Occurence of Protein-Bound Lysylpyrrolaldehyde in Dried Pasta[1]

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ABSTRACT

The extent of the advanced Maillard reaction (MR) involving protein-bound lysylketoses degradation that occurs in pasta drying was studied by evaluating furosine and lysylpyrrolaldehyde (LPA). By enzymatic hydrolysis followed by solid-phase extraction and ion pair reversed-phase high-performance liquid chromatography (IP-RP HPLC) with 297-nm detection, protein-bound LPA was separated without interference and quantified in pasta products using carboxy-2-pyrrolaldehyde as the external standard. Model doughs containing different amounts of cold or [U-14C]-labeled glucose or maltose and pasta processed under different heating conditions were considered. A close relationship between the accumulation behaviors of furosine and protein-bound LPA was observed, confirming that LPA is a main derivative of lysylketoses residues. Further degradation of LPA was detected only under thermal treatments not applicable in pasta drying. Under the pasta processing conditions of a semipilot plant, and with semolina of known origin and free sugar composition, LPA formation was enhanced (>12 mg/100 g of protein) when the drying cycle included temperatures close to 80°C or higher, and pasta moisture values were close to 15% or lower. Under low-temperature (50°C) conditions, no LPA was produced. Another key parameter was the concentration of reducing sugars, the most effective being glucose. Although other aminoketoses besides lysylketoses are probably formed and degraded, furosine and LPA evaluation seems to sufficiently describe the MR extent occurring on pasta protein. Considering the undesirable sensorial changes of pasta attributable to extensive MR, the possible antinutritional properties reported for LPA, and the wide range of LPA values (from 0 to over 40 mg/100 g of protein) found in commercial spaghetti, a better control of the advanced MR in this food is advisable.

Pasta under drying represents a relatively simple food system where nonenzymatic browning related to the Maillard reaction (MR) can easily occur. This is due to the type and concentration of the main reagents involved, the water activity (a_w) of the system in drying, and the time-temperature conditions of the thermal process (Kaanane and Labuza 1989). MR in pasta takes place both between water-soluble compounds and on the protein matrix. Among the soluble products, we recently identified and quantified the glucosylisomaltol, namely 2-acetyl-3-D-glucopyranosylfuran (AGPF), a stable degradation product arising in pasta drying from the reaction of maltose with some free amino acids, mainly glutamine (Resmini et al 1993).

The early MR on pasta protein was evaluated for the effects on reactive lysine (Dexter et al 1984, Acquistucci et al 1988), and more recently, on blocked lysine as determined by furosine (Resmini et al 1990a). This molecule derives from lysylketoses (Amadori rearrangement products) through a dehydration-cyclization reaction under acid-hydrolysis conditions (Finot and Mauron 1972). Furosine values of commercial spaghetti ranged from 50 to 700 mg per 100 g of protein, indicating that nearly 30% lysine of semolina could become biologically unavailable in pasta processed under some types of high-temperature drying cycles. The behaviors of the furosine formation rate suggested that, in pasta dried under high-temperature conditions, degradaditions, degradation of lysylketose residues can occur (Pagani et al 1992). Taking the reducing sugar composition of semolina into consideration (Lintas and D'Apollonia 1973, Kruger and Matsuo 1982, Resmini et al 1993), the lysylketoses formed in pasta protein are mainly maltulosyl-, fructosyl-, and maltulotriosyl-lysyl residues. The heating conditions are much milder than those applied for other cereal-based foods. In bakery products, for example, the advanced MR is reported to liberate soluble degradation compounds from protein (Rothe et al 1972, Nishibori and Kawakishi 1990). Therefore, we can suppose that in pasta tion of lysylketose residues can occur (Pagani et al 1992). Taking the reducing sugar composition of semolina into consideration (Lintas and D'Apollonia 1973, Kruger and Matsuo 1982, Resmini et al 1993), the lysylketoses formed in pasta protein are mainly maltulosyl-, fructosyl-, and maltulotriosyl-lysyl residues. The heating conditions are much milder than those applied for other cereal-based foods. In bakery products, for example, the advanced MR is reported to liberate soluble degradation compounds from protein (Rothe et al 1972, Nishibori and Kawakishi 1990). Therefore, we can suppose that in pasta the main derivatives of lysylketoses residues are nitrogen-containing compounds that remain protein-linked. The literature reports several possible compounds including pyrrolaldehydes, pyridiniumbetaines, and amino reductones (Ledl and Schleicher 1990). Among N-substituted pyrrolaldehydes, the presence of ε-(2-formyl-5-hydroxymethyl-pyrrol-1-yl)-L-norleucine, abbreviated as lysylpyrrolaldehyde (LPA), also named pyrraline (Hayase et al 1989), was reported to derive from several lysylketoses in heated-model systems and in food, either under free form (Nakayama et al 1980, Chiang 1988) or protein bound (Sengl et al 1989, Henle and Klostermeyer 1993). The amount of LPA was proposed as a parameter for evaluating the advanced MR extent in heat-treated or long-stored food. Furthermore, the weak mutagenic activity found in a heated lysine-glucose mixture, attributed to LPA (Omura et al 1983), and its enzyme-depressive properties (Oste et al 1987), indicate the interest in LPA formation in basic foods submitted to severe heating processing.

The objective of this work was to point out a sensitive and routine method for evaluating protein-bound LPA in pasta products. Subsequently, we studied the heat-processing conditions promoting LPA formation with respect to the early MR extent in model systems, experimental pasta, and commercial spaghetti.

MATERIALS AND METHODS

Semolina and Pasta Samples

Commercial durum wheat semolina was used to prepare manually purified gluten (Knight 1965) and experimental pasta (spaghetti Ø 17 mm). Commercial spaghetti (Ø 17 mm) was produced from semolina of durum wheat (cultivars Lira, Neodur, or Valnova). Both experimental pasta and commercial spaghetti were obtained in a semipilot plant (Braibanti Research Centre, Calliano, Italy) described in other work (Resmini and Pagani 1983). The experimental pasta samples were dried with cycles at 40°C,

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including steps at 75°C or 85°C; holding times ranged from 1 to 24 hr. During these heating steps pasta moisture was kept constant at 22, 15, or 12.5% (by weight), respectively, as described by Pagani et al (1992). Commercial spaghetti was dried using medium-temperature (MT) or high-temperature (HT) cycles (Fig. 1). Spaghetti made from Valnova was also dried with a low-temperature (LT) cycle at 50°C for 7 hr. Other spaghetti samples (Ø 17 mm) and cereal-based products were collected at local markets.

Standard and Reference Materials

Glucose and maltose were purchased from Sigma Chemical Co. (St. Louis, MO); furosine dihydrochloride was purchased from Neosystem (Strasbourg, France); carboxy-2-pyrrolaldehyde and HMF were purchased from Aldrich Chemical Co. (Milwaukee, WI); AGPF was purified from a heat-treated maltose-glutamine solution as described by Resmini et al 1993). LPA was synthesized according to Schussler and Ledl (1989b). Solutions of [U-14C] glucose (200 μCi/ml, 293 mCi/mmol) and [U-14C] maltose (200 μCi/ml, 630 mCi/m*M*) were purchased from Amersham (Buckinghamshire, UK). Pepsin (10 FIP-U/mg, 7192) and pronase E (4000 PU/mg, 7433) were purchased from Merck. Prolidase (46U/mg, 33562) was purchased from Serva. Leucine aminopeptidase (122U/mg, 61860) was purchased from Fluka.

Sugars and Amino Acids

Soluble reducing sugars were extracted as previously reported (Resmini et al 1993) and determined by HPLC. The International Dairy Federation (1991) method for lactulose evaluation was used. Furosine was determined according to Resmini et al (1990b). Amino acid composition of pasta was determined after either enzymatic or acid hydrolysis (1 mg of dried product per milliliter of 6*N* HCl kept at 110°C for 22 hr in a glass vial sealed under vacuum) with an amino acid analyzer under the previously reported chromatographic conditions (Resmini et al 1993). Moisture (method 44-15A) and protein (method 46-11A) (N × 5.70) of dough and pasta samples were determined by AACC methods (1983).

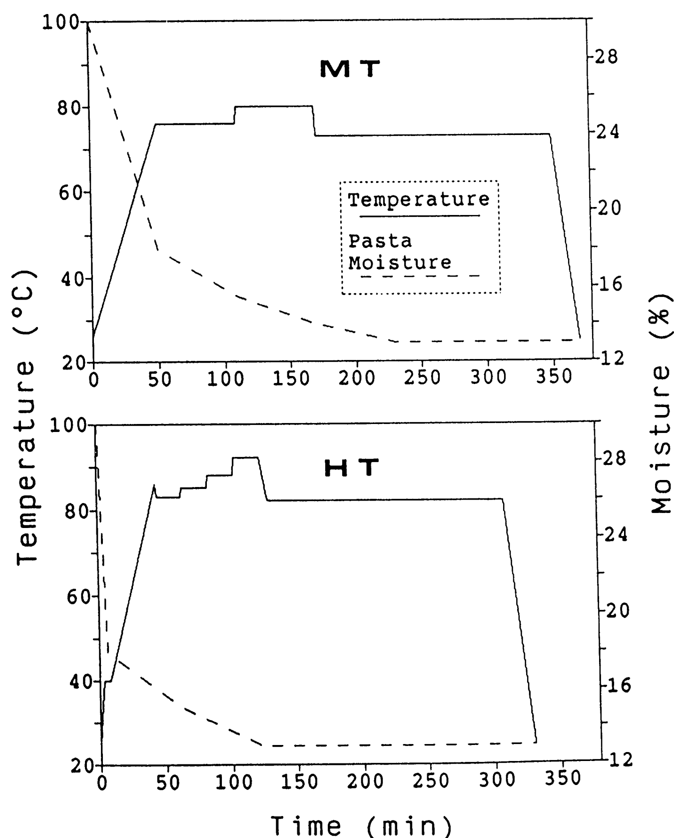

Fig. 1. Drying conditions of spaghetti processed in a semipilot plant with a medium-temperature (MT) or high-temperature (HT) drying cycle.

Model Doughs

One gram of purified freeze-dried gluten (reducing sugars not detectable by HPLC method, 88–90% protein on dry matter) was kneaded in a 25-ml glass vial with 0.8 ml of maltose or glucose solutions to obtain sugar concentrations of 0, 14, 28, 56, and 112 m*M*/100 g of protein in the doughs. The pH values were always between 6 and 7. Each dough was oven-dried at 70°C for 24 hr, 85°C for 7 hr, or 97°C for 5 hr. The heating conditions were chosen to always reach a complete dough dryness (11–12% moisture by wet weight). Doughs containing 56 m*M* of maltose or glucose per 100 g of protein were also dried at 85°C for holding times from 1 to 44 hr. Labeled doughs for LPA quantification were prepared in 2-ml glass vials by mixing 10 mg of freeze-dried gluten with 10 μl of glucose or maltose solution containing 6–15 μ*M* of cold sugar and 16–100 μCi of the corresponding labeled molecule; they were then dried at 80°C for 48 hr.

Hydrolysis Conditions

Enzymatic hydrolysis was performed according to the method proposed by Schmitz et al (1976) and used by Henle and Klostermeyer (1993) for LPA evaluation in milk protein, with some modifications. In a 10-ml screw cap vial, 200 mg of dried pasta or 50 mg of dried dough (previously ground to 200–400 mesh) were mixed with 2 ml of water and adjusted to pH 2.0. One crystal of thymol and 2.5 mg of pepsin (250 μl of a solution containing 10 mg/ml) were added. The closed vial was magnetically stirred in an oil bath at 40°C. After 24 hr, it was adjusted to pH 8.2 with 1*N* NaOH and 0.5 ml of 1*M* borate buffer, pH 8.2, containing 2.5 mg of pronase was added. After another 24 hr, 20U prolidase and 12U aminopeptidase M were added. Hydrolysis was usually interrupted after 24 hr (72 hr total), but longer incubation times for every enzyme were also tested; in these cases, an additional amount of the enzyme was introduced for every 24-hr incubation. The hydrolysate was made up to 5 ml with water, adjusted to pH 6.0–6.2, and centrifuged at 12,000 × *g* for 15 min. The supernatant was filtered on a 0.2 μm cellulose acetate membrane (MFS, Dublin, CA) and submitted to solid-phase extraction (SPE). Buffer and enzyme amounts were proportionally reduced for labeled dough hydrolysis.

Alkaline hydrolysis was performed according to Schussler and Ledl (1989a). The neutralizated hydrolysate was then submitted to purification by SPE.

Water-soluble MR compounds were extracted from pasta by the sample processing described for enzymatic hydrolysis, including pH adjustment but without using enzymes.

Hydrolysate Purification by SPE

Before injection into HPLC, the hydrolysate solution (4.5 ml) was clarified on a prewetted 200-mg silica cartridge (Extract-clean, Alltech). The first displaced 1 ml was discarded and the subsequent eluate was collected, adjusted to pH 7.0–8.0, and directly injected. If necessary, desalting and LPA concentration were achieved by submitting the eluate to a second SPE on a C18 cartridge (Sep-pak, Millipore). Solution volumes from 0.5 to 1 ml were added into the prewetted cartridge, washed with 10 ml water, and eluted with 5 ml of methanol. The eluate was dried under vacuum at 35°C and dissolved with 100-200 μl of water before injection. This double-SPE procedure was used for radioactive monitoring of LPA peak and for alkaline hydrolysate.

HPLC of Lysylpyrrolaldehyde

The ion pair reversed-phase (IP-RP) HPLC was adopted. Column type: C8, 250 mm length, 4.6 mm i.d., 5 μm (chemically unmodified batch for furosine-dedicated column) (Alltech Europe, Belgium). Solvent A: 0.4% acetic acid (v/v). Solvent B: 0.27% potassium chloride (w/v) in solvent A. Linear elution gradient expressed as solvent B proportion: 0–8 min, 0%; 8–22 min, 100%; 22–23 min, 100%; 23–24 min, 0%. Flow rate: 1.2 ml/min. Run-to-run time: 32 min. Column temperature: 32°C. Column washing

with pure methanol (0.1 ml/min) was performed overnight. Injected volume: 10 or 50 μl. HPLC apparatus: Waters 625-LC nonmetallic system. Detection: 297 nm with Milton Roy model SM 4000 and in the 210–400 nm range with Waters 996 diode array detector (DAD). Peak quantification was performed by area integration (valley-to-valley mode) with Waters 840 software.

For radioactivity monitoring, fractions of eluting solvent were collected in scintillation glass vials every 5 sec and submitted to radiocarbon counting by liquid scintillation (LSC) with 85% counting efficiency. To achieve radiochemical evaluation of the LPA response factor, quantification of the net radioactivity of the LPA peak was performed after the peak-area integration mode and subtracting the corresponding background to the gross-count value of each collected fraction. On the basis of the radiocarbon abundance of pyrrolic ring, the amount of LPA in the collected peak was stoichiometrically calculated.

Protein-Bound Radioactivity Split by Heating of Dough

Dough containing [U-14C] maltose (0.55 m*M* per gram of protein, 16 μCi per m*M* of maltose) was oven-dried at 70°C for 24 hr and then finely ground. To remove all water-soluble radioactivity, four subsequent washings with 50 ml of water per gram of dough were performed; each washing included 15 min of sonication, 5 min of homogenization, and a final centrifugation discarding the supernatant. The washed dough was freeze-dried and submitted to a second heat-treatment at either 70°C for 24 hr, 85°C for 7 hr, or 97°C for 5 hr. Before and after the second heat-treatment, total radioactivity was measured on dried dough dissolved with NaOH 1*N* and adjusted to pH 6.0. Free radioactivity split by the second heat-treatment was determined after sonication of 50 mg of dried dough with 10 ml of water for 15 min, followed by centrifugation at 3,000 × *g* for 10 min. Supernatant (2 ml) was submitted to LSC. Counting efficiency measured by the internal standard mode ranged between 50 and 70%.

RESULTS AND DISCUSSION

HPLC of Protein-Bound Lysylpyrrolaldehyde

Under ion-exchange chromatography, LPA peak is eluted together with phenylalanine. To avoid interference, either an additional RP-HPLC on a C18 column (Sengl et al 1989) or monitoring with DAD (Henle and Klostermeyer 1993) have been proposed. Under RP-HPLC, LPA elutes close to tryptophan (Schussler and Ledl 1989a). Because of the relatively high concentration in food, and despite its different UV spectrum, tryptophan can represent a seriously interfering peak as well. According to Chiang (1988), free LPA can be separated under isocratic HPLC conditions and monitored with high selectivity by electrochemical detector. Taking into account both the hydrophobic and ionic properties of this basic molecule, we prefered IP-RP HPLC for evaluating LPA in food hydrolysate. Under the adopted conditions, synthesized LPA was eluted with a 15–17 min retention time (RT), 5–7 min after the tryptophan tailing peak (Fig. 2). Some furan compounds arising from the advanced MR, such as AGPF and HMF, were satisfactorily separated as well. A slow decrease in absolute RT values with an increase in relative RT of LPA was observed during column lifetime. The acidic eluents probably produce a slow cleavage of C8 chains from the silica matrix of the stationary phase. This modification promotes reduction of hydrophobic interactions and increases polar interactions towards the eluted molecules.

LPA shows the UV spectrum (Fig. 3) reported by Nakayama et al (1980) as characteristic of pyrrole-2-aldehydes, and, in weakly acid or neutral aqueous solutions, it gave an additional tailing peak (24-min RT) and reconverted to the original one in the alkaline medium. Because of this behavior and its shifted spectrum, this additional compound could be a condensation product, probably a bispyrrole arising from one of the possible dimerization reactions assumed by Ledl and Schleicher (1990). This polymerization tendency of LPA suggested the possible promotion of protein cross-linking in aged human tissues (Hayase et al 1989).

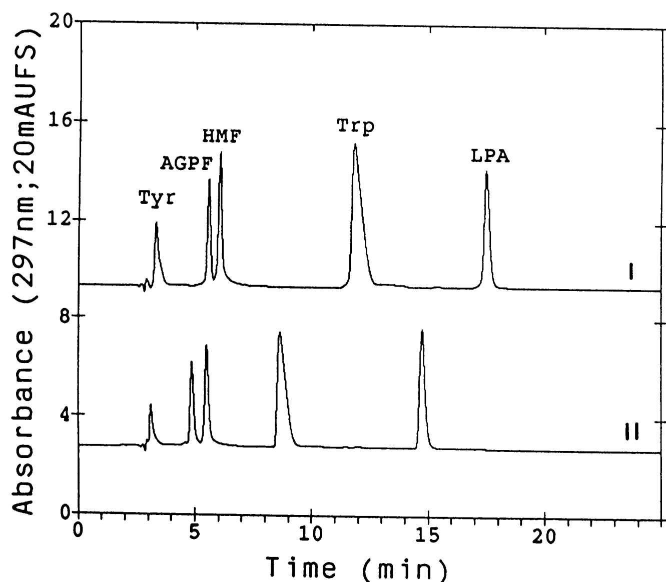

Fig. 2. Ion pair reversed-phase high-performance liquid chromatography of standard solution with injected 15 n*M* of tyrosine (Tyr), 150 p*M* of acetylglucopyranosylfuran (AGPF), 120 p*M* of hydroxymethylfurfural (HMF), 4 n*M* tryptophan (Trp), and 70 p*M* of lysylpyrrolaldehyde (LPA). Patterns were obtained on the same column after 100 and 600 injections, lines I and II, respectively.

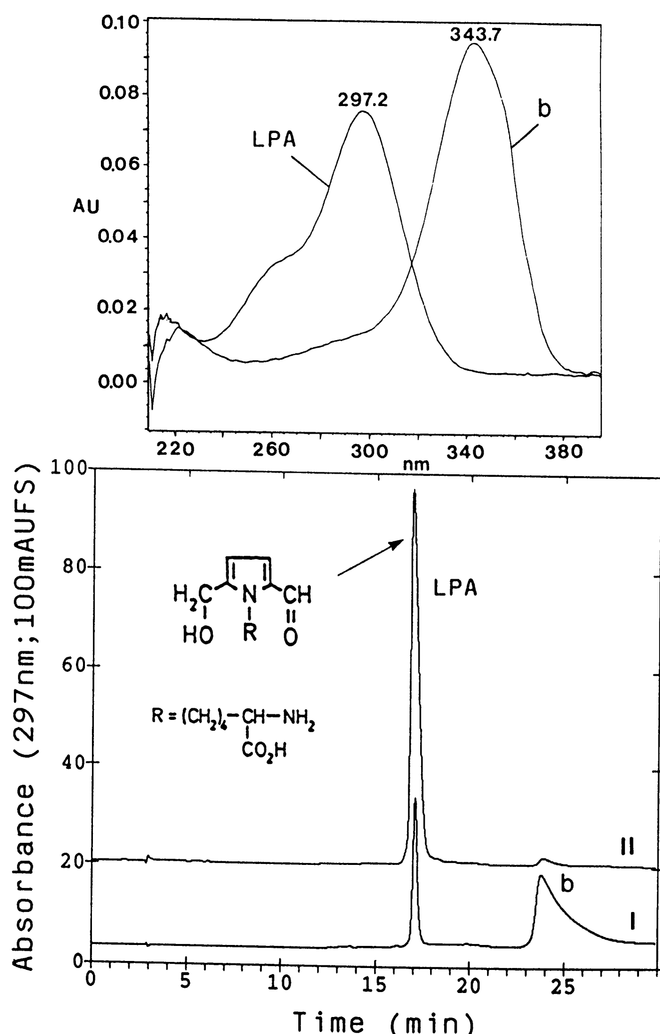

Fig. 3. High-performance liquid chromatography elution profiles of lysylpyrrolaldehyde (LPA, 1 n*M* injected) stored in a weakly acid solution (pH 4.0) and after adjustment to pH 8.0, lines I and II, respectively. The upper box shows the diode array detector spectra of the peaks.

Some functional properties of heat-treated foods may be affected by the same reaction mechanism.

Enzymatic hydrolysis followed by silica cartridge clarification and IP-RP HPLC allowed a selective and sensitive evaluation of protein-bound LPA in pasta processed under different heating conditions (Fig. 4). Furthermore, no LPA peak could be detected in LT-dried pasta, semolina, or the water-extract of pasta, even when heated under the most severe conditions. This proves the separation is interference-free. Due to the negligible amount of free lysine (P. Resmini, *unpublished data*), the lack of free LPA in pasta was expected and may be not in contrast to data of Chiang (1988), who reported free LPA ranging from 1 to 200 ppm in other food preparations. We observe that 1 ppm of LPA is easily detected by our method.

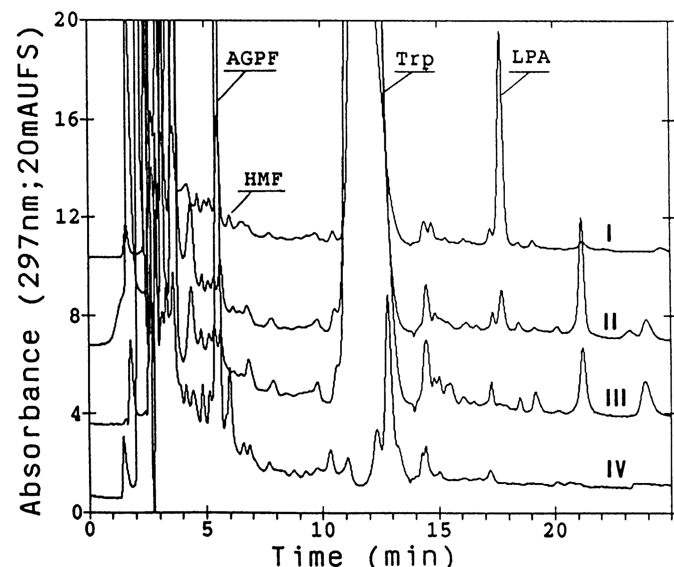

Fig. 4. High-performance liquid chromatography elution profiles of pasta processed under different heating conditions. Enzymatic hydrolysates of spaghetti dried with high temperature (line I) (12.1 mg of protein-bound lysylpyrrolaldehyde [LPA] per 100 g of protein); medium temperature (line II) (1.9 mg of protein-bound LPA); and low temperature (line III) cycles. Water extract of experimental pasta heat-treated at 85°C for 24 hr with 12.5% moisture and containing 120 mg of protein-bound LPA (line IV). AGPF = acetylglucopyranosylfuran, HMF = hydroxymethylfurfural, Trp = tryptophan.

Because the adopted enzymatic method was proposed for dairy protein, we could control whether or not the protein-bound LPA was completely released in HT-dried pasta (Fig. 5). Only an increase in the incubation times of prolidase plus aminopeptidase produced an LPA split slightly higher (>10%) than that obtained under hydrolysis conditions reported for milk protein (24 hr per enzyme, 72 hr total), but a prolonged step under acid condition negatively affected LPA recovery. However, the amount of most of the amino acids split by enzymatic hydrolysis (72 hr) was not less than 80% of that found after acid hydrolysis (Table I). Taking these results into consideration, we adopted a 72-hr incubation time for pasta products as well.

As shown in Figure 6, alkaline hydrolysis could not be adopted for evaluating protein-bound LPA in cereal products. Partial degradation of the molecule occurred during hydrolysis, markedly in presence of starch. Hugli and Moore (1972) reported lysine degradation under similar alkaline hydrolysis conditions.

LPA peak quantification was achieved by radiochemical monitoring of the HPLC pattern of labeled model doughs. Because the peak shape under radioactivity detection was the same as that under UV detection, and it lacked interfering compounds (Fig. 7), this approach is reliable and precise. According to the accepted mechanism, the same LPA molecule is reported to form from a reaction of a lysyl residue with either glucose, maltose, or lactose (Ledl and Schleicher 1990). Under the described hydrolysis conditions, LPA obtained from both [U-14C] glucose and maltose gave the same response factor, which was close to that of carboxy-2-pyrrolaldehyde (Table II). This commercially available compound (6-min RT) representing the main part of the pyrrole moiety in the LPA molecule has a similar UV spectrum and can be used as an appropriate standard for routine quantification. Overall performance of the whole analytical procedure is good. The coefficient of variation was 3.2 for 6 replicates. The minimum detectable injected amount is 100 femtomoles of LPA, corresponding approximately to the 0.03 mAU peak-height and

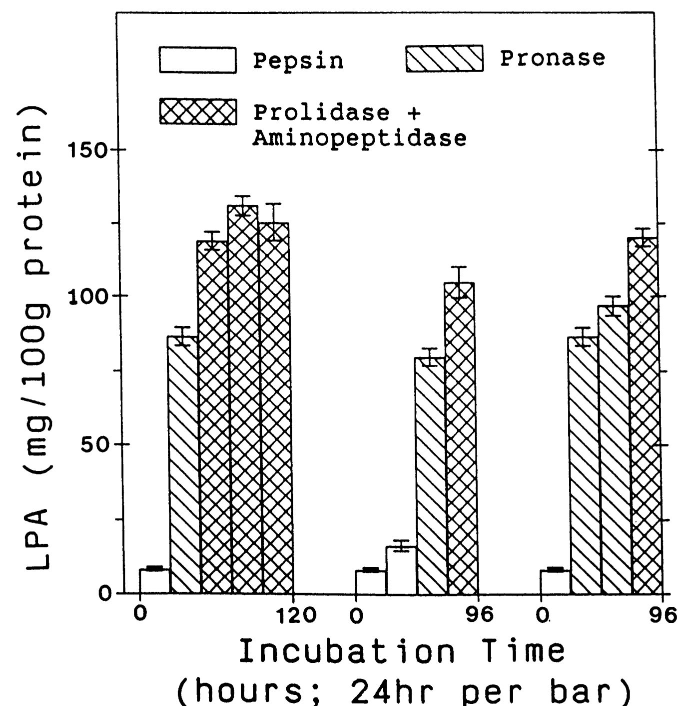

Fig. 5. Release of lysylpyrrolaldehyde (LPA) from high-temperature treated (85°C for 24 hr, 12.5% moisture) experimental pasta after different incubation times of each enzyme. Error-bar represents standard deviation of the mean.

TABLE I
Amino Acid Composition[a] Determined after Acid or Enzymatic Hydrolysis of High-Heated Pasta[b]

	Acid Hydrolysis	Enzymatic Hydrolysis
Amino Acids		
Aspartic Acid[c]	25.19 ± 0.40	6.94 ± 0.36
Threonine	23.88 ± 0.48	23.02 ± 0.36
Serine	49.21 ± 0.19	45.71 ± 0.46
Asparagine[c]	...	12.95 ± 0.18
Glutamic Acid[c]	238.55 ± 2.69	16.67 ± 0.22
Glutamine[c]	...	189.14 ± 0.71
Proline	105.02 ± 0.47	95.23 ± 1.92
Glycine	33.21 ± 0.71	26.49 ± 0.66
Alanine	35.21 ± 0.23	31.95 ± 1.36
Valine	34.93 ± 0.05	33.19 ± 0.30
Half Cystine	11.10 ± 1.19	10.47 ± 0.23
Methionine	7.45 ± 0.84	6.23 ± 0.21
Isoleucine	27.58 ± 0.43	31.84 ± 0.37
Leucine	57.01 ± 1.06	54.39 ± 0.59
Tyrosine	18.67 ± 0.75	16.07 ± 0.59
Phenylamine	31.66 ± 0.12	30.22 ± 0.15
Tryptophan[c]	...	4.24 ± 0.37
Lysine[c]	8.60 ± 0.20	6.11 ± 0.20
Histidine	12.84 ± 0.15	11.40 ± 0.42
Arginine	20.16 ± 0.26	18.97 ± 0.12
Ammonia[c]	299.72 ± 5.39	117.31 ± 1.91
Protein (N × 5.70)	11.38 ± 0.12	11.38 ± 0.12

[a] Given as m*M* per 100 g of protein. Mean values ± standard deviation ($n = 2$).
[b] Heated at 85°C for 24 hr with 12.5% pasta moisture.
[c] Due to degradation and rearrangement reactions these values are not comparable.

0.1 ppm of LPA in dried pasta. The response is linear in the range of 1–1,000 pM injected ($r^2 = 0.999$).

Lysylpyrrolaldehyde in Dried Model Doughs

The lysine content of durum wheat pasta protein may range from 13 to 19 m*M* per 100 g (Dexter et al 1984, Manser 1986), but that of free reducing sugar may range from less than 6 m*M* per 100 g of protein in semolina (Kruger and Matsuo 1982) to 100 m*M* in dried pasta (Dexter et al 1990). As a consequence, under the same drying conditions, the amount of free reducing sugars represents the key factor affecting the MR extent on pasta protein. The relative composition of reducing sugars is roughly 75% maltose, 10% maltotriose, 10% glucose, and 5% fructose (Resmini et al 1993). To evaluate the effect of maltose and glucose concentration on the early and advanced MR on gluten protein, model doughs containing sugar amounts of 0–110 m*M*/100 g of protein and heat-treated under different thermal conditions were analyzed for furosine and LPA. Data of Figure 8 clearly point out the effect of both heating and sugar concentrations on the MR patterns. The early MR extent at 70°C for 24 hr strictly depended on the sugar concentration and the effect of the sugar type was negligible. Due to the lysylketose stability under mild heating conditions (Hurrel and Carpenter 1981), only a small amount of LPA (<3 mg/100 g of protein) was produced. The reported stronger reactivity of glucose for the Amadori compounds production (Ludeman and Erbersdobler 1990) was seen at 85°C; the rate of furosine formation being faster in glucose-system. At this temperature, lysylketoses were degraded, producing LPA values that were 5–10 times higher than those found at 70°C. Because of the lower thermal stability of fructosyllysyl residue with respect to maltulosyllysyl residue (Kato et al 1989),

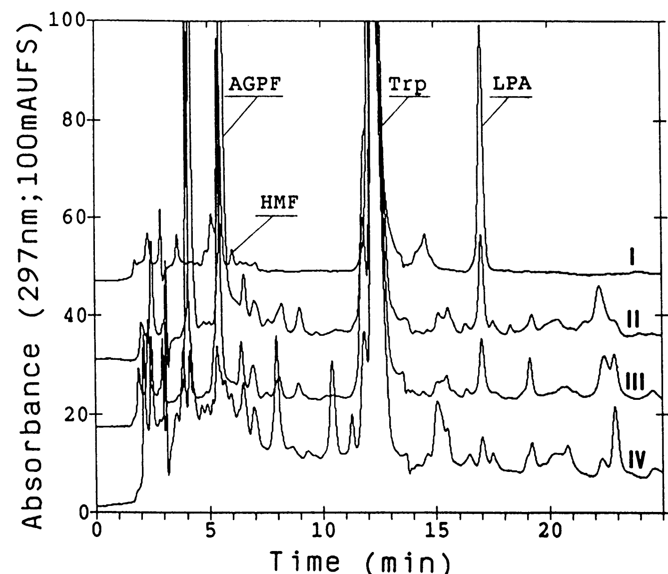

Fig. 6. High-performance liquid chromatography elution profiles of model doughs dried at 85°C for 18 hr in presence of 54 m*M* of maltose per 100 g of protein and submitted to different hydrolysis conditions. Line I, enzymatic hydrolysis (65 mg of lysylpyrrolaldehyde [LPA] per 100 g of protein); line II, alkaline hydrolysis after elimination of free maltose (21 mg of LPA); line III, 14 mg of LPA with free maltose; line IV, alkaline hydrolysis in presence of free maltose and 1 g of starch per gram of protein (6.5 mg of LPA). AGPF = acetylglucopyranosylfuran, HMF = hydroxymethylfurfural, Trp = tryptophan.

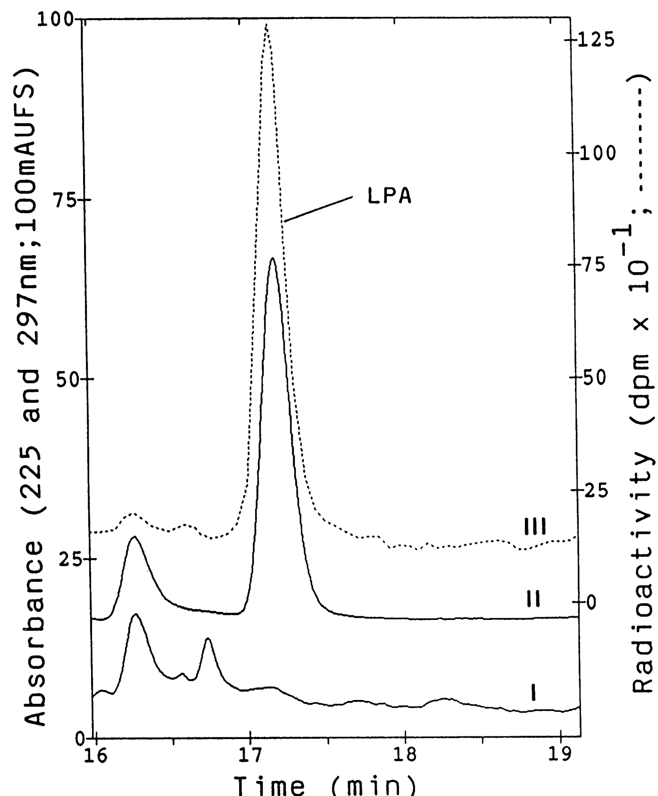

Fig. 7. High-performance liquid chromatography elution profiles of lysylpyrrolaldehyde peak (LPA) in enzymatically hydrolysed model dough containing [1U-14CJ] maltose and submitted to double solid-phase extraction (replicate 1 of Table II). Absorbance at 225 or 297 nm (lines I and II, respectively) and radioactivity detection (line III)

TABLE II
Response Factor of Labeled Lysylpyrrolaldehyde in Ion Pair Reversed-Phase High-Performance Liquid Chromatography with 297 nm Detection of Enzymatically Hydrolysed Model Doughs Dried with [U-14C] Reducing Sugars

				Lysylpyrrolaldehyde		
Labeled Sugar	Replicate	Specific Activity of Sugar Radiocarbon (dpm/nmol)	Injected Activity (dpm.e-6)	Peak Area (mV.sec.e-3)	Peak Activity (net dpm.e-3)	Response Factor (pmoles/mV.sec.e-3)
Glucose	1	452[a]	1.75	1,865	6.1	1.21
	2	2,494[b]	11.00	13,085	225.2	1.15
Maltose	1	968[c]	3.52	728	5.0	1.18
	2	2,473[d]	5.50	2,510	41.1	1.10
						1.16 ± 0.047[e]
Carboxy-2-	1		238 (p*M*)	208		1.14
-pyrrolaldehyde	2		120 (p*M*)	109		1.10
	3		15 (p*M*)	13		1.15
						1.13 ± 0.026[e]

[a] 2.32 mg of cold molecule + 16 μCi labeled molecule.
[b] 2.59 mg of cold molecule + 100 μCi labeled molecule.
[c] 2.16 mg of cold molecule + 33 μCi labeled molecule.
[d] 2.61 mg of cold molecule + 100 μCi labeled molecule.
[e] Mean values ± standard deviation.

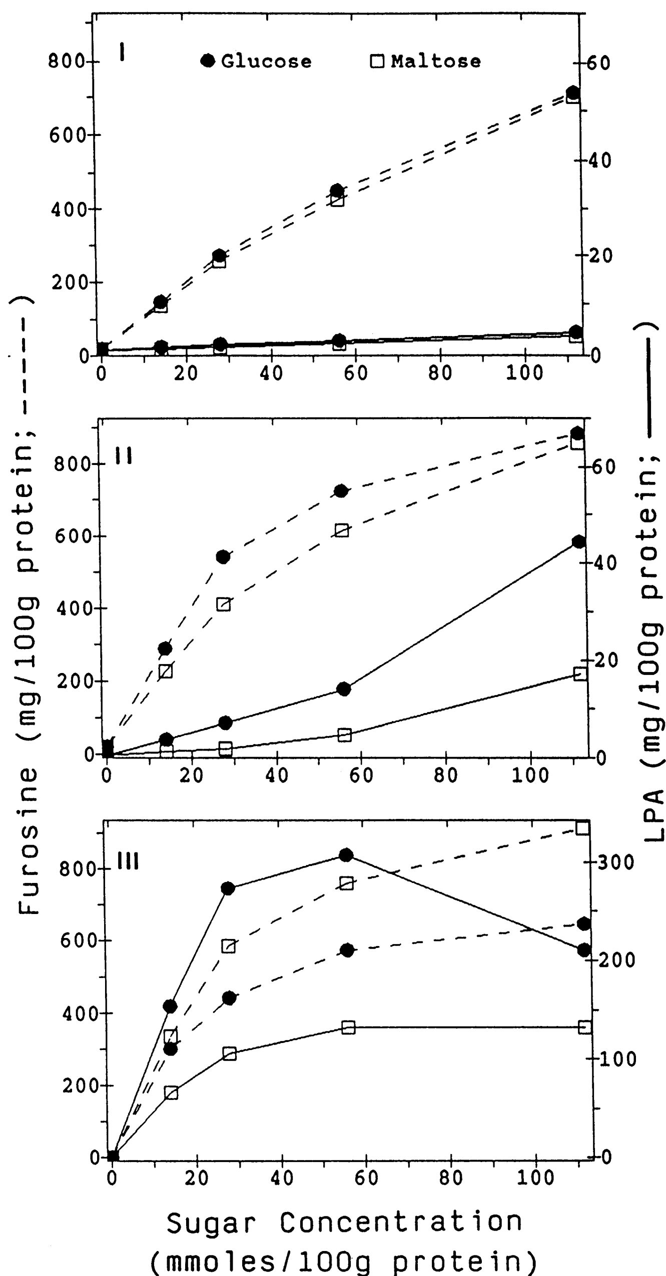

Fig. 8. Furosine (dotted line) and lysylpyrrolaldehyde (LPA) (solid line) accumulation in model doughs heat-dried at 70°C for 24 hr (I), 85°C for 7 hr (II), and 97°C for 5 hr (III) with different amounts of glucose or maltose.

LPA formation at 85°C was enhanced in the glucose system. This different thermal stability caused at 97°C furosine to accumulate more in the maltose system and LPA to accumulate much more in the glucose system. At this temperature, lysylketose formation was probably only slightly faster than its degradation; hence, the accumulation of furosine and LPA had roughly comparable behavior for up to 56 m*M* of sugar. For higher sugar concentrations, LPA underwent to further degradation (see the slope of both curves in Fig. 8).

Additional information on the advanced MR in model doughs was obtained by evaluating the radioactivity of a labeled maltose system that was submitted after drying to further heating in the absence of free sugar (Table III). Splitting of radioactive material was appreciable only at 97°C for 5 hr, indicating that the decomposition of LPA did not occur under milder conditions. On the basis of both the specific activity of reacted maltose (16 μCi/m*M*) and gluten-bound radioactivity (3,666 dpm per milligram of protein) detected after the first heating, 104 n*M* of maltose per milligram of protein were involved in the MR on gluten. On the basis of furosine value and the conversion factor (1.44) of furosine to blocked lysine reported for our hydrolysis conditions (Erbersdobler and Dehn-Muller 1989), only 71 n*M* of maltose per milligram of protein reacted with the lysine residues of gluten. It is reasonable to suppose that, in addition to protein-bound maltulosyllysine, other free maltulosyl-amino acids are formed and decomposed, even when heated at 70°C for 24 hr. Moreover, data of Table III suggest that other derivatives of maltulosyllysine are produced.

The same relationships between furosine and LPA patterns found by varying the sugar concentration were observed in doughs containing a constant amount of glucose or maltose and varied holding time at 85°C (Fig. 9). At the beginning of the heat treatment, the rate of ketosyl-lysine formation, as measured by furosine, was much faster than its decomposition; however, for prolonged heating of the dried dough, LPA accumulation was dominant, especially in glucose system. The behaviors of all curves of furosine and LPA are consistent with the occurence of a close relationship between lysylketose degradation and LPA accumulation. The formation of LPA from a different pathway, as suggested by Henle and Klostermeyer (1993) for skim milk powders heated a 110°C, seems less likely here. Taking the thermal conditions of the HT-drying process of pasta into consideration, data of model doughs indicate that LPA residues can easily form in commercial spaghetti. A subsequent appreciable decomposition is unlikely, even at the highest sugar concentrations and in presence of reducing monosaccharides.

Lysylpyrrolaldehyde in Experimentally Dried Pasta and Commercial Spaghetti

Modern pasta drying cycles can include heating steps with temperatures from 70 to over 90°C for holding times up to 12 hr. The pasta moisture decreases from 30 to 12% (Manser 1986). In HT-drying cycles, steps at 75–85°C for 2–6 hr, when pasta moisture is 15% or lower, are often performed to improve pasta

TABLE III
Stability of Labeled Residues of Gluten Maltulosylketoses in Dried Model Dough Submitted to a Second Heat Treatment After Elimination of Free Maltose

Heat treatment	Radioactivity of Maltose-Free Dough (dpm/mg of protein)[a] Total	Water-Soluble	Furosine (mg/100 g of protein)[b]	Lysylpyrrolaldehyde (mg/100 g of protein)[b]
First				
70°C/24 hr	3,666 ± 133	. . .	720	2.9
Second				
70°C/24 hr	3,688 ± 151	0	698	7.2
85°C/7 hr	3,765 ± 106	21 ± 6	650	16.7
97°C/5 hr	3,562 ± 172	805 ± 53	475	58.0

[a] Mean values ± standard deviation ($n = 3$).
[b] Mean values ($n = 2$), standard deviation < 5% of the mean.

cooking quality (Dalbon and Oehler 1983, Dexter et al 1984). At these moisture conditions, pasta a_w reaches values of 0.8–0.7 (Andrieu et al 1985), which were reported to be optimal for the MR (Kaanane and Labuza 1989). Therefore, significant formation of LPA must be expected. The behaviors of furosine formation in pasta samples heated under different HT low-moisture conditions suggested degradation of the Amadori compounds (Pagani et al 1992); hence, we studied LPA accumulation on the same samples. Its behavior was greatly affected by pasta moisture, both at 75 and 85°C, and the highest values were found in the range 15–12.5% (Fig. 10). Nevertheless, the processing temperature appeared to be the major parameter affecting LPA formation in drying. The amount formed at 85°C was always at least three times higher than at 75°C, with much smaller differences in the furosine values. The curve slope suggests no appreciable degradation of LPA residues and confirms the behaviors found on model doughs. Because of the thermal stability of protein-bound derivatives of aminoketoses in pasta drying, the protein-starch interactions we observed by electron microscopy of HT low-moisture dried pasta (Resmini and Pagani 1983) may be due to the advanced MR involving terminal glucose of starch fractions.

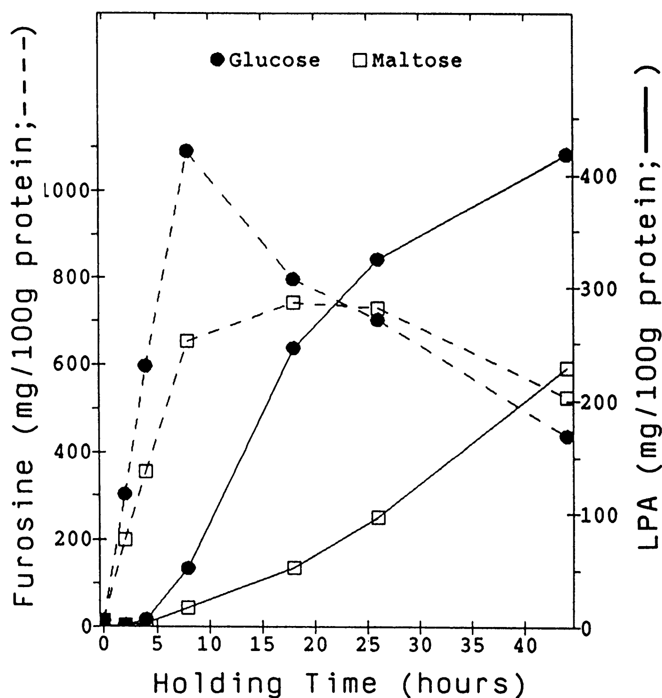

Fig. 9. Furosine (dotted line) and lysylpyrrolaldehyde (LPA) (solid line) accumulation in model doughs heat-treated at 85°C for different holding times in presence of 54 mM of glucose or maltose per 100 g of protein.

To evaluate LPA formation under real drying conditions, spaghetti from durum wheats Lira, Neodur, and Valnova produced with the cycles shown in Figure 1 were considered. The most severe heating step of the MT cycle (80°C for 1 hr) was performed when pasta moisture reached 16–15%; the temperature of the remaining drying period was 70°C. The HT cycle was

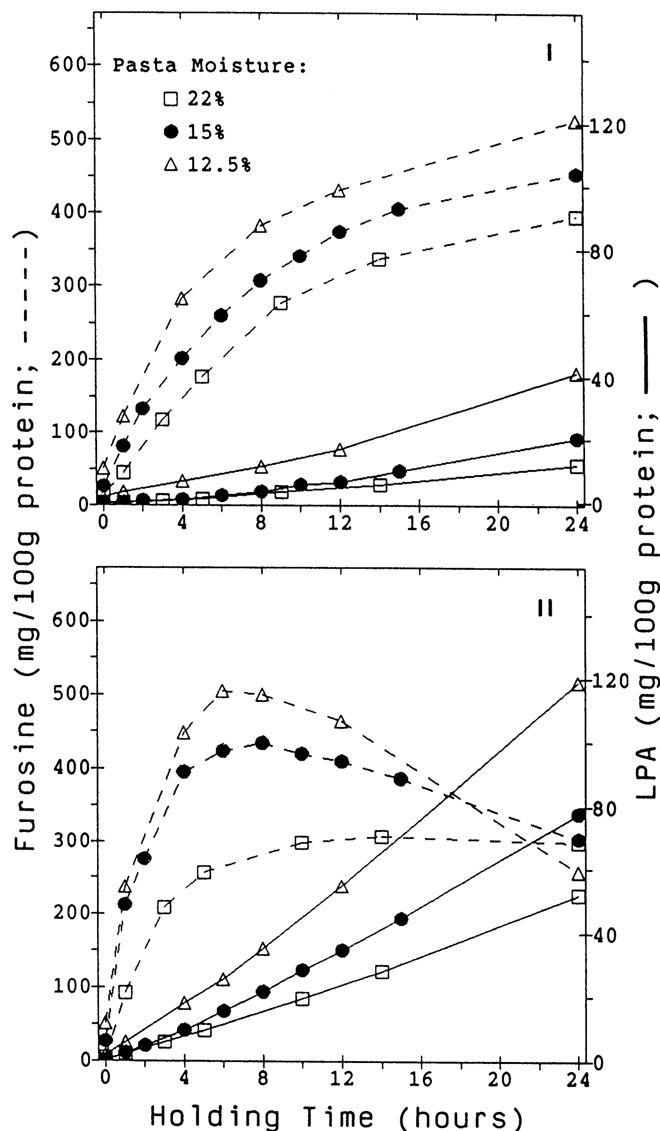

Fig. 10. Furosine (dotted line) and lysylpyrrolaldehyde (LPA) (solid line) accumulation in experimental pasta heat-treated at 75°C (I) or 85°C (II) for different holding times.

TABLE IV
Furosine and Lysylpyrrolaldehyde (LPA) (mg/100 g of protein) in Semolina (S) and Spaghetti from Durum Wheat Cultivars Lira, Neodur, and Valnova with Known Amount of Reducing Sugars (nmoles/kg of product)[a] and Processed with Low-Temperature (LT), Medium-Temperature (MT) or High-Temperature (HT) Drying Cycles

	Lira			Neodur			Valnova			
	S	MT	HT	S	MT	HT	S	LT	MT	HT
Furosine[b]	5.1	309	488	7.8	353	554	5.5	46	320	553
Blocked lysine[c]	7.3	445	702	11.2	508	798	7.9	66	461	796
LPA[b]	0	1.0	12.1	0	1.9	16.4	0	0	0.9	12.5
Blocked lysine[c]	0	0.6	7.0	0	1.1	9.5	0	0	0.5	7.2
Maltose	6.8		46.9	6.9		46.5	10.4			56.7
Maltotriose	10.3		12.0	8.2		9.8	8.8			10.3
Glucose	1.9		4.7	3.0		7.1	1.9			4.8
Fructose	1.3		3.1	2.1		4.8	1.4			3.1

[a] Data from Resmini et al 1993.
[b] Mean values ($n = 2$), standard deviation < 5% of the mean.
[c] Data expressed as corresponding blocked lysine (mg/100 g of protein), assuming 1.44 is the conversion factor for furosine and 0.58 is the conversion factor for LPA.

characterized by drying at 80°C with short steps in the 82–90°C range and a decrease in the spaghetti moisture from 15 to 12%. The amount of LPA produced was 1–2 mg/100 g of protein in the MT-dried spaghetti and over 12 mg in the HT-dried spaghetti (Table IV). Data of calculated blocked lysine indicate that only a small amount of the Amadori compounds is involved in LPA formation. Although lysine blockage looks relevant in HT-dried pasta, the adopted conversion factor of furosine (1.44) was reported for dairy products (Erbersdobler and Dehn-Muller 1989) and must be confirmed for cereal-based products. LT-drying (50°C for 7 hr) conditions included as a reference did not produce LPA. The enhancing effect of heating steps at 80°C or more on the advanced MR is evident under low-moisture conditions of pasta. The highest values of LPA and furosine in Neodur spaghetti can be related to the higher content of reducing monosaccharides. The effect of amylolytic activity and starch damage must be considered here. Sprout damage (Matsuo et al 1982), starch granule mechanical damage (Matz 1987), higher extraction rate of milling (Kruger 1981), and shearing occurring during pasta extrusion (Dexter et al 1990) have all been reported to affect the reducing sugar content of pasta. As a consequence, all these parameters may indirectly enhance the MR in drying, particularly when glucose is split.

LPA values found in commercial spaghetti, either collected at the local markets or produced in a semipilot plant, were fully in agreement with those reported for the experimental samples (Fig. 11). When the early MR was low, and the furosine was ~<300 mg/100 g of protein, the occurence of advanced stage was not significant, and LPA was always <2 mg. Under extensive early MR (500–700 mg of furosine), extensive advanced stage could occur, and LPA values ranged from 10 to >30 mg/100 g of protein, according to the adopted drying cycles and composition of free reducing sugars in pasta. Still, the amount of LPA is affected by prolonged storage of dried pasta, because the advanced MR can proceed under these conditions (Labuza et al 1982). The LPA value in an HT-dried spaghetti sample stored for two years at 30°C was 51.5 mg/100 g of protein. As reported by Chiang (1988), LPA determination appears to be a suitable tool for evaluating storage conditions of low-moisture heated foods. Although partial decomposition of LPA probably always occurs in baked products, it is surprising to note that the extent of the advanced MR in today's spaghetti can range from values found in a bread crumb up to that of cracker and biscuit samples.

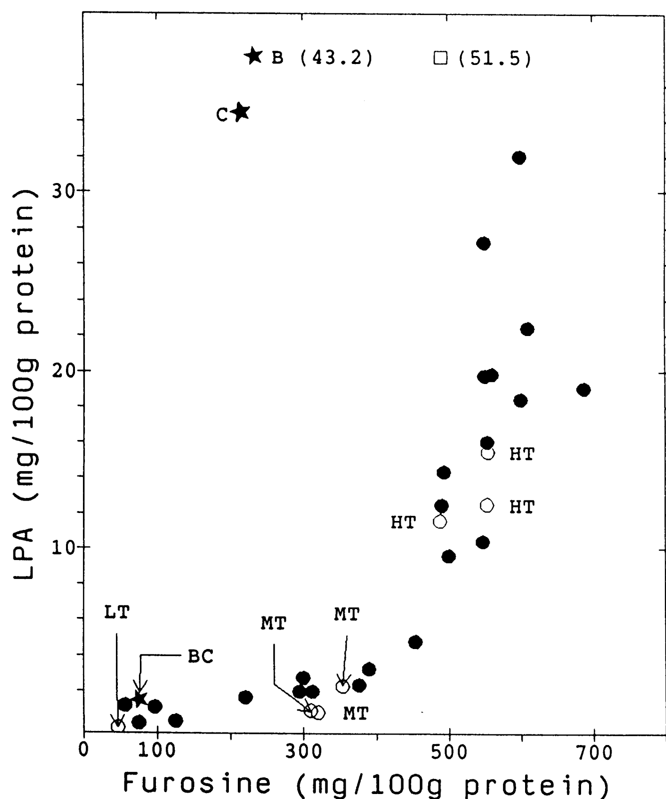

Fig. 11. Furosine and lysylpyrrolaldehyde (LPA) levels in different cereal-based foods. Commercial spaghetti analyzed after collection from the local market (●) and after storage for two years at 30°C (□). Spaghetti produced in semipilot plant (○) with low-, medium-, or high-temperature drying cycles (LT, MT, and HT, respectively). Commercial samples (★) of bread crumb (BC), cracker (C), and biscuit (B). The reported values are means of two determinations. Standard deviation < 5% of the mean.

CONCLUSIONS

The significant extent of the advanced MR on protein occurring in pasta drying has been established. Due to thermal process conditions, the decomposition of aminoketose residues produces mainly still protein-bound derivatives. A main degradation product of lysylketoses proved to be LPA. The IP-RP HPLC method proposed here allows sensitive and interference-free routine quantification of this protein-bound pyrrolaldehyde in dried pasta and other cereal-based products using pyrrole-2-carboxyaldehyde as the external standard.

The wide range of LPA values found in commercial spaghetti was in agreement with the data obtained on experimental samples. Drying temperatures higher than 75°C, when pasta moisture was 15% or lower, and the high concentration of free reducing sugars, the most effective being glucose, were the major factors responsible for LPA formation. Though other derivative compounds are likely to be involved in the MR occurring in pasta drying, the heat-damage of protein can be described by the evaluation of furosine and protein-bound LPA. Moreover, the level of these molecules is indicative of thermal processing conditions and of quality characteristics of raw materials affecting the amylolytic activity and, therefore, the reducing sugar content.

HT drying cycles present both positive and negative aspects in pasta processing. Shortening the drying times increases plant productivity and improves cooking quality of pasta, even that produced from raw material of poor quality (Resmini and Pagani 1983). These reasons account for the great diffusion of this technology in today's pasta industry. The negative aspects involve mainly the loss of available lysine related to furosine value, the abnormal formation for pasta of advanced MR-compounds (more characteristic of bakery products) to which some antinutritional properties have been attributed, and the undesirable sensorial changes resulting from the extensively developed MR (Buhler-Miag Inc. 1979). Further knowledge of the conditions and mechanisms regulating the MR occurring in pasta drying is required to better control all parameters involved in every step of production, without losing the advantages of HT-drying technology.

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