Application of the Microwave Hydrolysis to Furosine Determination in Cereal and Dairy Foods

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A hydrolysis procedure for the determination of furosine amount in cereals and dairy products has been applied. Results have been compared with those found by the traditional hydrolysis method. Data obtained by the microwave method correlated linearly with those found by the application of the traditional method ($r = 0.99$) and the relative standard deviation (1.0−12.0%) was close to that of the traditional hydrolysis (0.5−12.0%) for all of the samples. In addition, the method was applicable to different products having a wide range of furosine level (12.0−400.0 mg/100 g of protein for cereals; 6.0−900.0 mg/100 g of protein for dairy products).

Keywords: *Furosine; cereals; dairy products; microwave; hydrolysis*

INTRODUCTION

Heat treatment, which is often applied in food industry, is well-known to produce modifications of nutrients in foods. In particular, the development of the Maillard reaction (MR), which is considered to be the most evident effect induced by heat treatment, may be monitored by measuring the relative amount of new or transformed molecules present in end products (Resmini and Pellegrino, 1994). Among the latter, furosine [ϵ-*N*-(2-furoylmethyl)-L-lysine], found in acid hydrolysates of cereal and dairy products, has been indicated as a suitable marker of process and food quality. In fact, this molecule is related to the formation of intermediates produced by the condensation of reducing sugars with the ϵ-amino group of protein bound lysine (Bruggemann and Erbersdobler, 1968). Furosine has the disadvantage that it is formed from the Amadori products a rate of 30−40%. However, this recovery is reproducible if constant conditions are applied (Erbersdobler, 1995).

During the last years, the analytical improvement and the availability of a pure standard have enhanced the furosine determination. However, although several analytical procedures have been performed (Resmini et al., 1990; Buser and Erbersdobler, 1985; Tirelli and Pellegrino, 1995), the hydrolysis always remains the limiting step. In fact, this procedure is carried out in 8 N HCl for 23 h at 110 °C according to the amino acid determination as modified by Resmini et al. (1990). This method, though simple, is time consuming and not well suited to the on-line control. Considering the good results obtained on protein hydrolysis by a microwave system (Chiou et al., 1989; Engelhart et al., 1990; Marconi et al., 1995b), a specific microwave procedure for simple and rapid furosine determination was set up (Marconi et al., 1995a; Marconi et al., 1996).

Because the microwave energy penetrates deeply into food matrices and not just at the surface, the process is greatly accelerated, and this technique results in time and energy savings, allowing the precise process control of and improvement of working conditions (Copson, 1975). For this reason, the microwave application to food analysis has widely increased during the past years (digestion, extraction, etc.)

The goal of this study was to employ microwave hydrolysis in the determination of furosine in different cereals and dairy products having a wide range of furosine content so as to suggest an alternative method suitable for routine control. The results obtained were compared with those found by conventional hydrolysis.

MATERIALS AND METHODS

Chemicals. Hydrochloric acid and acetic acid were of HPLC grade and were purchased from Fluka (Buchs, Switzerland). Potassium chloride was from Merck (Darmstadt, Germany).

Deionized water was purified with a Milli-Q water purification system (Millipore, Bedford, MA). Sep-pak, C_{18} cartridges (Millipore) were used for the sample purifications.

Furosine standard was purchased from Neosystem Laboratoire (Strasbourg, France).

Samples. Different wheat and dairy products were purchased in local stores. Cereal samples were semolina, spaghetti, short pasta, pasta with eggs, biscuits, and rusks. They all were ground in a Buhler-Miag mod ML 1204 grinder (Uzwil, Switzerland) and carefully mixed. Samples were stored at 4 °C before analysis.

Milk samples were high-quality pasteurized milk (DA), UHT milk (DB), UHT skimmed milk (DC), in bottle sterilized milk (DD), milk powder (DE), and concentrated milk (DF). Concentrated milk was diluted 1:1 v/v with water, HPLC analytical grade. Cheese samples were mozzarella cheese (DG), imitation mozzarella cheese (DH) (ingredients: curd, milk proteins, butter, salt, citric acid), processed cheese (DI) (ingredients: cheese, butter, whey, casein, polyphosphate, and sodium citrate), and processed cheese (DL) (ingredients: whey, cheese, butter, polyphosphate, citrate, milk protein, salt). Mozzarella samples were frozen at −40 °C and then grated and mixed well. Processed cheeses were homogenized and mixed. Dairy products were stored at −40 °C before analysis.

Equipment. A closed vessels microwave system, Model MDS-2000 CEM Corp. (Matthews, NC) power max 630 ± 50 W, equipped with in board temperature and pressure system controls was used. A feedback control signal to regulate microwave power output was used as temperature control. PFA Teflon vessels (100 mL volume) were used. The oven was equipped with a turntable (3 rpm) and a mode stirrer which distributed the microwave energy to prevent uneven heating. This apparatus is commonly used in food analysis for determinations by atomic absorption spectroscopy.

A HPLC Waters Model 625 LC compatible system (Milford, MA) connected to a reversed-phase column C8, 250 mm × 4.6 mm i.d., Alltech, furosine dedicate (Deerfield, IL), was used. Furosine was detected on a UV−vis Waters Model 486 at 280 nm wavelength and 0.001 AUFS.

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Table 1. Furosine Content in Cereal Products (mg/100 g of Protein) and Statistical Evaluation of Results Obtained by Microwave (*A*) and Conventional (*B*) Procedure

sample	code	microwave (*A*)	RSD, $n = 3$	traditional (*B*)	RSD, $n = 3$	protein,[a] % w.b	*t*, exp	*t*, crit	$[(A - B)/A]$, %
semolina	CA	12.0	4.9	12.0	8.3	10.0 (N × 5.70)	0.50	2.78	0
spaghetti 1	CB	73.8	6.6	78.0	3.3	11.7 (N × 5.70)	1.52	2.78	6
spaghetti 2	CC	253.4	7.6	281.0	2.1	11.8 (N × 5.70)	2.43	2.78	11
short pasta 1	CD	113.6	8.3	103.1	3.7	11.4 (N × 5.70)	1.73	2.78	9
short pasta 2	CE	284.5	2.9	324.1	1.0	11.6 (N × 5.70)	*7.89*	2.78	14
pasta with eggs 1	CF	52.4	5.3	43.4	7.9	14.5 (N × 6.25)	*3.37*	2.78	17
pasta with eggs 2	CG	50.1	5.6	46.0	2.2	11.2 (N × 6.25)	2.04	2.78	8
rusks	CH	433.8	4.6	433.0	3.8	12.2 (N × 5.70)	0.08	2.78	< 1
biscuits	CI	186.7	7.2	181.3	3.9	7.5 (N × 6.25)	0.61	2.78	3

[a] Determined on raw materials.

Table 2. Furosine Content in Dairy Products (mg/100 g of protein) and Statistical Evaluation of Results Obtained by Microwave (*A*) and Conventional (*B*) Procedure

sample	code	microwave (*A*)	RSD, $n = 3$	traditional (*B*)	RSD, $n = 3$	protein[a] (N × 6.38), % w.b	*t*, exp	*t*, crit	$[(A - B/A)]$, %
pasteurized milk	DA	5.8	9.4	6.1	4.1	3.2	0.45	2.78	5
UHT milk	DB	43.4	7.7	51.8	12.8	3.1	1.95	2.78	20
UHT skimmed milk	DC	67.5	4.8	69.0	3.0	3.2	0.60	2.78	2
in bottle sterilized milk	DD	277.7	6.3	260.2	0.4	3.2	1.72	2.78	4
milk powder	DE	286.1	1.0	298.8	5.0	26.6	1.42	2.78	6
concentrated milk	DF	847.0	6.2	889.8	1.8	6.6	1.34	2.78	5
mozzarella cheese	DG	7.3	11.5	7.1	2.1	20.0	0.18	2.78	3
imitation mozzarella cheese	DH	5.7	8.0	6.5	2.4	19.6	*2.92*	2.78	12
processed cheese 1	DI	227.1	10.9	253.7	6.8	12.4	1.52	2.78	12
processed cheese 2	DL	224.7	8.4	251.9	6.1	11.0	1.92	2.78	14

[a] Determined on raw materials.

Protein. Protein determination was carried out according to the AOAC procedure (1984).

Traditional Hydrolysis. A sample amount of cereals, cheese, and milk powder containing 30−70 mg of protein was put in a screw-cap Pyrex vial and left to hydrolyze under nitrogen with 8 mL of 8 N HCl at 110 °C for 23 h. 2 mL of milk weighed in a Pyrex vial were added to 6 mL of 10.6 N HCl and left to hydrolyze under nitrogen at 110 °C for 23 h.

Microwave Hydrolysis. A representative amount of cereals (1.0 g), cheese (0.8 g), and milk powder (0.4 g) corresponding to about 60−140 mg of protein was put into a PFA Teflon (perfluoroalkoxy) digestion vessels and left to hydrolyze under nitrogen with 16 mL of 8 N HCl. Samples of 4 mL of milk were weighed and added to 12 mL of 10.6 N HCl in PFA digestion vessels and left to hydrolyze under nitrogen.

The experimental conditions selected, using six vessels, were as follows (Marconi et al., 1995a):

	first stage	second stage	third stage
power (% 630 W)	40	50	75
time (min)	2	5	10
temp (°C)	50	100	155
pressure max (psi)	80	80	130

HPLC Determination. After the hydrolysis, 0.5 mL of hydrolysate was purified on a Sep-pak (Millipore) cartridge, diluted, and determined according to the procedure of Resmini et al. (1990).

Chromatographic Conditions. The eluition solvent was water/acetic acid 0.4% v/v (solvent A) and potassium chloride 0.27% w/v in solvent A (solvent B) at a flow rate of 1.2 mL/min and a column temperature of 30 °C. The elution gradient started with 100% solvent A, remained isocratic for 11 min, reached 50% solvent B at 19 min, and remained isocratic until 22 min, and then 100% solvent A was restored. The injected volume was 50 μL, and the run time was 31 min.

Analyses were carried out in triplicate, and furosine is expressed as mg/100 g of protein.

Statistical Evaluation. Results are expressed as mean values, and the relative standard deviation (RSD) is reported. Significant differences were analyzed by the Student *t*-test.

RESULTS AND DISCUSSION

Cereals. The presence of furosine in pasta can be regarded as expression of nutritional damage being correlated to the lysine unavailability (Finot and Mauron, 1972) and as descriptor of the drying conditions applied during pasta making (Pagani et al., 1992). Actually, the furosine level in pasta is not subjected to the law regulation; however, according to several authors, a low furosine amount is related to a general high-quality of foods. For this reason, some researchers have underlined the importance of controlling the furosine amount in pasta (Resmini, 1995; Miraglia et al., 1994).

To test the versatility of the microwave system in cereal analysis, samples produced from different raw materials and having undergone different processings were chosen in addition to pasta samples.

It is well-known that the microwave efficiency is strictly related to physicochemical characteristics of samples such as the chemical composition (lipids, protein, sugars), thermal conductivity, surface/volume ratio, and density. However, to perform a uniform microwave energy distribution, cereal products having different chemical composition (pasta, biscuits, etc.) were hydrolyzed separately to prevent heating difformities among the vessels.

Data reported in Table 1 showed that samples, characterized by a protein content between 7.5% of sample CI and 14.5% of sample CF, exhibited a wide range of furosine amount (12.0−433.8 mg/100 g of protein). Results plotted on a regression line were described by the regression equation $Y = -4.37 + 1.06X$ and a correlation coefficient $r = 0.99$, $p \leq 0.01$. The two hydrolysis procedures gave comparable results as demonstrated by the mean values and the relative standard deviation. At a 95% level of probability the coupled groups did not differ significantly, with the exception of samples CE and CF that exhibited the

highest *t* experimental values. Anyway, the variability between the two sets of data gave results generally comparable to the reproducibility obtained by the same traditional method (Resmini et al., 1992).

The good agreement between the results is confirmed either in the presence of a low level of furosine or with a high furosine amount.

Protein content determined on the raw materials is also reported in Table 1. However, as suggested by Resmini et al., 1990, the protein determination was also carried out in the hydrolysate. Variability observed on the two data sets was comparable to that measured in the raw samples and lower than 5%. This means that the microwave procedure did not affect the protein recovery.

Dairy Products. Results obtained on dairy products by applying the two procedures are listed in Table 2.

Samples showed a wide range in furosine due to the different ingredients and processings applied (Resmini and Pellegrini, 1994). In these foods, the main method for the amino ketose formation is represented by the reaction between lactose and lysine to form the stable lactulose−lysine. The low level of furosine found in pasteurized milk (6 mg/100 g of protein) increased up to 278 mg/100 g of protein in bottle sterilized milk reaching about 850 mg/100 g of protein in concentrated milk. Although it had undergone a high-heat treatment, the imitation mozzarella cheese exhibited a neglegible furosine amount (about 7 mg/100 g of protein), comparable to that of mozzarella cheese, due to the low lactose content of the ingredients. The employment of milk products (dried whey, whey protein, etc.) and the presence of reducing sugars (4−7%) in the processed cheese were responsible for the relative high furosine level in these products (up to 200 mg/100 g of protein).

The agreement between the results was described by the regression equation $Y = 1.04 + 1.04X$ and the correlation coefficient $r = 0.99$, $p \leq 0.01$. The relative standard deviations among replicate analyses were equivalent, and they ranged between 1.0 and 11.5 for the microwave method and between 0.4 and 12.8 for the traditional one. As already described for cereals, the microwave hydrolysis did not affect the protein determination in the hydrolysates, and this procedure can be applied in a wide range of protein (3.1−26.6 g/100 g of products) and furosine content (6−800 mg/100 g of protein).

Conclusions. The proposed method represents a good alternative to the conventional procedure with results comparable to those of the traditional one. Data show that the microwave procedure did not affect the fructose−lysine moiety during the hydrolysis and its conversion to furosine, which is recognized to be influenced by the heating. The short hydrolysis time makes the method suitable for the routine analyses especially in the on-line control with practical advantages. The method is also versatile and can be applied to different foods having different chemical composition (lipids, sugars, etc.) also in presence of low levels of furosine (semolina and mozzarella cheese).

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