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Racemization of amino acids during classical and microwave oven hydrolysis — application to aspartame and a Maillard reaction system

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Abstract

The conventional acid hydrolysis routinely used in protein analysis induces partial racemization of the amino acids. An alternative method, using microwave heating, was evaluated with the aim of assessing its efficiency in preserving the amino acids in their original configuration. The method was employed on the dipeptide aspartame. The results showed that some advantages, implying less racemization, could be gained by using the microwave technique, but the method was applicable only at temperatures under 150°C. Mild, conventional hot oven-hydrolysis, involving a shorter hydrolysis time, could have the same mild effect. The different techniques were applied to aspartame treated at different pH, a soft drink and a Maillard reaction model system. The results showed that aspartame was insusceptible to racemization in acid and neutral environments, and not until very high pH did racemization start. The soft drink contained a small amount of D-amino acids, 0.27 mg D-asp and 0.14 mg D-phe/100 ml. The model system exhibited no racemization.

Keywords: Racemization; Amino acids; Oven hydroysis; Aspartame; Maillard reaction system

1. Introduction

L-amino acids are naturally occurring enantiomers in the protein of most living organisms, but the study of D-amino acids is of increasing interest in various fields. Since digestive proteolytic enzymes are specific for L-amino acids (Hayashi & Kameda, 1980; Man & Bada, 1987) and modern food technology uses a variety of processes which are potentially capable of generating detectable quantities of D-amino acids in foodstuffs (Brückner & Hausch, 1990; Bunjapamai, Mahoney, & Fagerson, 1982; Friedman & Liardon, 1985; van der Merbel et al., 1995), D-amino acid analyses are of great interest particularly in the field of nutrition.

The analysis of amino acids in proteins requires hydrolysis, and this is often carried out with 6 M HCl, at 110°C for 24 h (Hirs, Stein, & Moore, 1954). This will lead to a partial racemization, most pronounced for the acidic amino acids, and it is obviously impossible to distinguish between the naturally formed D-amino acids and those arising from racemization during hydrolysis (Man & Bada, 1987; Manning, 1970). Using deuterium chloride instead of HCl for hydrolysis makes it possible to label the amino acids that are racemized in the process, and they can then be identified by mass spectrometry (Liardon & Hurrell, 1983; Liardon & Lederman, 1986; Manning, 1970). These procedures are, however, complicated and there is a need for a simpler method for the analysis of D-amino acids in proteins.

Chen, Chiou, Chu, and Wang (1987) found that complete hydrolysis of peptides could be achieved after a few minutes in a microwave oven, utilizing pressure-resistant vials. This technique has been rapidly accepted (Acquistucci, Panfili, & Marconi, 1996; Joergensen & Thestrup, 1995; Margolis, Jassie, & Kingston, 1991). Microwave hydrolysis has also been shown to be advantageous in the analysis of racemized amino acids in a protein, since the rapid procedure is assumed to minimize hydrolysis-induced racemization (Peter, Laus, Tourw'e, Gerlo, & van Binst, 1993; van de Merbel et al., 1995).

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In the present study we have examined hydrolysis-induced racemization, comparing the conventional hydrolysis technique with the microwave technique. The substrate was the dipeptide aspartame (L-asp-L-phe methyl ester), a sweetener often used in the food industry. The constituting amino acids, aspartic acid and phenylalanine, are among those most prone to racemization (Man & Bada, 1987) and aspartic acid can be used as an index of racemization (Friedman, Zahnley, & Masters, 1981). The different techniques were performed on aspartame at different pH, a soft drink containing aspartame (diet Cola) and a Maillard reaction model system containing lysine and glucose.

The separation and detection techniques used were ion-exchange chromatography, with post-column derivatization using ninhydrin. The enantiomeric forms were analysed on a column with chiral crown ether as the stationary phase.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade. Those used in the preparation of buffers and ninhydrin reagents, perchloric acid, hydrochloric acid (30%), phenol and sodium hydroxide were obtained from Merck (Darmstadt, Germany). The L-amino acid standards, L-aspartame and L-polylysine, were purchased from Sigma Chemical Co. (St. Louis, USA) as was the α-aminobutyric acid used as the internal standard. All the D-amino standards were obtained from ICN Biomedicals Inc (Ohio, USA), L-asparty L-L-phenylalanine from Acros Oeganics (New Jersey, USA) and human serum albumin (HSA) from Kabi Vitrum (Stockholm, Sweden). D-Glucose was purchased from BDH (Poole, England). The membrane filter was from Spectra/Por (Los Angeles, USA) and had a molecular weight cut-off of approximately 3500 Dalton. The water used was purified by Milli-Q purification. The soft drink was bought in the local supermarket and was analysed one month before its expiry date.

2.2. Apparatus

The microwave oven was a Philips, model 8100, with three energy levels determined to be 330, 700 and 860 W. These values were found by heating a defined amount of water in a beaker for a given time, after which the effect was calculated using the equation $P_{watt} = c \times m \times \Delta T/t$, where c is (4.18×1000), m is water (kg), ΔT the temperature difference (°C) and t the heating time (s). The oven had a fixed plate.

The vessels, used for hydrolysis in the microwave oven, height 5 cm, outer diameter 3 cm and inner diameter 1 cm, were made of Teflon, at the workshop of the Department of Chemistry. The lid was 0.6 cm thick. The total volume was 3 ml. One Teflon vessel was prepared with a gold tube inserted into the wall, with the tip projecting into the centre of the vessel. The temperature of the liquid inside the vessel was measured by a Ni,Cr- Ni,Al thermocouple (accuracy ±1°C) in the gold tube.

Quantitative and chiral amino acid analyses were performed with an amino acid analyser, Model LC 5001 from Biotronik, München, Germany. The column used for the quantitative analysis (3.2×385 mm) was packed with the resin BTC 2710 (Biotronik). For the chiral separation, the column was replaced by a Crownpak CR (+), 150 × 4 mm, from Dancel Chemical Industries Ltd, Tokyo, Japan. The recorder used was a Shimadzu CR 3A (Tokey, Japan). The heating block (Multi-Block) was supplied by Lab-Line and the spectrophotometer used was an Ultrospec II from LKB.

2.3. Protein hydrolysis

To perform a complete hydrolysis of the substrate, 5–10 mg of the sample was hydrolysed in sealed glass tubes with 6 M HCl, containing 0.1% phenol, for 24 h at 110°C. After hydrolysis the sample was evaporated to dryness and dissolved in lithium buffer at pH 1.9, as recommended by Biotronik, before ion- exchange separation. The internal standard was added before evaporation.

In order to optimize the conditions for protein hydrolysis with respect to low racemization, the substrate was hydrolysed with 3 and 6 M HCl containing 0.1% phenol, either in a conventional hydrolysis oven, at 110°C, for 3, 7 and 24 h, or in special Teflon vessels in a microwave oven. Three heating regimes were employed:

1. Rapid heating: 60 s, to 185–190°C, effect 700 W and a volume of 2 ml.
2. Moderate heating: 120 s and 180 s, to 150 and 160°C, respectively, effect used was 330 W and a volume of 2 ml.
3. Slow heating: (1) 360 s to 115°C, effect 700 W for 60 s and 300 W for 300 s. The sample was allowed to cool to room temperature (22°C) between heating; (2) 600 s to 115°C, effect 330 W for 300 s twice with a cooling period in between. In both cases the volume was 1 ml.

To avoid possible variation in the irradiation, the vessel was placed in the centre of the microwave oven on a marked place. After hydrolysis, the samples were evaporated and dissolved in lithium buffer prior to separation.

As aspartame is an easily hydrolysed peptide, there was a need for an objective estimate of the efficiency of

the different hydrolysis methods on proteins. Therefore HSA was hydrolyzed under the same conditions as aspartame.

2.4. Quantitative analysis

The quantitative analysis was performed with a standard lithium citrate buffer and the instrument was programmed for physiological fluids. The elution temperature varied between 34 and 61°C. The injection volume was 50 μl and the post-column derivatization of amino acids was performed with ninhydrin. The buffer flow was 0.25 ml/min and the ninhydrin flow was 0.14 ml/min. The temperature of the reaction vessel was 125°C. The spectrophotometric estimate of the amino acids was made at 570 nm. Furosine demanded a higher pH in the last buffer for proper elution (3.80 instead of 3.49). Otherwise the parameters were the same. Furosine eluted after arginine.

The amount of amino acids was calculated using a standard solution of amino acids as reference. The furosine content was calculated using lysine as a reference (Desrosiers, Savoie, Begeron, & Parent, 1989).

2.5. Analysis of amino acid racemization

The amino acids originating from pure aspartame, the soft drink aspartame and the Maillard model system were separated on the chiral column connected directly to the amino acid analyser. Perchloric acid, pH 1.8 and a column temperature of 0°C (ice-water) were used for the elution of D- and L-aspartic acid and D- and L-lysine. Phenylalanine was eluted at room temperature (22°C) at pH 2, with perchloric acid mixed with 10% MeOH. The flow rates, the ninhydrin reaction and the method of detection were the same as in the quantitative analysis. The identification was confirmed by retention times and co-injection of amino acid standards. When necessary, some of the elution parameters were altered and the identification procedure was repeated.

A linear response ($r \geqslant 0.996$) was found in the concentration range 0.005 to 3.8 μmol /ml for all the amino acid forms studied, and the resolution was 1.4 for aspartic acid, 2.0 for phenylalanine (Fig. 1) and 2.4 for lysine. The amount of D-amino acid was calculated as a percentage of the total amount of the amino acid % $D = D/(D + L) \times 100$.

2.6. Sample preparation

For the heat and alkali/acid treatment, aspartame was dissolved in boiling NaOH, perchloric acid at different pHs and in distilled water and was boiled for 5 min. Aspartame was also dissolved in NaOH at pH 12, phosphoric acid at pH 3 and water and stored at room temperature for 1 and 96 h. The samples were then evaporated to dryness, dissolved in 6 M HCl, transferred to the hydrolysis vessel and hydrolysed with 6 M HCl for 120 s to 150°C.

The soft drink was degassed in an ultra-sound bath, evaporated and hydrolysed with 6 M HCl in a hot oven (3 and 24 h) and in a microwave oven (120 s/150°C)

The Maillard model system consisted of 4 mg polylysine (hydrobromide MW 20,700–29,800), 40 mg glucose and 100, 500 and 1000 μl distilled water in glass tubes equipped with screw caps. The samples were heated for 30, 60 and 120 min at 135°C in a heating block. After heating, the absorbance was measured at 420 nm and aliquots were hydrolysed for quantitative analysis of lysine and furosine (a product of acid hydrolysis of early Maillard reaction) in a microwave oven for 120 s to 150°C and for 180 s to 160°C, and in a hot oven for 3 and 24 h. The remainder was dialysed for 48 h to remove the sugar, which would otherwise interfere with the chiral analyses. The dialysates were then

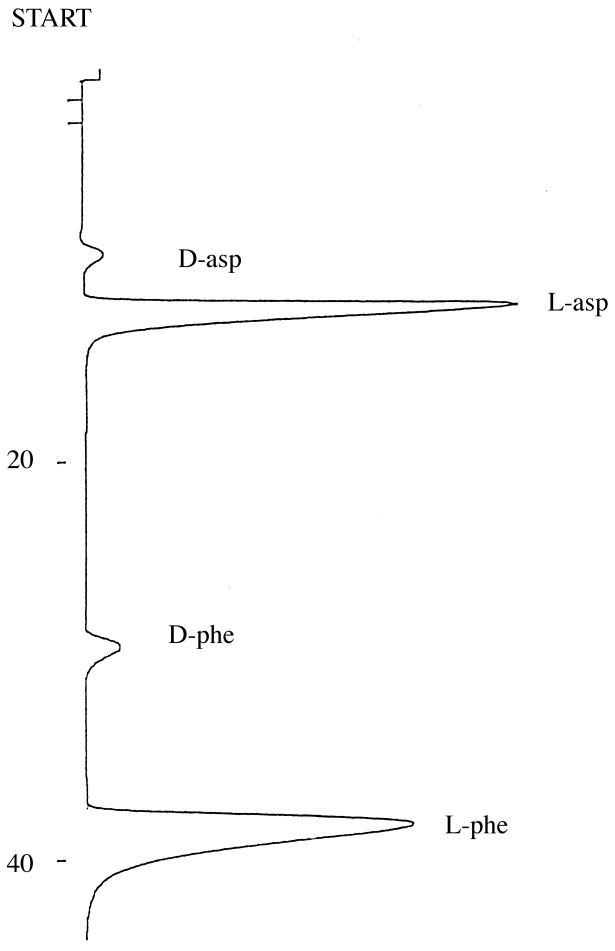

Fig. 1. Enantiomeric separation of aspartic acid and phenylalnine in hydrolysate of aspartame with the resolutions of 1.4 and 2.0, respectively. Eluation parameters: perchloric acid pH 1.8 and column temperature 0°C for 16 min, perchloric acid +10% MeOH pH 2 and column temperature 23°C for 35 min.

evaporated, dissolved in 6 M HCl and hydrolysed as described above, and then analysed chirally.

3. Results and discussion

3.1. Temperature in the Teflon vessel

The temperature generated inside the Teflon vessel in the microwave oven was measured in 2 ml distilled water, 3 M HCl and 6 M HCl. The power levels used were 330, 700 or 860 W and the treatment time between 5 and 180 s. Temperatures up to about 150°C could be measured. Above this the pressure inside the vessel was too high to maintain the gold tube in the correct position. There was consequently no practical way to determine higher temperatures inside the vessel. Temperatures above 150°C were estimated from curve fitting, using a first-order equation, $Y = 22 + B(1-e^{-kt})$, where 22 is the initial temperature (°C) (room temperature throughout the experiments), B the plateau value, k is a constant and t the time. Eight to 10 data points were used for each calculation. At a power of 330 W, the temperature of 2 ml distilled water rose to 140°C in 240 s. The curves for 2 ml 3 M and 6 M HCl were almost identical. After 90 s and ~150°C, extrapolations were performed which gave ~160°C in 180 s and 165–170°C in 240 s. In all cases the curves began to flatten out after ~120 s (Fig. 2). Temperatures given in this paper were either measured directly through the gold tube or calculated using the first-order model. With 1 ml acid in the vessel, the system was able to measure a temperature of ~160C because of the lower pressure. Since there is some uncertainty in the choice of extrapolation parameters, there may be a slight deviation from the real temperature values above 150°C.

3.2. Optimizing the hydrolysis with aspartame

In general, 3 M HCl was a more effective hydrolysing agent than 6 M HCl in microwave hydrolysis, and this pattern was the same for both constituent amino acids of aspartame (Table 1). With the conventional oven technique at 110°C there was no difference between hydrolysis using the two acid concentrations (Table 2). The rapid hydrolysis in the microwave oven is probably due to the combination of the heating effect and a possible increase in the rotational force on the peptide bonds caused by the microwaves (Margolis et al., 1991) and these effects may explain the differences in hydrolysis efficiency of the acid concentrations.

In the case of rapid hydrolysis, 60 s/185–190°C and 100% hydrolysis, the percentages of D-asp and D-phe were about 3 with both the acid concentrations. It should be noted that the percentage D-phe was as high as that of D-asp (Table 1), although generally aspartic acid is more prone to racemization than phenylalanine (Man & Bada, 1987).

In the moderate method, hydrolysis was complete for 3 M HCl, 180 s/160°C, but somewhat less for 6 M HCl, 93% (Table 1). For 120 s/150°C the relation was 93% and 88%. The chiral analyses showed that 3M HCl had a greater racemization effect on the substrate than 6 M HCl, especially in the case of aspartic acid. Treatment with 3 M HCl for 120 s gave 0.55% D-asp and 0.12% D-phe while 6 M HCl for 120 s gave 0.29% and 0.16%,

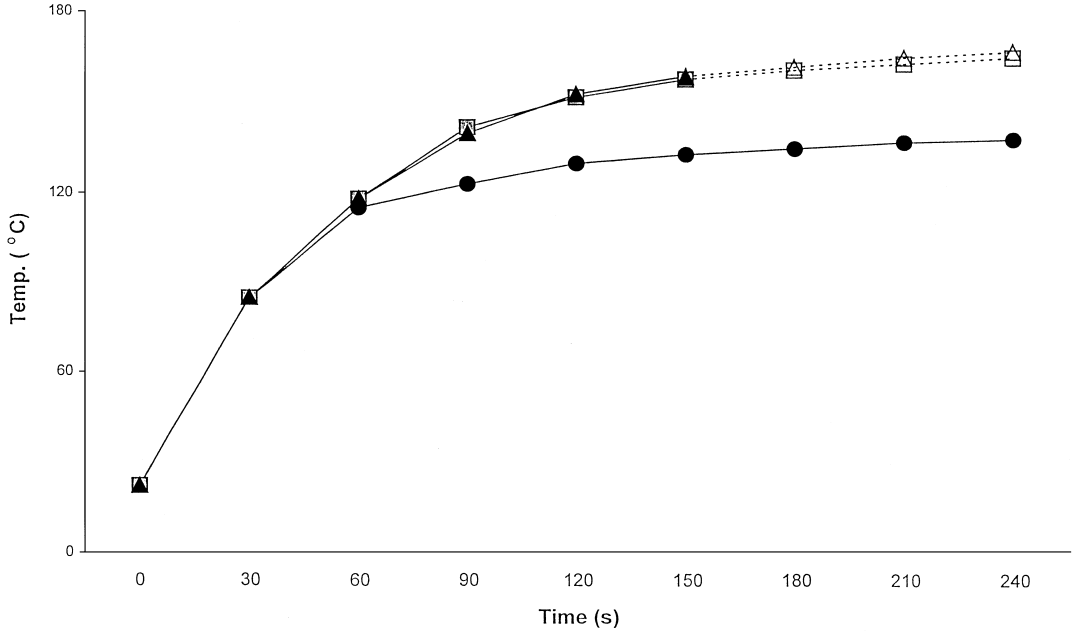

Fig. 2. Temperature rise in a Teflon vessel, containing 2 ml distilled water (●), 3 M HCl (■), or 6 M HCl (▲) heated in a microwave oven at an effect of 330W. Temperatures above ~150°C are estimates (broken lines) using a first-order equation for the temperature increase.

respectively. After 180 s the values were 4.8% D-asp and 2.0% D-phe for 3 M HCl while for 6 M the values were 2.1% and 1.4%, respectively (Table 1).

The slow heating 360 s/115°C and 600 s/115°C was performed only with 6 M HCl and generally led to racemization comparable to that in the moderate hydrolysis at 150°C, but the hydrolysis exchange was somewhat less, around 80% (Table 1).

With the conventional hydrolysis technique, 3 and 6 M HCl had the same low racemization effect in the 3 h treatment, 0.3–0.5% of both D-asp and D-phe, but increasing the treatment time enhanced the effect more with 6 M HCl than with 3 M HCl. Even then, the racemization of aspartic acid was more obvious than that of phenylalanine. With 3 M HCl and 24 h, the amount of D-asp was 2.8% and D-phe 0.7%, compared with 4.7% D-asp and 1.6% D-phe with 6 M HCl (Table 2).

There are two different ways to compare the results in Tables 1 and 2. With the hydrolysis of aspartame as the starting-point, 120 s/150°C gave the smallest racemization and only the 3 h hot oven-hydrolysis had comparably low values. This was observed with both the acid concentrations used. The second method of comparison focuses on the efficiency of the hydrolysis observed in the experiments with HSA. Conventional hydrolysis at 7 h/6 M HCl and at 24 h/3 M HCl was the superior hydrolysis method, while still exhibiting acceptably low racemization.

Accordingly, there was a negative correlation between increasing acid concentration and racemization in microwave hydrolysis, and a positive relation in hot-oven heating at 110C. The action of microwaves on the protein involves induced rotational forces, which influence the formation and/or stability of the carbanion intermediate, the starting point for amino acid inversion (Liardon & Ledermann, 1986). Thus, the effect of microwaves on amino acid racemization is not only a question of increased temperature, but also other mechanisms, possibly explaining the differences observed between the results of microwave heating and conventional oven heating.

The negative inductive strength of the amino acid side chain provides one explanation for the different rates of racemization of the amino acids in alkaline and neutral environments, but the correlation is generally poor at low pH (Liardon & Ledermann, 1986). However, under acidic conditions the rate of racemization of aspartic acid correlates with the negative inductive strength of its undissociated β-carboxylic group (Liardon & Hurrel, 1983, Liardon & Jost, 1981) and this may contribute to the increased tendency of aspartic acid to undergo hydrolysis-induced racemization as found by, among others, Man and Bada (1987).

Evidently, a method for D-amino acid analysis, useful as a screening method, must be a compromise between complete hydrolysis and minimal racemization. Since quantitative analysis could be performed in the traditional way, incomplete hydrolysis may be acceptable for racemization studies. Using the slow method 120 s/150°C 6 M HCl (hydrolysis 88 ± 6%) and 3 M HCl (hydrolysis 93 ± 9%) showed that, within each series, there was no correlation between more effective

Table 1
Hydrolysis-induced racemization in aspartame using microwave hydrolysis [The efficiency of hydrolysis is compared with human serum albumin (HSA) hydrolysis, performed in the same way]

HCl	Time (s)	End temp (°C)	*n*	% hydr. of aspartame	% D-asp	% D-phe	% hydr. of HSA (*n* = 2)
3M	60	185–190	2	100	~ 3	~ 3	
6M	60	185–190	2	100	~ 3	~ 3	
3M	120	150	5	93±9	0.55±0.11	0.12±0.10	50
6M	120	150	5	88±6	0.29±0.12	0.16±0.06	52
3M	180	160	3	100±1	4.83±1.10	1.98±0.50	70
6M	180	160	3	93±8	2.14±0.58	1.43±0.02	76
6M	360	115	3	82±4	0.19±0.05	0.15±0.03	47
6M	600	115	2	77	0.64	0.36	60

Table 2
Hydrolysis-induced racemization in aspartame using conventional hydrolysis for different time periods [The efficiency of the hydrolysis is compared with human serum albumin (HSA) hydrolysis, performed in the same way]

HCl	Time (h)	Temp°C	*n*	% hydr of aspartame	% D-asp	% D-phe	% hydr. of HSA (*n* = 2)
3M	3	110	2	98	0.3	0.3	65
6M	3	110	2	95	0.5	0.4	87
3M	7	110	2	98	1.3	0.4	82
6M	7	110	2	98	1.8	0.7	91
3M	24	110	2	103	2.8	0.7	95
6M	24	110	7	100	4.7 ± 0.59	1.59 ± 0.20	100

hydrolysis and increased racemization. In the first case ($n = 5$) the coefficient of correlation was 0.58 for D-asp and 0.33 for D-phe, and in the second ($n = 5$) 0.28 and 0.53, respectively. This supports the acceptance of incomplete hydrolysis as a compromise in the analysis of amino acid racemization in protein.

3.3. Applications

Since aspartame is frequently used as an artificial sweetener in the food industry, it is of interest to know its racemization tendency in different environments. Boiling in phosphoric acid at pH 1.5 and 3 and in distilled water for 5 min generated a degree of racemization of about 0.5% of each D-amino acid, insignificantly above the values for untreated aspartame (0.3% D-asp and 0.2% D-phe). After boiling in NaOH at pH 11, however, a slight increase was observed, between 0.5 and 1% of each D-amino acid, and at pH 11.5 the values had increased to slightly over 1%. At pH 12 a pronounced enhancement was seen, namely, about 5% for D-asp and 7.5% for D-phe (Fig. 3).

At room temperature, the aspartame experiment with NaOH at pH 12, resulted in 2.3% D-asp and 1.2% D-phe when the aspartame was analysed immediately after the addition of the hydroxide. One hour later, the racemization had increased to 2.5% D-asp and 1.8% D-phe, and after 96 h the values were 4.1 and 6.4% for D-asp and D-phe, respectively. The corresponding treatment with phosphoric acid at pH 3 and water gave no racemization. The results showed that aspartame was insusceptible to racemization in acid and neutral environments and racemization did no start until very high pH. This was observed both at room temperature and at 100°C.

Aspartame has limited stability in aqueous systems and the decomposition products are aspartic acid, phenylalanine, aspartylphenylalanine and diketopiperazine. To obtain the true value of the original aspartame content of a soft drink the calculations must therefore be based on the asp and phe contents and hydrolysis is necessary. The investigated drink was found to have an original aspartame content of 23 mg/100 ml. Besides the constituent amino acids in aspartame, aspartic acid (10.2 mg/100 ml) and phenylalanine (12.7 mg/100 ml), ammonium ions (9.25 mg/ml) and one unidentified constituent were present. The chiral analysis gave 3.1% D-asp and 1.2% D-phe following microwave hydrolysis, (120 s/150°C), 2.7% D-asp and 1.1% D-phe following the 3 h hot-oven hydrolysis and 10.1% D-asp and 2.9% D-phe the conventional 24 h hydrolysis. In all cases hydrolysis was complete. Assuming the minimum values, drinking 100 ml of the soft drink would mean a consumption of only 0.27 mg D-asp and 0.14 mg D-phe.

Polylysine, heated and unheated, was more difficult to hydrolyse. The short microwave hydrolysis (120 s/150°C) resulted in only approximately 50% hydrolysis, while 180 s/160°C led to 95% hydrolysis and 3 h hot-oven hydrolysis resulted in 80% hydrolysis (Table 3). The presence of Maillard products was demonstrated by colour development, loss of lysine and formation of furosine. It is well known that the maximum reaction rate in the Maillard reaction occurs at intermediate water activity levels (Whister & Daniel,

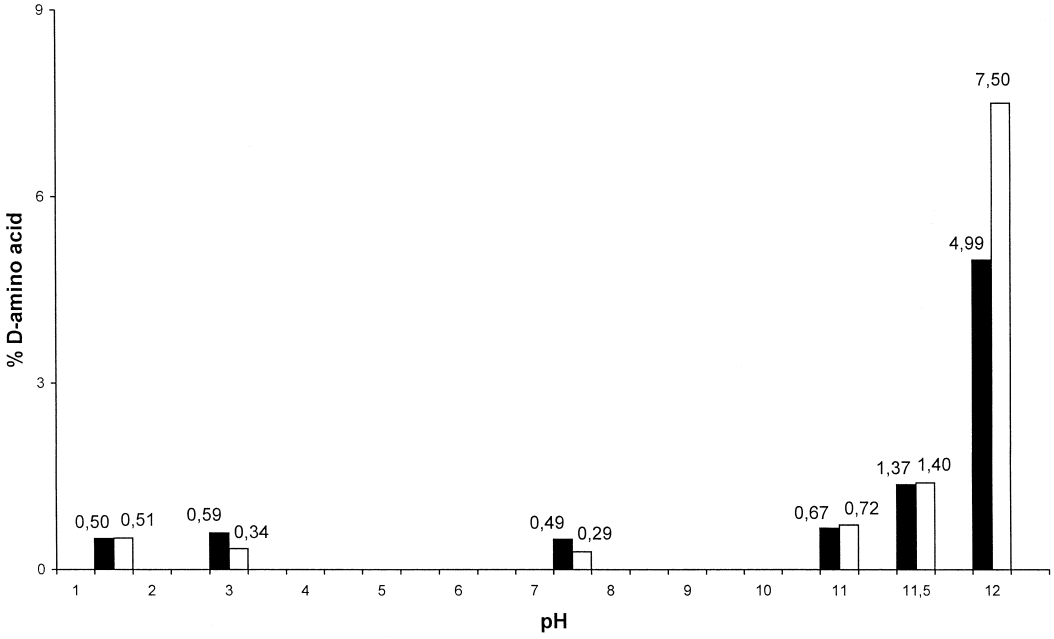

Fig. 3. Racemization of aspartic acid and phenylalanine as constituents of aspartame boiled for 5 min in dilute phosphoric acid, (pH 1.5 and 3) distilled water and dilute NaOH, (■) D-aspartic acid, (□) D-phenylalanine.

1985) and in the present experiment (using a concentration of 0.434 g sample/ml water) the lysine concentration was 45% of the original concentration after 30 min and 36% after 1 h. In both cases the presence of furosine was notable, 5.3 mg/ml (30 min) and 2.1 mg/ml (1 h) (Table 3). In the tests with lower concentrations (0.09 g and 0.045 g sample/ml water) 72 and 88% of the lysine remained after 30, 60 and 120 min heating (not significantly different) and the furosine content was less than 0.1 mg/ml. The enantiomeric analyses of both unheated and heated polylysine showed a pattern similar to that obtained from the test series performed with aspartame. Short hydrolysis times, with both the microwave oven (120 s/150°C) and the conventional oven (3 h) gave the lowest content of D-lysine, $<0.9\%$, compared with about 2.0% for the longer hydrolysis times (180 s/160°C and 24 h) (Table 3).

The results of the furosine analyses and the absorbance measurements indicated the presence of early and advanced Maillard products in the polylysine tests. In spite of this, there was no evidence of any racemization beyond that induced by hydrolysis and/or that based on enantiomeric contamination.

Research on racemization in Maillard products is not very extensive. Our results agree with those obtained by Miller, Olsson, and Pernemalm (1984) and Liardon and Hurrell (1983). Miller et al. observed slow racemization in lysine after refluxing for 30 h in acetate buffer at pH 4, but presumed extensive racemization under conditions comparable to roasting and frying. Liardon and Hurrell examined spray-dried milk and found no significant racemization, even after relatively severe Maillard reaction treatment (9 weeks at 70°C and 1 h at 120°C). On the other hand, Hayase, Kato, and Fujimaki (1979) reported strongly racemized amino acids, above all, aspartic acid and glutamic acid, after roasting casein with glucose, but gave no information on lysine.

The results concerning racemization of aspartic acid can be compared with those obtained in our earlier study on HSA hydrolysis (van de Merbel et al., 1995). In that study 24 h conventional hydrolysis of HSA generated 10.6% D-aspartic acid, while the microwave hydrolysis (180 s/160°C) resulted in 4.4%. The corresponding values for pure aspartame were 4.7% and 2.1% D-asp. Although the amount of the material is small, a certain pattern is perceptible, namely, conventional hydrolysis (24 h) produces about double the amount of D-aspartic acid as produced by 180 s/160°C microwave hydrolyses. With regard to phenylalanine and lysine no such comparison is possible, since information of D-phe and D-lyst is missing. This study shows that the advantages of microwave hydrolysis are negligible, unless 120 s/150°C is used. However, these parameters were not sufficient to give a satisfactory degree of hydrolysis for polylysine. For this sample shorter conventional hydrolysis is preferable.

In the experiments performed by P'eter et al. (1993), it was shown that microwave hydrolysis caused limited racemization. Since the equipment used for microwave hydrolysis was different from ours, it is only possible to compare trends and not quantitative results. The trends were the same, i.e. racemization increased with reaction time, but was generally lower than with the conventional technique (110°C/24 h). P'eter et al. concluded that incomplete hydrolysis must sometimes be accepted and no experiments were carried out for reduced times in a conventional oven.

In contrast to our microwave experiences (higher temperatures resulting in higher racemization and with a critical temperature of about 150°C), Csapo et al.

Table 3
Racemization in polylysine after heating with glucose, a comparison between hydrolysis in a microwave oven and conventional hydrolysis

Mixture	135°C min	mg lys/ml (% of initial lysine)	mg fur/ml (% of initial lysine)	abs 420 nm	Microwaves 120 s/150°C Hydr. 47% (% D-lys)	Microwaves 180s/160°C Hydr.95% (% D-lys)	110°C3 h Hydr. 80% (% D-lys)	110°C 24 h Hydr. 100% (% D-lys)
Untreated polylysine					0.9	1.3	0.5	2.1
					0.6	1.5	0.4	2.1
0.434 g Sample/ml water[a]	0	29.1	0	0				
	30	13.1 (45)	5.3 (18)	0.114	0.6	2.2	n.d.	1.8
	60	10.6 (36)	2.1 (7)	0.253	n.d.	1.6	n.d.	1.0
0.090 g Sample/ml water[a]	0	6.0	0	0				
	30	4.4 (73)	0.07 (1.2)	0	0.7	1.9	0.3	1.6
	60	4.2 (70)	0.03 (0.5)	0	0.5	2.3	0.7	2.1
	120	4.3 (72)	0.06 (1.0)	0	0.9	2.2	0.4	1.5
0.045 g Sample/ml water[a]	0	2.6	0	0				
	30	2.3 (88)	0.01 (0.4)	0	0.9	2.0	0.4	2.4
	60	2.2 (85)	0.05 (1.9)	0	0.8	1.6	0.4	1.9
	120	2.3 (88)	0.03 (1.2)	0	0.5	1.8	0.2	1.8

[a] Polylysine/Glucose 1:10 by weight.

(1997) found decreased racemization at temperatures between 160 and 180°C with reduced hydrolysis times (<60 min) in a conventional oven. Under conditions ensuring total hydrolysis racemization was 1.5 times lower than that obtained with the traditional method of hydrolysis. The fact that the protein hydrolyses more rapidly at high temperatures and the free amino acids obtained are more resistant to racemization than peptide bound acis, may explain the lower degree of racemization at high temperature (Csapo et al., 1997). In our experiments, no investigations were carried out on free amino acids using the microwave method. The high pressure in the vessels and/or the forces causing molecular rotation might, in some way, also influence free amino acids and perhaps explain the difference between the results.

4. Conclusions

The result of this study shows that it is not possible to obtain both complete hydrolysis and minimal racemization of peptide-bound amino acids. If, however, a compromise can be accepted it is possible to obtain minimal hydrolysis-induced racemization with both the conventional and the microwave techniques. However, it is important to evaluate different hydrolysis techniques, in each individual case before conclusions on racemization are drawn.

Furthermore, it was observed that not even the conventional 110°C hydrolysis induced racemization to a very high degree and with knowledge of this significant natural racemization should be found even without hydrolysis optimization.

Aspartame, both pure and as a constituent in a soft drink, showed only a limited tendency towards racemization as did lysine in a Maillard model mixture using polylysine.

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