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Furosine: a Suitable Marker for Assessing the Freshness of Royal Jelly

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Fifteen commercial samples of royal jelly, consisting of 10 imported samples, and 5 samples of known origin obtained freshly harvested from beekeepers, were analyzed for protein, lysine, and furosine content. In addition, a commercial sample of royal jelly, at the beginning of its commercial shelf life, was stored for 10 months both at 4 °C and at room temperature in order to assess the development of the Maillard reaction (furosine) and relative nutritional damage (blocked lysine). The commercial royal jelly products contained different amounts of furosine, ranging from 37.1 to 113.3 mg/100 g protein, evidence of different storage times and conditions. The average furosine content of the royal jelly samples of known origin and harvesting was significantly lower than that of the imported samples (41.7 versus 73.6 mg/100 g protein, respectively). With regard to shelf life, furosine content increased significantly from 72.0 mg/100 g protein to 500.8 mg/100 g protein after 10 months of storage at room temperature, while it increased to a much lower level (100.5 mg/100 g protein) when the royal jelly was stored at 4 °C. However, nutritional damage, expressed as blocked lysine (calculated indirectly from the furosine content), was minor or negligible, 11.9 and 2.3% of total lysine, in samples stored at room temperature and at 4 °C, respectively. Lysine was determined by an innovative procedure based on high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC−PAD). The results showed that furosine is a suitable index for assessing the quality and freshness of royal jelly.

KEYWORDS: Royal jelly; furosine; freshness; Maillard reaction; blocked lysine; nutritional damage

INTRODUCTION

Royal jelly (RJ) is a viscous substance secreted by the hypopharyngeal and mandibular glands of nurse worker bees (*Apis mellifera* L.) that constitutes the essential food for the larvae of the queen bee.

In modern diets, the use of RJ has significantly increased because of the trend toward use of healthy, organic, nutraceutical, and functional foods, and dietary supplements. The Republic of China is the world's largest producer and exporter of royal jelly. Most of the Chinese RJ production (400−500 t) is distributed in Europe, Japan, and the United States (*1*). Because RJ is a rapidly perishable matrix, it has to be stored at 4 °C or below −17C° in order to maintain its functional/nutraceutical properties (*1*−*6*). For this reason, RJ would lose its high market value (approximately 0.5−1.0 U.S. $/g) if improperly harvested, collected, packaged, and stored.

The authenticity or the adulteration of RJ is ascertained by the presence of specific carbohydrates (di- and trisaccharides) or fatty acids (10-hydroxy-2-decenoic acid and 10-hydroxydecanoic acid) (*7*−*11*).

Various physical and chemical markers have been identified and proposed to assess the freshness and quality of RJ (*2*−*6*, *12*−*14*). All these indices, such as qualitative−quantitative variations in viscosity, water-soluble proteins, titratable acidity, browning (color), free amino acids, and enzymatic activity, that are mainly due to the Maillard reaction (MR) and lipid hydrolysis and oxidation, are not very reliable because they are influenced by a variety of factors.

Although several works indicate the development of the MR in RJ (*2*, *3*, *12*, *15*), no data are available on the presence of the Amadori compound.

The furosine assay, which is specific for ϵ-N-deoxy-ketosyllysine (Amadori compound), remains the most sensitive and most accepted method for determining the extent of "early" Maillard reactions (*16*, *17*). Although high temperatures favor the reaction, even low temperatures over a long period can bring on an initial step, which is why furosine can be used as a shelf life index for food products normally stored at room temperature.

For these reasons, furosine is one of the most commonly used markers for evaluating the raw materials, the type and conditions of heat treatment, and the storage conditions (shelf life) of milk, milk powder, mozzarella cheese, pasta, eggs, soymilk, tomato products, parboiled rice, cooked ham, and honey, etc. (*16*−*28*).

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The aim of this paper is to assess the MR by measuring furosine content in several commercial RJ samples, and during the RJ storage at different times and temperatures, in view of proposing furosine as an index for RJ freshness.

MATERIALS AND METHODS

Samples. Ten commercial products imported from the Republic of China and Taiwan characterized by different shelf life levels were purchased from health-food stores in Rome, Campobasso, and Bologna, Italy. The indications "best before (date)" and to "store refrigerated after purchase" were given on the labels.

Five commercial samples of known origin and harvesting times were obtained from beekeepers near Bologna and Campobasso, Italy.

A commercial RJ sample consisting of 10 10-g pots at the beginning of their shelf life were purchased from a health food store in Rome. First, the 10 pots were carefully homogenized and divided into 14 aliquots of 3 g each placed in airtight glass vials (4 mL). Two aliquots were immediately analyzed for protein and furosine content. The other 12 samples were divided into two groups: one group was stored at 4 °C and the other group was stored at room temperature. One pair of vials from each group was subjected to different storage times of 3, 6, and 10 months. Refrigerated samples were returned to room temperature and then homogenized before being analyzed.

To get absolute fresh samples of RJ, queen cells produced during May, June, and July 2001 were obtained from Piana Miele beekeeper (Bologna, Italy). Three days after grafting (commercial harvesting time (*1*, *8*)), the queen cells containing RJ were removed from the frame and their wax tops were pinched shut. They were immediately wrapped in aluminum foil and taken to the laboratory. The RJ of 10 cells from a total of 3 harvests was extracted with a microspatula, pooled, and immediately analyzed for furosine content.

Water Activity. Water activity, a_w, of the RJ samples used for the storage test was measured at 20 °C as vapor pressure through the dew point using an Aqualab CX-2 instrument (Decagon Devices, Pullman, WA).

Protein. Protein content (N × 6.25) was determined according to the Dumas combustion method, AOAC methods 990.03 and 992.23 (*29*), using a Leco nitrogen determiner, model FP-528 (Leco Corp., St. Joseph, MI). An amount of 0.20 g of RJ sample was weighed in tinfoil (Leco tinfoil cups 502-186-100), using a foil holder (Leco 604-493), and twisting the ends of the foil to form a teardrop-shaped packet.

Furosine. An aliquot of sample (0.35 g), corresponding to about 30−70 mg of protein, was hydrolyzed in nitrogen with 8 mL of 8 N HCl at 110 °C for 23 h. After hydrolysis, 0.5 mL of the hydrolysate was purified on a Sep-pak cartridge (Waters Corp., Milford, MA), diluted, and determined according to the HPLC procedure of Resmini et al. (*30*).

A Waters HPLC system (Milford, MA) consisting of two pumps (model 510), a diode array detector (model 991), and an injector with a 50-μL loop (model 9125-080; Rheodyne, Cotati, CA) were used. Analytical separation was performed with an Alltech furosine dedicated column (Deerfield, IL) at 280 nm wavelength. The furosine standard was purchased from Neosystem Laboratoire (Strasbourg, France).

Lysine. A RJ sample corresponding to 25 mg of protein was placed in a flask with an acid-resistant rubber stopper. Then, 25 mL of 6 N HCl was added and the flask was put under vacuum in an oven at 110 °C for 24 h. The flask was then cooled, and the sample was evaporated to dryness under vacuum and dissolved in 0.1 N HCl.

Lysine was determined by an innovative procedure based on high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC−PAD), using a Dionex system (Dionex Corporation, Sunnyvale, CA) composed of a gradient pump (model GP50) with on-line degas and an electrochemical detector (model ED40). The instruments control, data collection, and total quantification were managed using Peak Net chromatography software (Dionex). The flow-through electrochemical cell (Dionex) consisted of a 1-mm diameter gold working electrode, a pH reference electrode, and a titanium body of the cell as the counter electrode. A controlled injector with a 25-μL sample loop was used for sample injection. Separations were performed with an AminoPac PA10 analytical column 250 mm × 2 mm i.d., particle size 8.5 μm (Dionex). The quantitative determination of lysine was carried out by isocratic elution using 50 mM NaOH with a flow rate of 0.25 mL/min (*31*). The column wash, followed by re-equilibration, was made with 125 mM NaOH and 0.5 M sodium acetate. The optimized waveform time-potential used was the same as the one reported by Clarke et al. (*32*). The amino acid standard for hydrolysate analysis was from Beckman Coulter (Fullerton, CA).

Table 1. Protein and Furosine Values (mean ± SD) of Commercial RJ Samples[a]

sample	protein (% wb)	furosine (mg/100 g protein)
	imported samples	
CRJ 1	14.3 ± 0.31	71.1 ± 0.01
CRJ 2	13.9 ± 0.10	80.9 ± 0.30
CRJ 3	13.9 ± 0.23	61.0 ± 1.21
CRJ 4	14.2 ± 0.03	52.5 ± 0.10
CRJ 5	14.3 ± 0.03	87.9 ± 3.82
CRJ 6	14.2 ± 0.01	49.6 ± 0.13
CRJ 7	14.0 ± 0.06	47.3 ± 1.63
CRJ 8	13.8 ± 0.08	91.8 ± 0.11
CRJ 9	12.4 ± 0.04	80.9 ± 1.70
CRJ 10	12.1 ± 0.00	113.3 ±1.23
mean	13.7[a]	73.6[a]
CV%	5.8	29.0
	freshly harvested samples	
FRJ 1	13.8 ± 0.08	37.1 ± 0.50
FRJ 2	12.0 ± 0.03	44.9 ± 2.95
FRJ 3	12.7 ± 0.13	38.5 ± 1.12
FRJ 4	13.5 ± 0.01	41.6 ± 1.23
FRJ 5	12.0 ± 0.11	46.6 ± 1.06
mean	12.8[a]	41.7[b]
CV%	9.0	9.7

[a] Different superscript letters within a column indicate statistically significant differences ($P < 0.01$)

Blocked Lysine. Blocked lysine was indirectly measured from the furosine content (*33*−*35*). The Amadori compound, after 7.8 N acid hydrolysis, generates about 45% lysine (regenerated lysine), 12% pyridosine, and 30% furosine (*35*, *36*). The lysine was regenerated at a similar level (about 45%) after both 6 N and 7.8 N HCl hydrolysis (*36*). From the furosine and lysine values, determined by the above-mentioned chromatographic methods, it is possible to calculate blocked, available, and total lysine as follows:

$$\text{blocked lysine} = \left(\frac{1}{0.30} \times \text{furosine}\right) \times \frac{\text{MW lysine}}{\text{MW furosine}}$$

$$\text{available lysine} = \text{chromatographed lysine} - (0.45 \times \text{blocked lysine})$$

$$\%\ \text{blocked lysine} = \frac{\text{blocked lysine} \times 100}{(\text{blocked lysine} + \text{available lysine})}$$

$$\text{total lysine} = \text{available lysine} + \text{blocked lysine}$$

All other chemicals were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, MO).

Statistical Analysis. Results were expressed as mean values ± standard deviations of two separate determinations. Data sets were evaluated using Student's *t* test to compare the differences between imported and freshly harvested RJ samples.

RESULTS AND DISCUSSION

The protein and furosine contents of commercial RJ samples (imported and freshly harvested) are reported in **Table 1**. Protein content ranged from 12.0 to 14.3% wb, producing values similar to those found in the literature (*1*, *5*, *8*, *10*, *37*, *38*).

The furosine content of commercial RJ varied from 47.3 to 113.3 mg/100 g protein in the imported samples and from 37.1

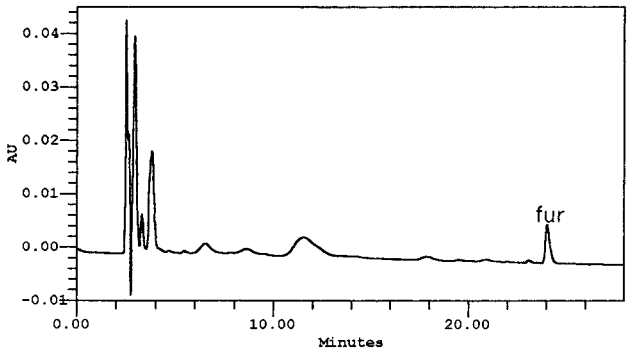

Figure 1. HPLC separation of furosine of RJ hydrolysate.

to 46.6 mg/100 g protein in the freshly harvested samples. The amount of furosine in the samples of known origin and harvesting (mean value = 41.7 mg/100 g protein) were significantly lower than that (mean value = 73.6 mg/100 g protein) of the imported products. The wide variability of furosine content found in the imported samples (CV = 29.0%) could be due to their different storage times and/or conditions. The repeatability (standard deviation) of furosine analysis in RJ samples was similar to that found in other matrixes (*18*, *22*, *23*, *25*, *30*). The HPLC chromatograms of RJ hydrolysate showed a well separated peak of furosine without interfering peaks (**Figure 1**).

The presence of furosine in RJ confirms the assumption of various authors (*2*−*8*, *13*, *14*) that some physicochemical and nutritional modifications (color, browning, glycated proteins, etc.) are to be imputed to the MR. With regard to the shelf life of fresh RJ, the optimal storage temperature recommended is 0−5 °C, which will preserve its efficacy for 12−18 months (*1*−*5*). These conditions were then assessed to determine whether they were truly appropriate to contain the MR and maintain the physicochemical and nutritional properties of the product unaltered.

Figure 2 reports the furosine values in RJ during storage at 4 °C and at room temperature for 10 months. The experimental points for each storage time were interpolated by the best fitting curve. **Figure 2** indicates that storage at 4 °C contains the MR, in fact furosine content increased very little (from 72.0 mg/100 g protein to 100.5 mg/100 g protein) after 10 months. In contrast, at room temperature, the linear increase in furosine was significant, reaching 500.8 mg/100 g protein after 10 months.

This intense development of the MR in RJ is due to the presence of large amounts of reducing sugars (glucose and fructose ≈30.5% db (*3*, *11*, *39*)), α- and ϵ-aminogroups of amino acids and proteins (free amino acids ≈2.5% of the nitrogen compounds (*1*, *12*); lysine ≈3.0% db (*5*, *8*, *37*)), and to the favorable values of pH (3.5−4.5 (*2*)), and water activity, a_w = 0.97. The wide availability of both reactants enables a kinetic of linear furosine formation to be obtained during prolonged storage at room temperature, which further attests to the suitability of this marker for the assessment of the quality/freshness of RJ.

The higher furosine values (432−1362 mg/100 g protein) found in honey by Villamiel et al. (*28*) were due to the heating treatment used in its manufacture and its very low protein/amino acid content (0.4%). For this low protein content, the widely acknowledged process marker in honey is hydroxy-methyl-furfural (HMF) (*40*), because it is mostly linked to the glycidic component.

To quantify nutritional damage due to inadequate storage conditions of RJ, the % blocked lysine and available lysine were measured from the furosine content and chromatographed lysine. The HPAEC−PAD procedure allows a complete separation of lysine in less than 5 min (**Figure 3**). The analytical performances (precision, accuracy, and sensitivity) of this innovative method were very good. The precision of the system expressed as RSD for 15 consecutive injections was <2.5% for lysine and <1% for retention time, whereas the accuracy expressed as lysine recovery was 98−102% for adding 2 mg lysine standard to RJ sample. The range of linearity was between 2.5 and 15 μM (R^2=0.999), whereas the detection limit (3 times noise level) was 56 pmol. The validation of the method by comparison with IEC and biosensor procedures (relative error <10%) was reported in a previous work (*31*).

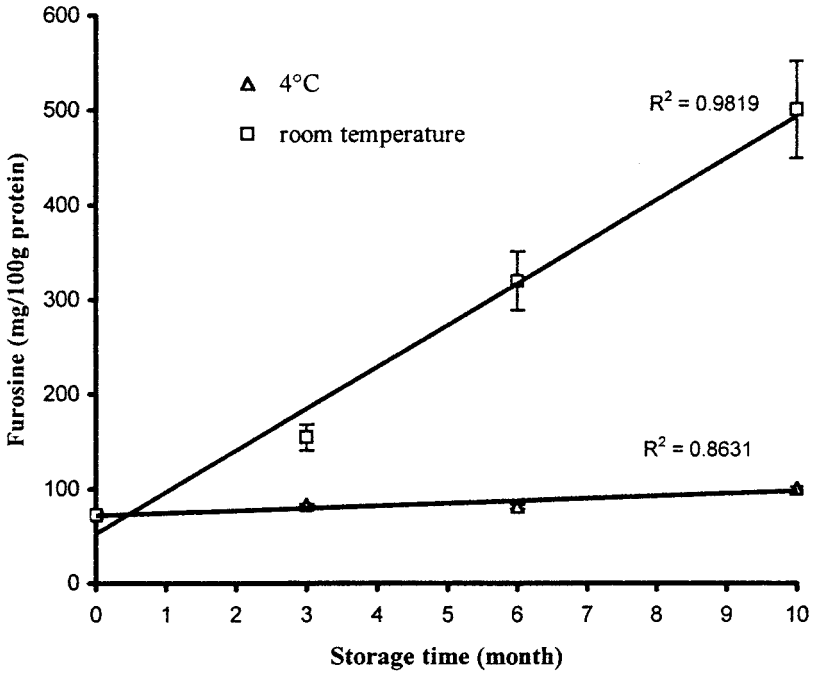

Figure 2. Furosine values in RJ during storage at 4 °C and at room temperature for 10 months.

Table 2. Furosine Content and Lysine Fractions of RJ Sample Stored for 10 Months at 4 °C and Room Temperature (RT)

			lysine fractions					
	furosine		chromatographed		available[a]	blocked		total[d]
sample	g/100 g wb	g/100 g protein	g/100 g wb	g/100 g protein	g/100 g wb	g/100 g wb[b]	% total lysine[c]	g/100 g wb
RJ 4 °C	0.012	0.100	0.98	7.90	0.969	0.023	2.3	0.99
RJ RT	0.062	0.500	0.93	7.49	0.876	0.119	11.9	1.00

[a] Chromatographed lysine = (0.45 × blocked lysine); *b* = (1/0.30 × furosine)(MW lys/MW fur); *c* = (blocked lysine × 100)/(blocked lysine + available lysine); *d* = available lysine + blocked lysine.

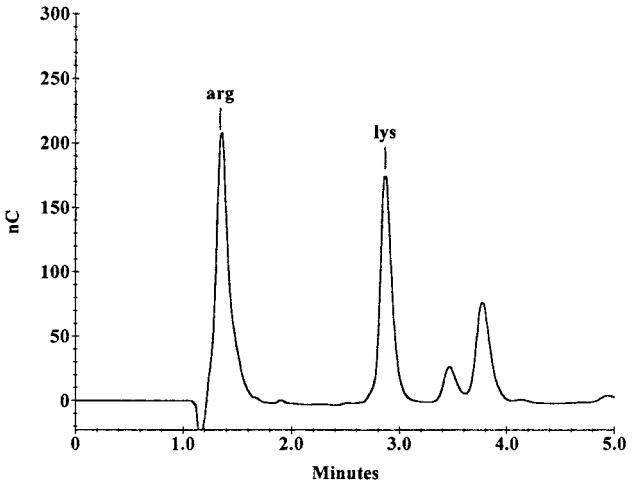

Figure 3. HPAEC–PAD separation of amino acids of RJ hydrolysate.

Table 2 reports the furosine and the lysine fractions (chromatographed, blocked lysine, available and total lysine) in the RJ samples stored for 10 months at 4 °C and at room temperature. The storage of RJ at room temperature for 10 months (500.8 mg furosine/100 g protein) produced blocked lysine that amounted to 11.9% of total lysine. The storage at 4 °C produced a negligible amount of blocked lysine (2.3% of total lysine). Therefore, storing RJ at 4 °C (as stated on food labels) appears to be sufficient to maintain the nutritional properties of RJ for the considered storage time. Total lysine, available plus blocked, should be close to the initial amino acid level when products have not undergone any browning reactions. The content of chromatographed lysine (7.5−7.9 g/100 g protein) in RJ was similar to values found by other authors (*5*, *8*, *37*).

A neoformation substance such as furosine, to be used as a good process indicator, should be absent or present only at low levels in the unprocessed product (*17*). To verify the reliability of furosine as a suitable marker for RJ quality assessment, furosine was immediately determined in absolute fresh RJ samples just of three harvests. Furosine content in recently produced samples was quite moderate, varying from 15 to 18 mg/100 g protein.

In conclusion, furosine seems to be a very sensitive indicator for measuring freshness and preserving genuineness of raw RJ. A quality control parameter for RJ, that would then provide a standard for harvesting, packaging, and storage procedures, would be of considerable importance, as RJ is a valued and expensive product (approximately 0.5−1.0 U.S. $/g product). These findings allow a hypothesis to be contemplated, the determination of a furosine threshold for RJ products, in order to protect specific products, such as those having a protected designation of origin (PDO) and protected geographical indication (PGI) (EEC Council Regulation 2081/92 of 14 July 1992) and differentiate them from other products.

The establishment of quality parameters would guard consumers against purchasing perished RJ products that are depleted of their highly praised healthy and nutritional properties.

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LITERATURE CITED

(1) FAO. *Value-Added Products from Beekeeping.* FAO Agricultural Services Bulletin 124; FAO: Rome, Italy, 1996.
(2) Baggio, A.; Dainese, N. La qualità della gelatina reale nella conservazione. *Ind. Aliment.* **1998**, *37*, 1290−1294.
(3) Chen, C.; Chen, S.-Y. Changes in protein components and storage stability of royal jelly under various conditions. *Food Chem.* **1995**, *54*, 195−200.
(4) Fik, M.; Firek, B.; Leszczynska-Fik, A.; Macura, R.; Surowka, K. Effect of refrigerated storage on the quality of royal jelly. *Pszczelnicze Zesz. Nauk.* **1992**, *36*, 91−101.
(5) Karaali, A.; Meydanoglu, F.; Eke, D. Studies on composition, freeze-drying and storage of Turkish royal jelly. *J. Apic. Res.* **1988**, *27*, 182−185.
(6) Yatsunami, K. Changes in quality of royal jelly. *Honeybee Sci.* **1988**, *9*, 67−71.
(7) Bloodworth, B. C.; Harn, C. S.; Hock, C. T.; Boon, Y. O. Liquid chromatographic determination of *trans*-10-hydroxy-2-decenoic acid content of commercial products containing royal jelly. *J. AOAC* **1995**, *78*, 1019−1023.
(8) Howe, S. R.; Dimick, P. S.; Benton, A. W. Composition of freshly harvested and commercial royal jelly. *J. Apic. Res.* **1985**, *24*, 52−61.
(9) Jia, L.; Zhang, H. X.; Kou, X. L.; Hu, Z. D. Separation and determination of 10-hydroxy-2-decenoic acid in royal jelly by capillary electrophoresis. *Chromatographia* **1995**, *41*, 605−609.
(10) Lercker, G.; Capella, P.; Conte, L. S.; Ruini, F. Components of royal jelly: I. Identification of the organic acids. *Lipids* **1981**, *16*, 912−919.
(11) Lercker, G.; Savioli, S.; Vecchi, M. A.; Sabatini, A. G.; Nanetti, A.; Piana, L. Carbohydrate determination of royal jelly by high-resolution gas chromatography (HRGC). *Food Chem.* **1986**, *19*, 255−264.
(12) Boselli, E.; Caboni, M. F.; Sabatini, A. G.; Lercker, G. Determination and changes of free amino acids in royal jelly during storage. In *Proceedings of the XXXVII International Apicultural Congress: Apimondia 2001 "Beekeepers meet Africa"*. Durban, South Africa, October 28−November 1, 2001.
(13) Chen, C.; Chen, S.-Y. Changes in UV−Vis spectroscopic spectra and quality stability of royal jelly under various storage conditions. *J. Chin. Agric. Chem. Soc.* **1996**, *34*, 257−265.
(14) Choi, Y. S.; Kim, M. K.; Lee, K. H. Quality of variation of nonprocessed fresh royal jelly in different storage temperatures and periods. *Korean J. Apic.* **1994**, *9*, 1−4.
(15) Takenaka, T.; Yatsunami, K.; Echigo, T. Changes in quality of royal jelly during storage. *Nippon Shokuhin Kogyo Gakkaishi* **1986**, *33*, 1−7.

(16) Erbersdobler, H. F.; Dehn, B.; Nangpal, A.; Reuter, H. Determination of furosine in heated milk as a measure of heat intensity during processing. *J. Dairy Res.* **1987**, *54*, 147−152.
(17) Pellegrino, L.; Resmini, P.; Luf, W. Assessment (indices) of heat treatment of milk. In *Heat-Induced Changes in Milk*; 2nd ed.; Fox, P. F., Ed.; IDF,: Brussels, Belgium, 1995; pp 409−457.
(18) Acquistucci, R.; Panfili, G.; Marconi, E. Application of the microwave hydrolysis to furosine determination in cereal and dairy foods. *J. Agric. Food Chem.* **1996**, *44*, 3855−3857.
(19) Hidalgo, A.; Pompei, C. Hydroxymethylfurfural and furosine reaction kinetics in tomato products. *J. Agric. Food Chem.* **2000**, *48*, 78−82.
(20) Hidalgo, A.; Rossi, M.; Pompei, C. Furosine as a freshness parameter of shell eggs. *J. Agric. Food Chem.* **1995**, *43*, 1673−1677.
(21) Hurrel, R. F.; Finot, P. A.; Ford, J. E. Storage of milk powders under adverse conditions. 1. Losses of lysine and other essential amino acids as determined by chemical and microbiological methods. *Br. J. Nutr.* **1983**, *49*, 343−354.
(22) Marconi, E.; Acquistucci, R.; Panfili, G. Furosine determination in commercial samples of soymilk. Cost Action 98 Effect of antinutrients on the nutritional value of legume diet. In *Proceedings of the 6th Workshop "in vitro and in vivo" models for the evaluation of nutritional quality of legumes*, Roma, 8−10 maggio 1997; European Communities, Luxembourg, 1999; pp 39−45.
(23) Marconi, E.; Panfili, G.; Acquistucci, R. Microwave hydrolysis for the rapid analysis of furosine in foods. *Ital. J. Food Sci.* **1997**, *9*, 47−55.
(24) Nardi, S.; Calcagno, C.; Zunin, P.; Evangelisti, F. Determinazione mediante HPLC della furosina in risi parboilizzati. *Riv. Sci. Aliment.* **1998**, *27*, 29−34.
(25) Pagani, M. A.; Resmini, P.; Pellegrino, L. Technological parameters affecting the Maillard reaction in pasta processing. *Tec. Molitoria* **1992**, *43*, 577−592.
(26) Pompei, C.; Spagnolello, A. Influence of tumbling and dextrose on furosine content in cooked ham. *J. Agric. Food Chem.* **1997**, *45*, 4080−4083.
(27) Sanz, M. L.; del Castillo, M. D.; Corzo, N.; Olano, A. Presence of 2-furoylmethyl derivatives in hydrolyzates of processed tomato products. *J. Agric. Food Chem.* **1999**, *48*, 468−471.
(28) Villamiel, M.; del Castillo, M. D.; Corzo, N.; Olano, A. Presence of furosine in honeys. *J. Sci. Food Agric.* **2001**, *81*, 790−793.
(29) AOAC. *Official Methods of Analysis*, 17th ed.; Association of Official Analytical Chemists: Gaithersburg, MD, 2000.
(30) Resmini, P.; Pellegrino, L.; Battelli, G. Accurate quantification of furosine in milk and dairy products by a direct HPLC method. *Ital. J. Food Sci.* **1990**, *2*, 173−183.
(31) Messia, M. C.; Panfili, G.; Marconi, E.; Cubadda, R.; Acquistucci, R. Innovative methods for the determination of lysine in pasta. *Tec. Molitoria* **2001**, *52*, 844−848.
(32) Clarke, A. P.; Jandik, P.; Rocklin, R. D.; Liu, Y.; Avdalovic, N. An integrated amperometry waveform for the direct, sensitive detection of amino acids and amino sugars following anion-exchange chromatography. *Anal. Chem.* **1999**, *71*, 2774−2781.
(33) Finot, P. A.; Deutsch, R.; Bujard, E. The extent of the Maillard reaction during the processing of milk. *Prog. Food Nutr. Sci.* **1981**, *5*, 345−355.
(34) Erbersdobler, H. F. Influence of food production intensity and processing on protein quality and amino acid availability. Cost Action 98 Effect of antinutrients on the nutritional value of legume diet. In *Proceedings of the 6th Workshop "in vitro and in vivo" models for the evaluation of nutritional quality of legumes*, Roma, 8−10 maggio 1997; European Communities, Luxembourg, 1999; pp 7−12.
(35) O'Brien, J. M.; Morrissey, P. A. The Maillard reaction in milk products. *IDF* **1989**, *238*, 53−61.
(36) Finot, P. A.; Mauron, J. Le blocage de la lysine par la réaction de Maillard. II. Propriétés chimiques des dérivés N-(désoxy-1-D-fructosyl-1) et N-(désoxy-1-D-lactulosyl-1) de la lysine. *Helv. Chim. Acta* **1972**, *55*, 1153−1164.
(37) Bonomi, A.; Marletto, F.; Lucchelli, L.; Anghinetti, A.; Bonomi, A.; Sabbioni, A. Composizione chimico-bromatologica della gelatina reale in rapporto alla flora nettarifera e pollonifera. *Riv. Ital. Sci. Aliment.* **1986**, *15*, 53−62.
(38) Lercker, G.; Caboni, M. F.; Vecchi, M. A.; Sabatini, A. G.; Nanetti, A. Caratterizzazione dei principali costituenti della gelatina reale. *Apicoltura* **1993**, *8*, 27−37.
(39) Palma, M. S. Composition of freshly harvested Brazilian royal jelly: identification of carbohydrates from the sugar fraction. *J. Apic. Res.* **1992**, *31*, 42−44.
(40) Singhal, R. S.; Kulkarni, P. R.; Rege, D. V. Honey: quality criteria. In *Handbook of Indices of Food Quality and Authenticity*; Woodhead Publishing Limited: Cambridge, England, 1997; pp 358−385.

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