Microwave-Assisted Synthesis and Extraction of Selected Maillard Reaction Products

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To take advantage of the chemical diversity of the products formed during Maillard reaction, a focused microwave system under atmospheric pressure was used to synthesize and extract selected Maillard reaction products. L-Phenylalanine or glycine/D-glucose mixtures were sequentially treated with microwave irradiation in an aqueous media to initiate the synthesis step followed by irradiation in an microwave-transparent solvent to perform extraction of the products formed. The phenylalanine model system generated a mixture consisting of benzeneacetaldehyde (48%), 2-(5′-(hydroxymethyl)-2′-formylpyrrol-1′-yl)-3-phenylpropionic acid lactone (34%), 3,5-diphenylpyridine (4%) and an unknown compound (14%). The glycine model system, on the other hand, generated a mixture consisting of 1,6-dimethyl-2(1*H*)-pyrazinone (4%), 1,5,6-trimethyl-2(1*H*)-pyrazinone (19%), 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone (62%), and two unknowns with molecular weights of 204 (10%) and 152 (5%).

Keywords: *Microwave-assisted synthesis; Maillard reaction; glycine; phenylalanine*

INTRODUCTION

Chemical changes initiated by the Maillard reaction during thermal processing of food are considered to be important contributors to the modification of color and sensory properties of processed foods (Labuza et al., 1995). The complexity of the Maillard reaction precludes its complete analysis through classical organic chemistry and requires the use of isotopically labeled starting materials such as sugars and amino acids to establish the origin and the fate of a multitude of short-chain reactive intermediates that form during the process. Identification of such reactive intermediates can help to predict the formation of certain end products and eventually can lead to the classification of the Maillard reaction into its underlying elementary processes. Such approaches have been successfully applied on a microscale level by the use of pyrolysis/GC/MS as an integrated reaction, separation, and identification system (Yaylayan and Keyhani, 1996a). However, many of the products formed during the Maillard reaction are still unidentified and require the production of analytically pure samples for spectroscopic identification and sensory characterization. The ability of focused microwave irradiation under atmospheric pressure conditions to selectively synthesize and quantitatively separate Maillard reaction products was investigated using a two-stage microwave-assisted process (MAP) (Paré et al., 1991, 1994; Paré 1994, 1995, 1996). MAP has been applied successfully to various liquid-phase and gas-phase extractions and is currently used extensively as a sample preparation tool (Bélanger et al., 1996). The first stage—microwave-assisted synthesis (MAS)—could be carried out in a microwave-active solvent such as water, ethanol, or water–ethanol mixtures depending on the energy requirements of the reaction, and the second stage—microwave-assisted extraction (MAE)—could be carried out in an microwave-transparent solvent such as petroleum ether, hexane, or mixtures of hexane and acetone to selectively extract a minimum number of products formed in the first stage. After evaporation of the solvent, the residue could be further purified, if necessary, by chromatography and characterized by spectroscopic techniques. Changing the type and composition of the solvents used in the MAS and MAE stages, in principle, can produce different products formed in the Maillard reaction. In addition, production of quantitative amounts of extracted products could be achieved by multiple extractions of up to 5 g of reactants. In this paper we report the preliminary findings of the application of the MAS/MAE technique in the area of the Maillard reaction using model systems consisting of D-glucose and glycine or L-phenylalanine.

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EXPERIMENTAL PROCEDURES

Petroleum ether (boiling range 60–80 °C, analytical reagent) was purchased from BDH (Montréal, Canada). D-Glucose, glycine, and L-phenylalanine were purchased from Aldrich Chemical Co. (Milwaukee, WI). The Soxwave 100 (focused microwave extraction system at atmospheric pressure) was obtained from Prolabo (Fontenay-Sous-Bois, France). The basic apparatus consists of a command box and a microwave module operating at an emission frequency of 2450 MHz and a 300 W full power. It is equipped with a 250 mL quartz vessel, a Graham type refrigerant column (400 mm length), and a bent extraction tube.

Microwave-Assisted Synthesis and Extraction of Maillard Reaction Products from D-Glucose/Glycine Model System. A D-glucose (1.00 g, 0.005 mol) and glycine (1.25 g, 0.016 mol) mixture was transferred into the 250 mL quartz

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Table 1. Mass Spectrometric Data of Compounds Produced by MAS/MAE

compound	*m/z* (relative intensity)
1,6-dimethyl-2(1*H*)-pyrazinone[a]	124 (95), **95 (100)**, 81 (14), 68 (27), 56 (48)
1,6-dimethyl-2(1*H*)-pyrazinone[b]	124 (98), **95 (100)**, 81 (15), 68 (30), 56 (56)
1,6-dimethyl-2(1*H*)-pyrazinone	**124 (100)**, 95 (97), 81 (14), 68 (27), 56 (48)
1,5,6-trimethyl-2(1*H*)-pyrazinone[a]	138 (75), **109 (100)**, 95 (33), 82 (8), 68 (20), 56 (42)
1,5,6-trimethyl-2(1*H*)-pyrazinone[b]	138 (80), **109 (100),** 95 (42), 82 (8), 68 (22), 56 (43)
1,5,6-trimethyl-2(1*H*)-pyrazinone	138 (87), **109 (100),** 95 (41), 82 (7), 68 (19), 56 (40)
3,5-diphenylpyridine[c]	232 (19), **231 (100)**, 230 (24), 203 (5), 202 (12), 115 (3), 102 (10), 101 (6), 77 (4), 76 (6)
3,5-diphenylpyridine	232 (18), **231 (100)**, 230 (26), 203 (6), 202 (13), 115 (2), 102 (12), 101 (7), 77 (4), 76 (6)
lactone[c]	256 (11), 255 (27), 211 (4), 210 (5), 193 (2), 182 (2), 180 (2), 167 (2), 148 (5), 147 (5), 131 (11), 120 (9), 108 (12), 104 (5), 103 (4), 92 (11), **91 (100)**, 78 (7), 77 (7), 63 (5), 51 (8)
lactone	256 (5), 255 (27), 211 (5), 210 (5), 193 (0), 182 (2), 180 (2), 167 (2), 148 (4), 147 (4), 131 (10), 120 (9), 108 (13), 104 (6), 103 (5), 92 (9), **91 (100)**, 78 (6), 77 (8), 63 (4), 51 (8)
Y_1	**190 (100),** 162 (28), 161 (20), 134 (15), 133 (40), 119 (23), 93 (10), 92 (16), 65 (10), 56 (30), 51 (18)
Y_2	153 (8), 152 (82), 124 (43), **123 (100),** 109 (52), 82 (12), 68 (31), 56 (38)
Y_3	205 (14), **204 (100),** 176 (22), 175 (35), 161 (10), 148 (17), 147 (44), 133 (9), 120 (10), 92 (12), 77 (8), 74 (7), 66 (8), 65 (10), 56 (29), 51 (12)
X_1	223 (16), 222 (97), 221 (44), 207 (10), 205 (12), 204 (20), 203 (12), 193 (13), 191 (13), 189 (11), 179 (11), 178 (31), 165 (14), 131 (22), 116 (21), **115 (100),** 105 (10), 103 (66), 102 (12), 91 (71), 89 (16), 77 (17), 65 (31), 63 (15), 51 (17)

[a] Oh et al., 1992. [b] Keyhani and Yaylayan, 1996b. [c] Keyhani and Yaylayan, 1996a. Lactone = 2-(5′-(hydroxymethyl)-2′-formylpyrrol-1′-yl)-3-phenylpropionic acid lactone. Y_1 = 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone.

extraction vessel of the Soxwave 100 microwave extraction system. Water (2 mL) was then added. The vessel was inserted inside the extraction cavity and fitted with a condenser. The irradiation was carried out in the following sequence at full power (300 W): 2 min on, 30 s off, 2 min on, 30 s off, 2 min on, 30 s off, and 2 min on (a total of 8 min of irradiation). At the end of the irradiation sequence a dark brown and dry slurry was formed that had characteristic baked bread notes. The extraction step was carried out with 40 mL of petroleum ether (60−80 °C) using the following sequence of irradiation: 40 s on, 30 s off, and 90 s on. The solvent was decanted and dried over sodium sulfate and analyzed by GC/MS. Further extraction with the same solvent did not yield any additional product.

Microwave-Assisted Synthesis and Extraction of Maillard Reaction Products from a D-Glucose/L-Phenylalanine Model System. A D-glucose (0.5 g, 0.0027 mol) and L-phenylalanine (1.37 g, 0.083 mol) mixture was transferred into the 250 mL quartz extraction vessel of the Soxwave 100 microwave extraction system. Water/methanol (1:1) (2 mL) was then added to solubilize L-phenylalanine. The vessel was inserted inside the extraction cavity and fitted with a condenser. The irradiation was carried out in the following sequence at full power (300 W): 2 min on, 30 s off, and 2 min on (a total of 4 min of irradiation). At the end of the irradiation sequence a dark brown and dry slurry was formed that had a characteristic flowery notes. The extraction step was carried out with 40 mL of petroleum ether (60−80 °C) using the following sequence of irradiation: 40 s on, 30 s off, and 90 s on. The solvent was decanted and dried over sodium sulfate and analyzed by GC/MS. Further extraction with the same solvent did not yield any additional product.

GC/MS Analysis. A Hewlett-Packard GC/mass selective detector (5890 GC/5971B MSD) was used for GC/MS analysis. The He flow rate was 0.8 mL/min for a split ratio of 92:1 and a septum purge of 3 mL/min. The injector temperature was set at 250 °C. Capillary direct MS interface temperature was 180 °C; ion source temperature was 280 °C. The ionization voltage was 70 eV, and the electron multiplier was 1682 V. The mass range analyzed was 50−550 amu. The column was a fused silica DB-5 column (30 m length × 0.25 mm i.d. × 25 μm film thickness; Supelco, Inc.). The column initial temperature was 25 °C and was increased to 250 °C at a rate of 7.5 °C/min; immediately the temperature was further increased to 300 °C at a rate of 25 °C/min and kept at 300 °C for 10 min.

RESULTS AND DISCUSSION

The Maillard reaction, under controlled experimental conditions, could become a potential source of compounds with vast molecular diversity that would, otherwise, have required complex multistep synthetic approaches for their production. A broad spectrum of heterocyclic, aromatic, carbocyclic, and bicyclic flavor-active compounds are known to be formed during Maillard reaction (Yaylayan and Despointes, 1994). As such, Maillard reaction mixtures could be viewed as potential "combinatorial flavor chemistry" libraries. However, under reflux conditions, the complexity and the low yields of diverse products formed makes it next to impossible to isolate specific components for spectroscopic and sensory analysis. To investigate the feasibility of simultaneous microwave-assisted synthesis and extraction of selected Maillard reaction products from sugar−amino acid mixtures, using focused microwave irradiation at atmospheric pressure conditions, two Maillard model systems were selected consisting of D-glucose and glycine or L-phenylalanine. Both systems were recently investigated using pyrolysis/GC/MS with ^{13}C-labeled glucoses and amino acids (Keyhani and Yaylayan, 1996a,b; Yaylayan and Keyhani, 1996a,b). By controlling the irradiation time and temperature (dependent on solvent mixture) during the MAS stage certain products could be made to be formed preferentially, thus producing mixtures rich in specific products, further selectivity could be obtained during the MAE stage whereby controlling the solvent polarity and extraction time specific products formed in the MAS stage could be extracted sequentially, by varying solvent composition. Table 1 lists the mass spectrometric data of all the compounds identified in both model systems.

L-Phenylalanine/D-Glucose Mixture. The phenylalanine glucose model system has been shown to produce a complex mixture of products under various experimental conditions such as refluxing in a solvent, autoclaving (Kunert-Kirchhoff and Baltes, 1990), or pyrolysis. However, application of the MAS/MAE technique generated a relatively simple mixture (Figure 1 and Table 2) consisting of benzeneacetaldehyde, 2-(5′-(hydroxymethyl)-2′-formylpyrrol-1′-yl)-3-phenylpropionic acid lactone (Kunert-Kirchhoff and Baltes, 1990), 3,5-diphenylpyridine, and an unknown compound X_1. All of these products have been detected in the pyrolysates of the D-glucose/L-phenylalanine mixture and L-phenylalanine Amadori compound except X_1, which

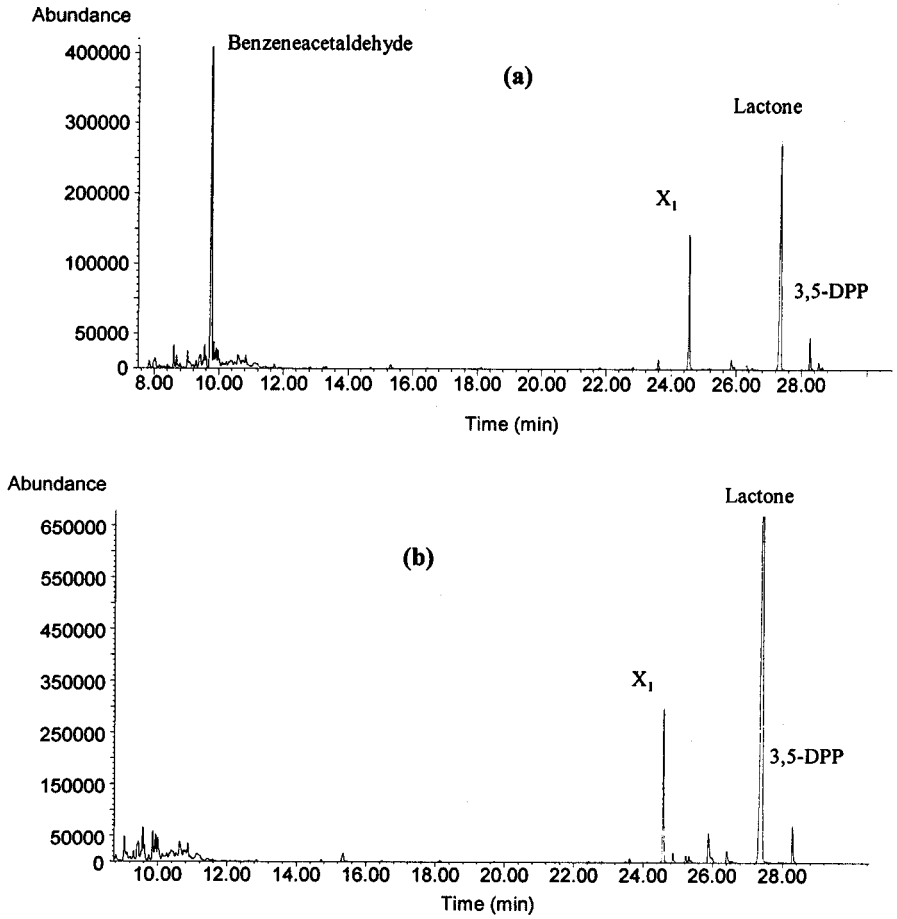

Figure 1. GC/MS chromatogram of D-glucose/L-phenylalanine extract in (a) reflux mode (in focused microwave) and (b) open vessel (in domestic microwave). Lactone = 2-(5′-(hydroxymethyl)-2′-formylpyrrol-1′-yl)-3-phenylpropionic acid lactone. 3,5-DPP = 3,5-diphenylpyridine. X_1 = unknown.

Table 2. Products Identified in L-Phenylalanine/D-Glucose Mixture[a]

	BAC		X_1		lactone		3,5-DPP	
	open	reflux	open	reflux	open	reflux	open	reflux
absolute area	2.1×10^6	3.1×10^7	8.5×10^5	9.2×10^6	1.2×10^7	2.2×10^7	tr	2.0×10^6
% area	14	48	6	14	80	34	tr	4

[a] BAC = benzeneacetaldehyde. Lactone = 2-(5′-(hydroxymethyl)-2′-formylpyrrol-1′-yl)-3-phenylpropionic acid lactone. 3,5-DPP = 3,5 diphenylpyridine. X_1 = unknown.

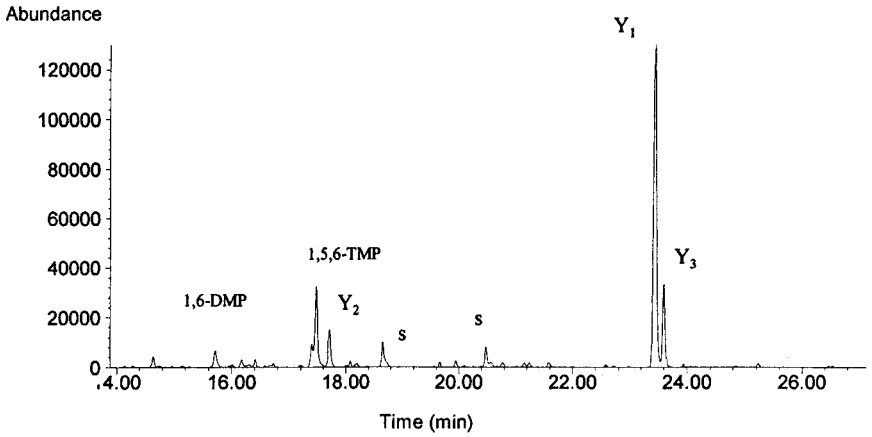

Figure 2. GC/MS chromatogram of D-glucose/glycine extract in reflux mode. 1,6-DMP = 1,6-dimethyl-2(1*H*)-pyrazinone. 1,5,6-TMP = 1,5,6-trimethyl-2(1*H*)-pyrazinone. Y_1 = 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone. Y_2 and Y_3 are unknown componds. s = solvent.

has been detected only during pyrolysis of the L-phenylalanine Amadori product, which indicates the possible formation of an Amadori intermediate during the synthesis stage (Yaylayan, 1996). In addition, the synthesis stage was also carried out in a domestic microwave oven using an open vessel, to compare the product distribution and the relative yields. According to Table 2, the advantage of using a reflux system is the higher yields obtained, whereas using the open system will generate fewer products in lower yields. The

Table 3. Products Identified in Glycine/D-Glucose Mixture[a]

	1,6-DMP	1,5,6-TMP	Y_1	Y_2	Y_3
absolute area	2.6×10^5	1.2×10^6	4.2×10^6	3.3×10^5	7.2×10^5
% area	4	19	62	5	10

[a] 1,6-DMP = 1,6-dimethyl-2(1*H*)-pyrazinone. 1,5,6-TMP = 1,5,6-trimethyl-2(1*H*)-pyrazinone. Y_1 = 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone. Y_2 and Y_3 are unknown compounds.

more volatile component such as benzeneacetaldehyde can evaporate under the open vessel conditions, as shown in Figure 1.

Glycine/D-Glucose Mixture. The glycine/D-glucose model system produced five major components (Figure 2 and Table 3), two of which (1,6-dimethyl- and 1,5,6-trimethyl-2(1*H*)-pyrazinones) have been detected and identified previously (Oh et al., 1992) in D-glucose glycine model systems. The mechanism of formation of alkyl-substituted pyrazinones has been determined by ^{13}C-labeling studies (Keyhani and Yaylayan, 1996b; Yaylayan and Keyhani, 1996b). Both pyrazinones can be formed from heated D-glucose glycine mixtures or from their corresponding Amadori product. Interestingly, all of the components detected in the MAS/MAE extract, including the three unknowns termed Y_1, Y_2, and Y_3, were also detected in the pyrograms of D-glucose glycine systems (Keyhani and Yaylayan, 1996b), Y_1 being the major product formed when 3 mole of glycine was reacted with 1 mol of D-glucose. All attempts to generate Y_1 by the classical reflux method followed by extraction with different solvents had failed. The success of MAS/MAE to produce Y_1, provided the opportunity to characterize the structure of an important Maillard product. A total of 50 g of glucose/glycine mixture was subjected to MAS/MAE in 3 g batches, the extracts were combined, and the solvent was evaporated. The resulting yellowish oil was chromatographed on a silica gel thick-layer plate using ethyl acetate as the solvent. Spectroscopic analysis indicated Y_1 to be 5-hydroxy-1,3-dimethyl-2(1*H*)-quinoxalinone. Details of the chromatographic separation and complete spectroscopic analysis will be published elsewhere (Keyhani and Yaylayan, 1997).

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