, 1992; Hidalgo and Zamora, 1993), and most of them have been shown to have antioxidant properties (Alaiz et al., 1996, 1997; Zamora et al., 1997).

If reaction products between aldehydes and protein residues are produced naturally during the life of living beings, and they have been shown to have antioxidant properties, these compounds should constitute a new group of natural antioxidants that might be present in fresh foods and that have not been previously considered. The present investigation was undertaken to determine the presence of these compounds in foods. However, because many aldehydes are produced from the different fatty acids, and there are also different amino acid residues involved, the number of potential aldehyde/protein products is high. The present study has been dedicated to determine one product of these reactions, the amino acid ϵ-*N*-pyrrolylnorleucine, in food products.

Previous work from this laboratory has shown that ϵ-*N*-pyrrolylnorleucine is a final product in the reaction of the 4,5-epoxy-2-alkenals produced in the oxidation of $n-3$ and $n-6$ fatty acids with the ϵ-amino group of lysine (Zamora and Hidalgo, 1994, 1995), and, more recently, pyrroles of this type have also been shown to be produced as a consequence of oxidative stress (Hidalgo et al., 1998). In addition, this amino acid can be determined by capillary electrophoresis in hydrolyzed proteins (Zamora et al., 1995).

EXPERIMENTAL PROCEDURES

Materials. ϵ-*N*-Pyrrolylnorleucine was synthesized as described previously (Hidalgo and Zamora, 1995). Fresh foods were purchased in different local markets. Twenty-two different products were selected to be analyzed. They were 5 fishes, 3 meats, 10 vegetables, and 4 nuts. The fishes were cod (*Gadus morhua*), cuttlefish (*Sepia officianalis*), salmon (*Salmo salar*), sardine (*Sardina pilchardus*), and trout (*Salmo gairdnerii*). The meats were beef (fillet), chicken (breast), and pork (chop). The vegetables were broad bean (*Vicia faba*), broccoli (*Brassica oleracea*), chickpea (*Cicer arietinum*), garlic (*Allium sativum*), green pea (*Pisum sativum*), lentil (*Lens esculenta*), mushroom (*Agaricus bisporus*), soybean (*Glycine max*), spinach (*Spinacia oleracea*), and sunflower (*Helianthus annuus*). The nuts were almond (*Prunus dulcis*), hazelnut (*Corylus avellana*), peanut (*Arachis hypogaea*), and walnut (*Juglans regia*). All chemicals were purchased from Aldrich (Milwakee, WI), Sigma (St. Louis, MO), Fluka (Buchs, Switzerland), or Merck (Darmstadt, Germany).

Determination and Characterization of ϵ-*N*-Pyrrolylnorleucine in Fresh Sardine. Because the method used to

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determine ϵ-*N*-pyrrolylnorleucine by capillary electrophoresis was previously employed only in model systems or oxidized microsomes (Zamora et al., 1995, 1997), before determination of this compound in real foods, its identity was confirmed by mass spectrometry. Sardine muscle (0.35 g) was homogenized at 0 °C in 3.5 mL of 50 mM sodium phosphate buffer, pH 7.4, containing 1 mM EDTA. The protein was precipitated by treating the homogenate with trichloroacetic acid (10%) at 0 °C for 15 min and, then, centrifuging at 2250*g* for 15 min. The pellet was suspended in 8 mL of 2 N NaOH and hydrolyzed at 120 °C for 20 h. The hydrolysate was then neutralized with HCl until pH 7−9, the NaCl produced was filtered, and the clear solution was diluted to a final volume of 6 mL. This solution was fractionated by solid-phase extraction chromatography using 3 mL silica gel columns (J. T. Baker Inc., Phillipsburg, NJ). Samples (500 μL) were taken to dryness and extracted two times with 2-propanol/methanol (1:1). The obtained extract was evaporated, dissolved in 300 μL of 2-propanol/methanol (1:1), and passed through the column, which was previously equilibrated with 2-propanol. The column was washed with 3 mL of 2-propanol, and the fraction enriched in ϵ-*N*-pyrrolylnorleucine was extracted with 6 mL of 2-propanol/water (9:1). Later, the column was washed with 3 mL of 2-propanol/water (8:2) and dried. This sequence was used repeatedly until the whole hydrolysate was fractionated. Purified fractions were combined, taken to dryness, dissolved in 200 μL of water, and studied by HPLC/MS. HPLC/MS conditions were analogous to those described previously (Zamora and Hidalgo, 1995).

Determination of Pyrrolyzed Proteins in Fresh Foods. As an additional confirmation that ϵ-*N*-pyrrolylnorleucine was present in food proteins prior to their hydrolysis, some food proteins were derivatized with *p*-(dimethylamino)benzaldehyde according to a previously described procedure (Hidalgo et al., 1998), which was slightly modified. Briefly, chickpeas, soybeans, and peanuts (1.5 g of chickpeas and soybeans were previously soaked overnight in distilled water) were homogenized at 0 °C in 5 mL of 50 mM sodium phosphate buffer, pH 7.4, containing 1 mM EDTA. The protein was precipitated by treating 1 mL of homogenate with 334 μL of 30% of trichloroacetic acid at 0 °C for 15 min and, then, centrifuging at 2250*g* for 15 min. The pellet was suspended in 700 μL of 50 mM sodium phosphate buffer, pH 7.4, and treated with 110 μL of Ehrlich reagent prepared according to the method of Hidalgo et al. (1998), and the resulting suspension was incubated at 45 °C for 30 min. The protein was collected by centrifugation at 2250*g* for 15 min, treated with 1 mL of 6 M guanidine−HCl with 20 mM potassium phosphate−trifluoroacetic acid, pH 2.3, and, finally, left for 30 min at 37 °C with vortexing. The absorbance spectra of 200 μL of the solution obtained after new centrifugation at 2250*g* for 15 min diluted with 800 μL of potassium phosphate−TFA−guanidine buffer were obtained in the next 2 h against a blank prepared under the same conditions but without *p*-(dimethylamino)benzaldehyde.

Determination of ϵ-*N*-Pyrrolylnorleucine in Fresh Foods. Fish, meat, vegetables, and nuts were homogenized at 0 °C in 5 mL of 50 mM sodium phosphate buffer, pH 7.4, containing 1 mM EDTA. The protein was precipitated by treating 1 mL of homogenate with 334 μL of 30% trichloroacetic acid at 0 °C for 15 min and, then, centrifuging at 2250*g* for 15 min. The pellet was suspended in 3 mL of 2 N NaOH and hydrolyzed at 120 °C for 20 h. The hydrolysate was then neutralized with HCl until pH 7−9, the NaCl produced was filtered, and the clear solution was diluted to a final volume of 5 mL. A quantity of this solution was diluted to 200 μL with 1 M sodium borate, pH 9.0, then mixed with 20 μL of homoarginine solution (0.33 mg/mL in water), which was used as internal standard, and, finally, derivatized with 10 μL of diethyl ethoxymethylenemalonate solution (40 μL/mL in ethanol). The mixture was shaken, and 40 μL of the protected amino acids was diluted with 110 μL of water and submitted to capillary electrophoresis according to a previously described procedure (Zamora et al., 1995).

The quantity of the food used in the homogenization depended on the type of food analyzed and was 0.5 g for fish, 0.3 g for meat, and 1.5 g for vegetables and nuts. In addition, the quantity of the hydrolysate used in the derivatization reaction also depended of the food analyzed. Thus, it was 30 μL for fish, meat, broad bean, chickpea, green pea, lentil, soybean, sunflower, almond, peanut, and walnut. A volume of 50 μL was used for garlic and hazelnut. A volume of 100 μL was used for broccoli, mushroom, and spinach. Chickpea, lentil, and soybean were soaked overnight in distilled water and peeled prior to homogenization.

Statistical Analysis. ϵ-*N*-Pyrrolylnorleucine values are expressed as mean values ± standard deviation (SD). The number of experiments is given in parentheses. Statistical data analysis was performed with the SPSS for Windows (v. 7.5.2S) statistical package.

RESULTS

Determination and Characterization of ϵ-*N*-Pyrrolylnorleucine in Fresh Sardine. Muscle from fresh sardines was homogenized, and their proteins were first precipitated with trichloroacetic acid and then hydrolyzed with 2 N NaOH. The derivatization of the produced amino acids with diethyl ethoxymethylenemalonate allowed the determination of the ϵ-*N*-pyrrolylnorleucine, arginine, and lysine content in these proteins. Figure 1A shows the electropherogram obtained for the hydrolysate of sardine proteins. The peaks corresponding to the amino acids ϵ-*N*-pyrrolylnorleucine (Pnl), arginine (Arg), and lysine (Lys) were identified according to their migration times and by addition of standard amino acids. Arginine, after basic hydrolysis, appeared as ornithine.

The peak corresponding to ϵ-*N*-pyrrolylnorleucine was also characterized by HPLC/MS. This technique required an increase in the concentration of this amino acid in the mixture, and a procedure had to be developed to obtain amino acid mixtures from hydrolysates with a high proportion of ϵ-*N*-pyrrolylnorleucine. This procedure, which was carried out by solid-phase extraction chromatography, was not quantitative and recovered only 80−85% of the ϵ-*N*-pyrrolylnorleucine present in the mixture (data not shown). However, this procedure allowed a considerable increase in the proportion of ϵ-*N*-pyrrolylnorleucine. Figure 1B shows the electropherogram obtained for the protein hydrolysate of sardine muscle after concentration by solid-phase extraction chromatography. The peaks corresponding to ϵ-*N*-pyrrolylnorleucine and lysine were confirmed by addition of their standards (Figure 1C). When the mixture enriched in ϵ-*N*-pyrrolylnorleucine was studied by HPLC/MS using the conditions described previously (Zamora and Hidalgo, 1995), the retention times and mass spectra obtained for the ϵ-*N*-pyrrolylnorleucine present in the sardine hydrolysate (Figure 2A) and the standard ϵ-*N*-pyrrolylnorleucine (Figure 2B) were analogous. The peaks at *m*/*z* 134 ($M^+ - CO_2 - NH_4$), 122 ($M^+ - C_2H_4NO_2$), 94 (ethylpyrrole − H), and 81 (methylpyrrole) were diagnostic of the proposed structure (Zamora and Hidalgo, 1994).

Determination of Pyrrolyzed Proteins in Fresh Foods. A further confirmation of the presence of pyrrolyzed proteins in fresh food products was obtained by treating their precipitated proteins with *p*-(dimethylamino)benzaldehyde. Figure 3 shows the absorbance spectra obtained for chickpea, soybean, and peanut proteins treated with the Ehrlich reagent. Although these proteins had very low solubility and their absorption spectra were very poor, it was possible to observe

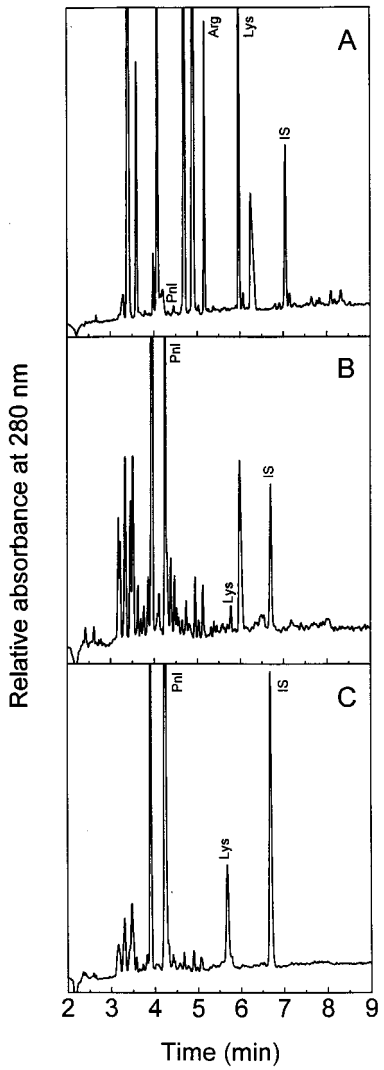

Figure 1. Electropherograms obtained for amino acid analysis after basic hydrolysis and derivatization of (A) fresh sardine muscle, (B) fresh sardine muscle after enrichment in ϵ-*N*-pyrrolylnorleucine by solid-phase extraction chromatography, and (C) sample B after the addition of standard ϵ-*N*-pyrrolylnorleucine and lysine. Peaks corresponding to ϵ-*N*-pyrrolylnorleucine (Pnl), arginine (Arg), lysine (Lys), and internal standard (IS) have been marked.

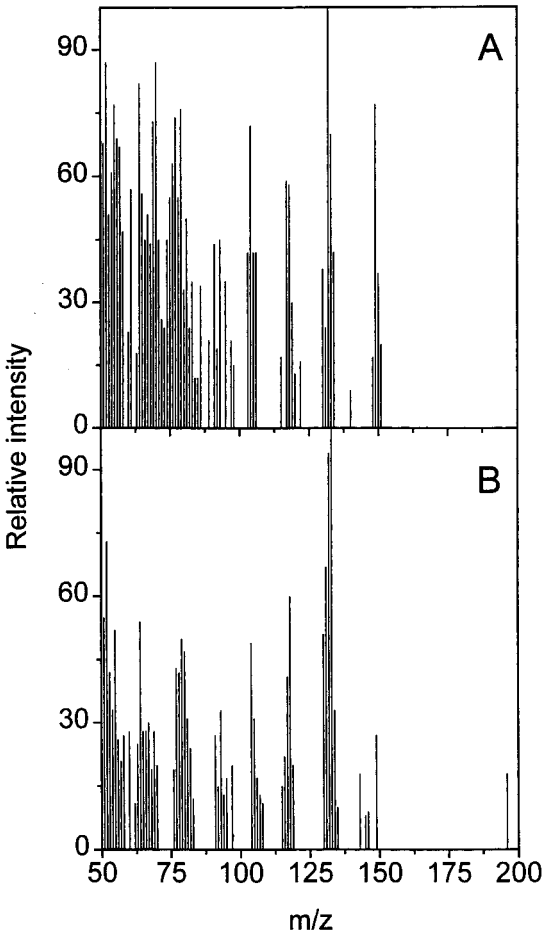

Figure 2. Mass spectra obtained for (A) ϵ-*N*-pyrrolylnorleucine concentrated by solid-phase extraction chromatography obtained from a hydrolysate of fresh sardine muscle and (B) standard ϵ-*N*-pyrrolylnorleucine. Both compounds eluted at the same retention time, and the mass spectrometer was coupled to the HPLC.

a small band corresponding to the Ehrlich adduct that appeared at ∼580 nm. This band was higher in soybean, less clear in peanut, and much more difficult to appreciate in chickpea. The intensity of this band was related to both the solubility of the proteins and their content in ϵ-*N*-pyrrolylnorleucine.

Determination of ϵ-*N*-Pyrrolylnorleucine in Fresh Foods. Analogously to sardine muscle, fresh foods were homogenized and their proteins precipitated with trichloroacetic acid, and the basic hydrolysates of these proteins were used to determine ϵ-*N*-pyrrolylnorleucine by capillary electrophoresis. All of the tested foods showed the presence of ϵ-*N*-pyrrolylnorleucine, but the concentration of this amino acid depended on the food analyzed. Figure 4 shows the electropherograms obtained for three different foods: salmon (A), spinach (B), and walnut (C). The peaks corresponding to ϵ-*N*-pyrrolylnorleucine (Pnl), arginine (Arg), and lysine (Lys) have been marked in the figure.

The concentrations of the amino acids ϵ-*N*-pyrrolylnorleucine, arginine, and lysine determined after basic hydrolysis for the 22 foods studied are collected in Table 1. ϵ-*N*-Pyrrolylnorleucine concentration ranged from 0.24 to 6.36 μmol/g and was higher in protein-rich foods. In fact, there was a correlation ($r = 0.687$, $p = 0.00041$) between the determined ϵ-*N*-pyrrolylnorleucine content and the protein content described for the same foods in the literature (Scherz and Senser, 1994; Salunkhe and Kadam, 1998). Figure 5A shows this correlation, which was not observed between ϵ-*N*-pyrrolylnorleucine concentration and the lipid content of the tested foods (Figure 5B).

On the contrary, when the ϵ-*N*-pyrrolylnorleucine/lysine ratio obtained for the different foods was plotted against the protein and the lipid contents of those foods (parts A and B of Figure 6, respectively) the correlation was observed between the ϵ-*N*-pyrrolylnorleucine/lysine ratio and the lipid content ($r = 0.7632$, $p < 0.0001$).

DISCUSSION

The amino acid ϵ-*N*-pyrrolylnorleucine was previously found to be produced in model systems exposed to oxidative stress (Zamora et al., 1995), and its formation has been suggested as a protective mechanism of living beings to eliminate toxic aldehydes produced during lipid oxidation and to produce endogenous antioxidants (Zamora et al., 1997). Because all living beings are continuously exposed to free radicals, the presence of

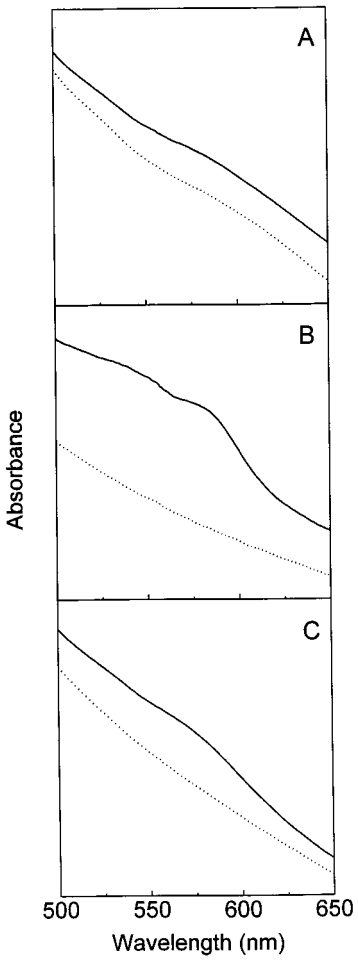

Figure 3. Absorbance spectra of Ehrlich adducts of untreated (A) chickpea, (B) soybean, and (C) peanut proteins (solid lines). Blank spectra are also included for comparison (dotted lines).

this amino acid might be expected in many, if not all, organisms. The present study has screened 22 fresh foods, and all of them have shown the presence of ϵ-*N*-pyrrolylnorleucine in their proteins when they were submitted to basic hydrolysis. The identity of this amino acid was confirmed in sardine muscle by HPLC/MS, and its presence in unhydrolyzed proteins was verified by treating chickpea, soybean, and peanut proteins with *p*-(dimethylamino)benzaldehyde.

The concentration of ϵ-*N*-pyrrolylnorleucine for each food was correlated with its protein content and was independent of its lipid content. However, when the ϵ-*N*-pyrrolylnorleucine/lysine ratio for each food was plotted against its protein and lipid contents, the correlation was observed between the ϵ-*N*-pyrrolylnorleucine/lysine ratio and its lipid content. By using this ϵ-*N*-pyrrolylnorleucine/lysine ratio, the results are not so dependent on the protein content of the food and the differences observed for ϵ-*N*-pyrrolylnorleucine are increased because this amino acid is thought to be produced from lysine (Hidalgo and Zamora, 1993). All of these results are in agreement with the origin proposed for this amino acid, which should be produced more easily if the food is easily oxidized, and this should be easier in foods rich in lipids.

Figure 6B also shows that some foods with a low lipid content (lentil, broad bean, green pea, and chickpea) have a relatively high ϵ-*N*-pyrrolylnorleucine/lysine ratio. This suggests that other variables should be considered to better understand the values obtained for

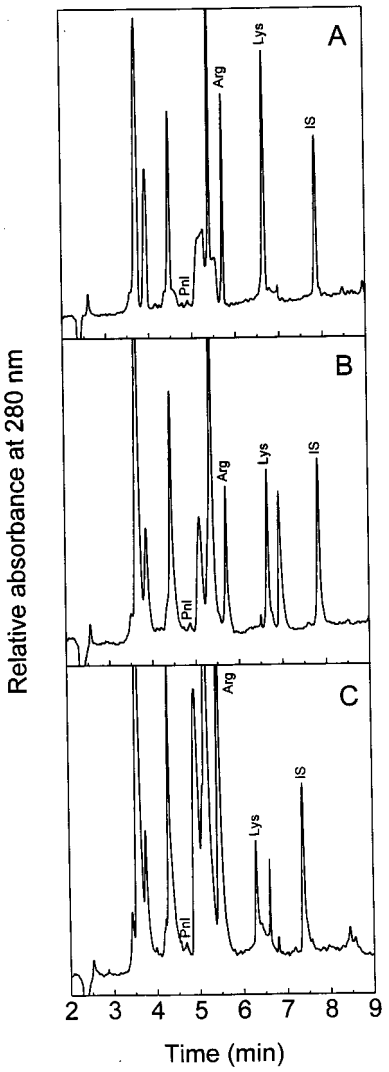

Figure 4. Electropherograms obtained for amino acid analysis after basic hydrolysis and derivatization of (A) salmon, (B) spinach, and (C) walnut. Peaks corresponding to ϵ-*N*-pyrrolylnorleucine (Pnl), arginine (Arg), lysine (Lys), and internal standard (IS) have been marked.

the ϵ-*N*-pyrrolylnorleucine/lysine ratio of each food. In particular, the above four vegetables with a low lipid content but with a high ϵ-*N*-pyrrolylnorleucine/lysine ratio are rich in iron, which should increase the lipid oxidation in these foods.

When the contribution of lipid, protein, and iron contents [obtained from Scherz and Senser (1994) or Salunkhe and Kadam (1998)] to the ϵ-*N*-pyrrolylnorleucine/lysine ratio was studied by using lineal regression, a much better correlation was obtained ($r = 0.881$, $p < 0.0001$). The ϵ-*N*-pyrrolylnorleucine/lysine ratio was found to be a function of these three variables according to the equation

$$\text{Pnl/Lys ratio} = 0.104 + (0.01304 \times \text{Fe}) + (0.003174 \times \text{Lip}) - (0.002888 \times \text{Prot})$$

where Fe is the concentration of iron in the food expressed in mg/100 g, Lip is the lipid content of the food expressed in g/100 g, and Prot is the protein content of the food expressed in g/100 g. This equation suggests a positive contribution of lipid and iron content to the production of ϵ-*N*-pyrrolylnorleucine and a negative contribution of the protein content, which is in agree-

Table 1. ϵ-*N*-Pyrrolylnorleucine, Arginine, and Lysine Composition of Food Products

food	code	μmol/g of food			amino acid ratio
		Pnl[a]	Arg[a]	Lys[a]	Pnl/Lys[a]
cod	1	2.84 ± 0.62 (8)	35.83 ± 3.72 (8)	52.60 ± 6.54 (8)	0.054 ± 0.007 (8)
cuttlefish	2	0.97 ± 0.25 (4)	19.20 ± 0.62 (4)	17.20 ± 1.02 (4)	0.057 ± 0.012 (4)
salmon	3	5.11 ± 0.62 (4)	46.74 ± 0.81 (4)	79.52 ± 5.60 (4)	0.064 ± 0.007 (4)
sardine	4	4.78 ± 0.54 (8)	46.97 ± 3.74 (8)	75.63 ± 3.37 (8)	0.063 ± 0.007 (8)
trout	5	4.45 ± 0.72 (8)	40.55 ± 7.08 (8)	69.13 ± 5.62 (8)	0.065 ± 0.012 (8)
beef	6	5.03 ± 1.66 (6)	46.06 ± 7.59 (6)	67.21 ± 10.89 (6)	0.073 ± 0.017 (6)
chicken	7	4.49 ± 1.05 (6)	43.87 ± 8.58 (6)	63.21 ± 8.81 (6)	0.071 ± 0.015 (6)
pork	8	6.36 ± 1.06 (6)	64.08 ± 7.18 (6)	87.58 ± 8.79 (6)	0.073 ± 0.012 (6)
broad bean	9	1.84 ± 0.68 (10)	11.50 ± 4.56 (10)	9.21 ± 2.02 (10)	0.202 ± 0.052 (10)
broccoli	10	0.61 ± 0.14 (6)	7.27 ± 1.57 (6)	7.46 ± 1.32 (6)	0.085 ± 0.027 (6)
chickpea	11	2.10 ± 0.34 (4)	14.54 ± 2.52 (4)	11.92 ± 1.79 (4)	0.177 ± 0.019 (4)
garlic	12	0.24 ± 0.15 (5)	6.15 ± 2.55 (5)	1.91 ± 0.95 (5)	0.123 ± 0.020 (5)
green pea	13	2.26 ± 0.35 (10)	13.65 ± 2.15 (10)	12.02 ± 1.43 (10)	0.190 ± 0.034 (10)
lentil	14	3.08 ± 0.48 (6)	18.08 ± 5.46 (6)	14.34 ± 3.65 (6)	0.221 ± 0.031 (6)
mushroom	15	0.30 ± 0.06 (6)	3.58 ± 0.39 (6)	3.73 ± 0.42 (6)	0.082 ± 0.022 (6)
soybean	16	3.52 ± 0.37 (5)	37.31 ± 12.78 (5)	25.78 ± 5.73 (5)	0.140 ± 0.022 (5)
spinach	17	0.41 ± 0.13 (6)	3.04 ± 1.04 (6)	3.25 ± 1.03 (6)	0.126 ± 0.016 (6)
sunflower	18	2.51 ± 0.58 (5)	56.52 ± 6.91 (5)	16.25 ± 1.59 (5)	0.153 ± 0.030 (5)
almond	19	2.04 ± 0.32 (6)	42.98 ± 5.06 (6)	6.43 ± 0.74 (6)	0.321 ± 0.063 (6)
hazelnut	20	0.96 ± 0.05 (5)	22.08 ± 1.64 (5)	2.99 ± 0.16 (5)	0.321 ± 0.015 (5)
peanut	21	2.89 ± 0.95 (5)	68.28 ± 9.89 (5)	13.01 ± 1.65 (5)	0.222 ± 0.059 (5)
walnut	22	2.45 ± 0.80 (5)	60.39 ± 12.53 (5)	6.99 ± 0.90 (5)	0.344 ± 0.069 (5)

[a] Abbreviations: Pnl, ϵ-*N*-pyrrolylnorleucine; Arg, arginine; Lys, lysine.

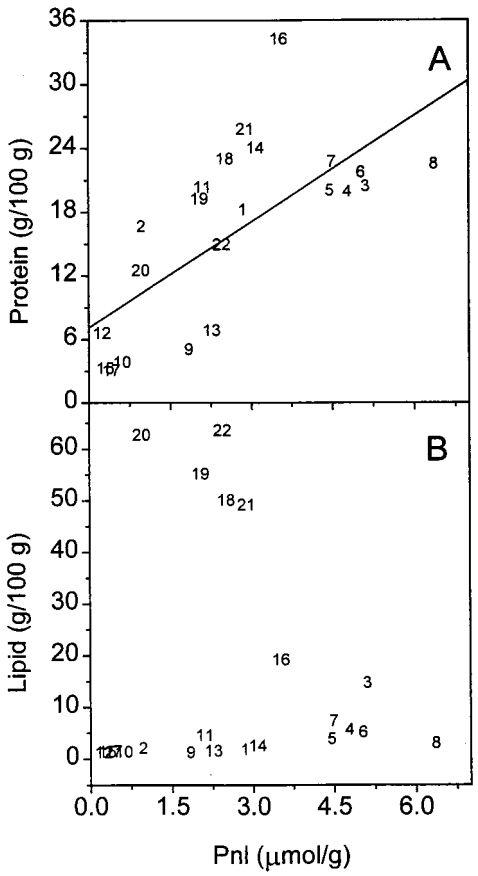

Figure 5. Plots of ϵ-*N*-pyrrolylnorleucine (Pnl) concentration versus (A) protein content and (B) lipid content of each food. Protein and lipid contents for the different foods were obtained from Scherz and Senser (1994) or Salunkhe and Kadam (1998). The number used for each food is indicated as "code" in Table 1.

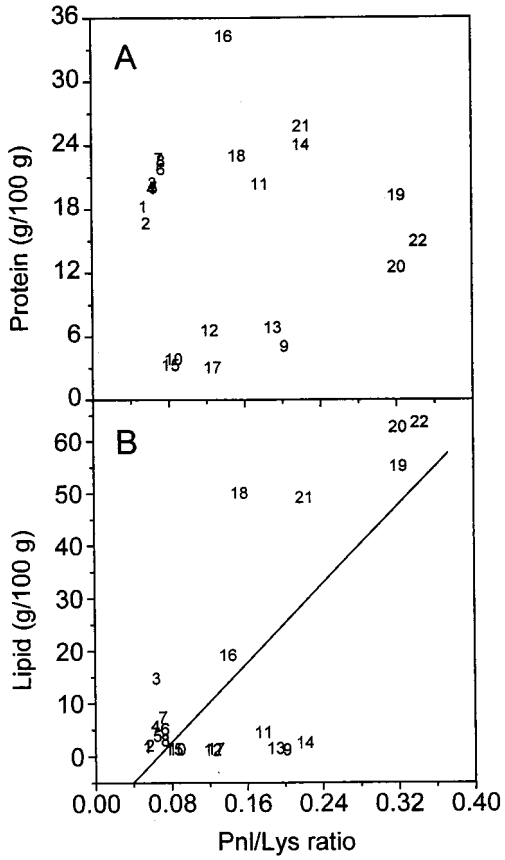

Figure 6. Plots of ϵ-*N*-pyrrolylnorleucine/lysine (Pnl/Lys) ratio versus (A) protein content and (B) lipid content of each food. Protein and lipid contents for the different foods were obtained from Scherz and Senser (1994) or Salunkhe and Kadam (1998). The number used for each food is indicated as "code" in Table 1.

ment with the proposed origin for ϵ-*N*-pyrrolylnorleucine. Both high lipid and iron contents should favor lipid peroxidation, and the protein might be acting as antioxidant as suggested previously (Alaiz et al., 1997, and references cited therein).

The plot of the ϵ-*N*-pyrrolylnorleucine/lysine ratios calculated according to the above equation versus the experimental values is shown in Figure 7A. For most of the foods the experimental and calculated values were very similar. However, both values were very different for the sunflower, suggesting that in this seed other

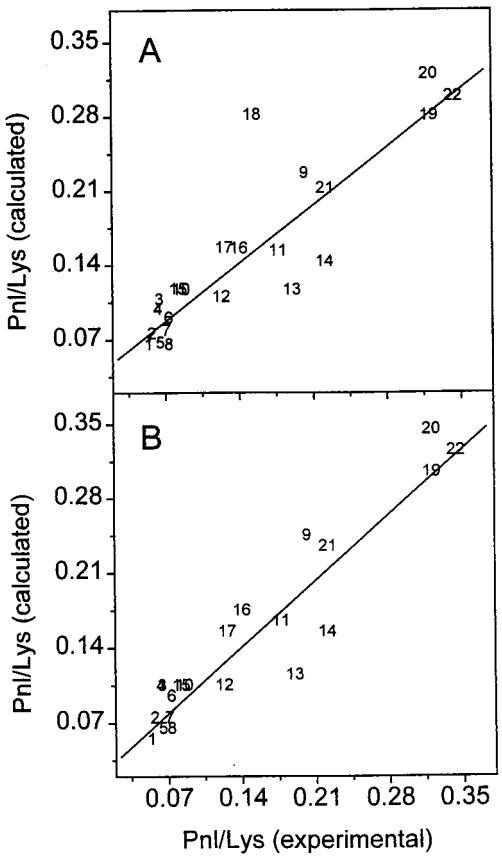

Figure 7. Plots of calculated versus experimental ϵ-*N*-pyrrolylnorleucine/lysine (Pnl/Lys) ratios for (A) the 22 food products analyzed in the present study and (B) when the data obtained for sunflower were eliminated in the regression analysis. The number used for each food is indicated as "code" in Table 1.

variables should be considered to calculate the ϵ-*N*-pyrrolylnorleucine/lysine ratio. If the results obtained for this seed are eliminated from the calculations, the correlation between the experimental and calculated ϵ-*N*-pyrrolylnorleucine/lysine ratios increased considerably ($r = 0.941$, $p < 0.0001$). Figure 7B shows this correlation.

All of the above results suggest that ϵ-*N*-pyrrolylnorleucine is a normal component of many fresh food products, in which it may be acting as a natural antioxidant. In addition, the ϵ-*N*-pyrrolylnorleucine/lysine ratio is characteristic for each food, and it is related to its lipid, iron, and protein contents. Additional studies are needed to analyze if this ϵ-*N*-pyrrolylnorleucine/lysine ratio may be changing as a function of processing and storage, therefore constituting a marker of the changes produced in the food during these processes. These studies are being developed at present in this laboratory.

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