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Isolation and structural analysis of Maillard polymers: caramel and melanoidin formation in glycine/glucose model system

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A water–methanol solution of glycine and D-glucose was refluxed at 65°C for seven h. The solvent was evaporated under vacuum at room temperature and the residue was dialyzed against distilled water. After dialysis, the solvent was evaporated under vacuum at room temperature. The non-dialyzable fraction was further fractionated by gel filteration. The process yielded three polymeric materials ($10\,000 < MW < 20\,000$) termed A, B1 and B2. The isolated polymers were further analyzed by UV-VIS and FTIR spectroscopy and by pyrolysis/GC/MS. Elemental analysis indicated that polymer A has the following empirical formula $C_7H_{11}N_1O_4$ and polymers B1 and B2 have the same empirical formula as D-glucose ($C_1H_2O_1$). The origin of nitrogen containing polymer A was assigned to Amadori intermediate or to its derivatives and the origin of polymers B1 and B2 was assigned to glucosone and/or to 3- or 1-deoxyglucosones; common non-nitrogen containing reactive intermediates formed during the Maillard reaction. Plausible mechanisms were proposed for the formation of polymers based on spectroscopic data.

INTRODUCTION

The complex process of polymeriztion during Maillard reaction, produces a variety of polymeric materials with different molecular weights, structures and elemental compositions. Most of the reported polymers (Wedzicha and Kaputo, 1992) from Maillard model systems, incorporate nitrogen. The composition of the polymers will depend on the ratio and the type of reactants, temperature, time, pH control and the solvent used (Wedzicha and Kaputo, 1992). However, it is simplistic to assume that only one polymeric material could be produced in a model system under specific conditions. A hypothetical sequence of polymerization reactions under Maillard condition, is shown in Scheme 1. The initial backbone polymeric materials could be formed by the linkage of several oligomeric units such as a, b, and c in Scheme 1, to produce a series of differently linked n initial polymers (PB_n; n = integer) which further undergo elimination type reactions (dehydrations, decarboxylations, intramolecular substitutions followed by elimination, etc.) to produce a series of n derivative polymers designated as PE_n (n = integer). Alternatively, the reactive sites on the initial polymers can interact with other components in the Maillard mixture to produce a more complex series of derivative polymers designated as PA_n (n = integer). Both series can undergo either addition or elimination processes to produce $P(A+E)_n$ and/or $P(E+A)_n$ series of polymers as shown in Scheme 1. Accordingly, dialysis alone of Maillard model systems can produce a mixture of different polymers. In this paper, non-dialyzable fraction of a D-glucose/glycine mixture was further fractionated by gel filteration to produce three polymers, two of which did not incorporate any nitrogen atoms and hence could be classified as caramels.

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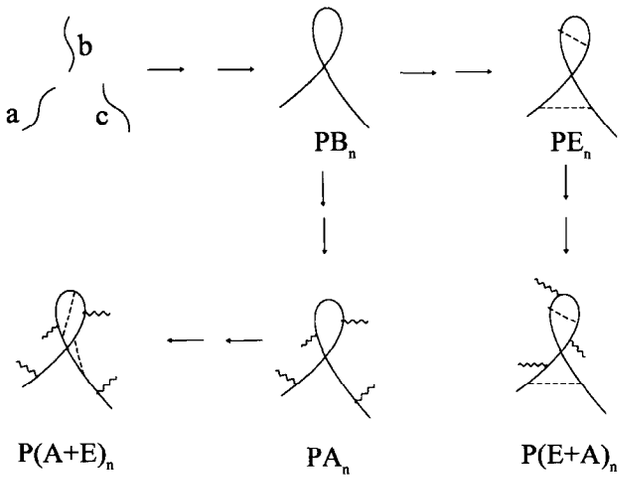
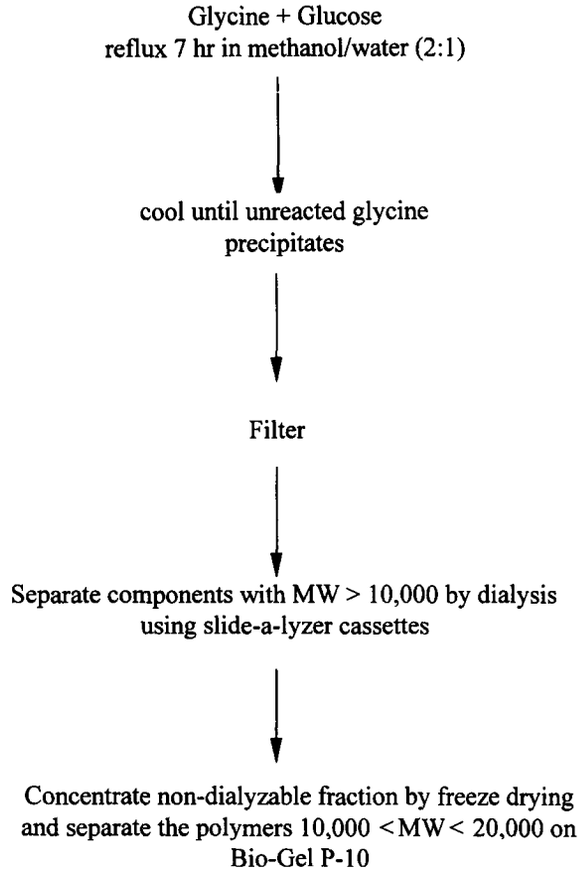
MATERIALS AND METHODS

D-Glucose was purchased from BDH Inc., glycine flom Fisher Scientific, BIO-GEL P-10 (exclusion limit 1500–20 000 Da) and BIO-GEL P-2 (exclusion limit 100–1800 Da) were purchased from Bio-Rad Laboratories (Richmond, CA). Slide-a-lyzer cassettes (3.0–15 ml, capacity, nominal molecular weight cutoff = 10 000) from Pierce (Rockford, Illinois, USA).



Scheme 1. Hypothetical polymerization process during the Maillard reaction, where a, b and c are oligomeric units, PB, PE, PA, P(E + A), and P(A + E) are polymers with different structures.



Scheme 2. Procedure for the isolation of Maillard polymers.

Water was purified using Milli-Q water purification system from Millipore Corp. Infrared spectra were recorded in D_2O on Nicolet 8210 Fourier transform spectrometer. UV/VIS spectra were recorded in water on a Beckman DU-64 spectrophotometer. Hunter tristimuls values were calculated using Color® add-on application for GRAMS/386 software (Galactic Industries, New Hampshire). Elemental analysis was performed by Guelph Chemical laboratories Ltd. (Ontario, Canada). Scheme 2 summarizes the process of isolation of polymers.

Polymer preparation

A solution of glycine (1.46 g, 0.66 M) and D-glucose (3.1 g, 0.66 M) in 30 ml, of water/methanol (1:2, v/v) was refluxed at 65°C for seven h. (If the solution is cooled and filtered unreacted glycine ~1 g can be precipitated). The solvent was evaporated under vacuum at room temperature and the residue (4.65 g—before filteration of unreacted glycine) was dialyzed against distilled water (12 h), using the slide-a-lyzer cassettes (3.0–15 ml, capacity, nominal molecular weight cutoff = 10 000). After dialysis, the solvent was evaporated under vacuum at room temperature. The non-dialyzable fraction (0.3 g) (molecular weight > 10 000 Da) was further fractionated by gel filteration as described below (see Scheme 1). The dialyzable fraction (4.3 g) was analyzed by GC/MS using the same chromatographic conditions as Py/GC/MS.

Gel filteration

Gel filteration was carried out on the non-dialyzable fraction, using a column (diameter, 2.5 cm) packed with Bio-Gel P-10 (30 cm, height) and water as the eluent at a flow rate of 15 ml/h. Fractions (10 ml) were collected and analyzed by UV-VIS spectroscopy. Fractions exhibiting similar spectra were pooled and the solvent was evaporated. The purity of the separated polymers was verified by HPLC. The pure samples were analyzed by FTIR and Py/GC/MS and submitted for microanalysis. Fractions 1–10 contained only solvent, fractions 11–21 contained pure polymer A, fractions 22–35 contained mixed A, B1 and B2, fractions 36–50 contained B1 and B2. B1 and B2 were further separated by repeated gel filterations on Bio-Gel P-10 and analyzed by HPLC.

HPLC analysis

HPLC system used was a Beckman System Gold, consisting of a variable wavelength UV detector model 166 and a 110B solvent delivery module controlled by Beckman System Gold software. The column was Progel TSK G2500PWXL (0.78 cm × 30 cm from Supelco) and mobile phase consisted of 100% water. The flow rate was set at 1.0 mL/min. The wavelength was set at 290 nm.

Pyrolysis/GC/MS analysis

A Hewlett-Packard GC/mass selective detector (5890 GC/5971B MSD) interfaced to a CDS pyroprobe 2000 unit was used for the Py/GC/MS analysis. Samples

(1–4 mg) were introduced inside a quartz tube (0.3 mm thickness) and plugged with quartz wool and inserted inside the coil probe. The Pyroprobe was set at the desired temperature (350°C) at a heating rate of 50°C/ms and with a THT (total heating time) of 20 s. The pyroprobe interface temperature was set at 350°C. The GC column flow rate was 0.8 ml/min for a split ratio of 92:1 and a septum purge of three ml/min. 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 30–300 amu. The column was a fused silica DB-5 column (30 m length×0.25 mm i.d.×25 um film thickness; Supelco, Inc.). Unless otherwise specified, the column initial temperature was −5°C for 3 min and was increased to 50°C at a rate of 30°C/min; immediately the temperature was further increased to 270°C at a rate of 8°C/min and kept at 270°C for 5 min.

RESULTS AND DISCUSSION

To generate structurally simple polymers (PB in Scheme 1), an equimolar mixture of glycine/glucose was refluxed under mild conditions at 65°C in methanol/water (2:1, v/v) for seven hrs. After cooling for 48 hours unreacted glycine (~70%) crystallized out of the solution. After filteration and evaporation, the residue was dialyzed against distilled water. The non-dialyzable fraction was analyzed by HPLC using a gel column. The analysis indicated the presence of three polymeric materials with retention times of 2.7 (polymer A), 3.5 (polymer B1) and 5.9 minutes (polymer B2). The three polymers (10 000 < MW < 20 000) were separated by repeated gel filtration as detailed under experimental section.

Microanalysis data of the isolated polymers are shown in Table 1. The polymers were further analyzed by UV-VIS and FTIR spectroscopy and their Hunter tristimulus values were calculated (see Table 2). In addition, the dialyzable fraction and the purified polymers were subjected to pyrolysis/GC/MS analysis (Tables 3–5). Elemental analysis indicated that polymer A (eluted first from the Bio-Gel column) has the following empirical formula $C_7H_{11}N_1O_4$ and polymers B1 and B2 have the same empirical formula as D-glucose $C_1H_2O_1$ (see Table 1). This is consisted with the fact that almost 70% of the glycine was recovered after the reaction, which indicates that under the experimental conditions the amino acid was mainly involved to catalyze the conversion of the sugar into a reactive form which is able to polymerize. During Maillard reaction Amadori intermediate is converted into reactive sugar derivatives such as glucosone and deoxyglucosones and amino acid is released during the process. According to Scheme 3, the origin of nitrogen containing polymer A is assigned to Amadori intermediate or to some of its derivatives and the origin of polymers B1 and B2 is

Table 2. Spectroscopic data of polymers

Polymer	UV-VIS (nm)	IR (cm^{-1})	Color*
Polymer A	219, 326	2937 (w), 1607 (s), 1463 (s), 1384 (m), 1090 (w)	X = 0.1527 Y = 0.1347 Z = 0.6005
Polymer B1	466 (sh), 347 (sh), 295, 209	2936, 2890, 1620, 1458, 1410, 1321, 1152, 1086, 1038	X = 0.089 Y = 0.076 Z = 0.356
Polymer B2	293	2936, 2890, 1620, 1458, 1389, 1321, 1336, 1152, 1086, 1038	X = 0.0429 Y = 0.036 Z = 0.1562

w = Weak, s = strong, m = medium, sh = shoulder. *Hunter tristimulus values.

Table 3. Composition of dialyzable fraction by GC/MS

% Area	Compound
1.38	2,3-Butanedione
20.93	Acetic acid
1.64	2-Propanone, 1-hydroxy-
0.54	2-Butanone, 3-hydroxy-
0.76	1H-Pyrrole, 1-methyl-
0.47	3-Butene-1,2-diol
2.26	2-Furancarboxaldehyde
0.77	2-Furanmethanol
4.38	Cyclopent-2-ene, 1,4-dione
2.49	Ethanone, 1-(2-furanyl)-
1.21	4-Hydroxybut-2-enoic acid lactone
2.32	2-Furancarboxaldehyde, 5-methyl-
3.45	Pyrazine, trimethyl-
4.2	1,2,3-Propanetriol
0.49	2-Cyclopenten-1-one, 2-hydroxy-3-methyl
0.74	Ethanone, 1-(2-pyridinyl)-
1.06	3-Furanone, 2,3-dihydro-4-hydroxy
8.03	Ethanone, 1-(1H-pyrrol-2-yl)-
0.54	Ethanone, 1-(1-methyl-1H-pyrrol-2-yl)
21.63	**4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-2-methyl**
0.57	Dimethylpyrazinone
0.76	Trimethylpyrazinone
0.37	5-Hydroxy-1,3-dimethyl-2-[1H]quinoxalinone

Table 1. Microanalysis data[a] of the purified Maillard polymers (10 000 < MW < 20 000) on Bio-Gel P-10

Sample	% Carbon	% Hydrogen	% Nitrogen	% Oxygen	Empirical formula
Polymer A	46.10 ± 0.11	6.43 ± 0.00	8.08 ± 0.00	37.43	$C_7H_{11}N_1O_4$
Polymer B1	36.50 ± 0.02	7.50 ± 0.11	0.00	53.84	$C_1H_2O_1$
Polymer B2	38.22 ± 0.15	7.28 ± 0.09	0.00	51.90	$C_1H_2O_1$

[a]Average of dublicate measurements, detection limit 0.01%.

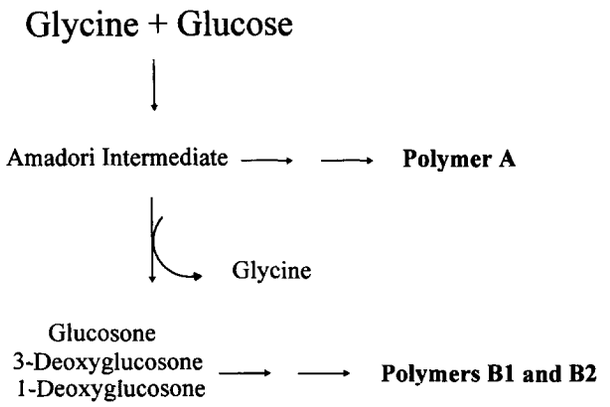
Table 4. Pyrolysis products of sucrose and polymers B1 and B2

% Area			Compound
B1	B2	Sucrose	
2.18	2.36	1.71	Formic acid
7.47	5.30	5.33	Acetic acid
0.00	1.82	0.11	2-Methylfuran
1.60	4.30	0.85	1-Hydroxy-2-propanone
7.65	10.21	39.50	2-Furancarboxaldehyde
0.48	0.73	0.78	2-Furanmethanol
0.36	0.40	0.35	2(3H)-Furanone-5-methyl
2.67	1.24	0.0	Cyclopent-2-en-1,4-dione
0.60	0.70	0.57	1-(2-Furanyl)-ethanone
0.00	1.49	0.45	1,3-Cyclopentanedione
6.91	2.35	0.33	5-Methyl-2-furancarboxaldehyde
0.00	0.13	0.0	2,2′-Bifuran
0.00	0.25	0.0	2-Hydroxy-3-methyl 2-cyclo-penten-1-one
2.46	1.46	0.0	2-Furancarboxylic acid
0.00	0.84	1.43	3-Furancarboxylic acid, methyl ester
0.00	0.26	0.0	2H-Pyran-2-one
0.02	0.06	0.13	3-Hydroxy-2-methyl-4H-pyran-4-one (maltol)
8.64	4.32	2.07	2,3-Dihydro-3,5-dihydroxy-2-methyl-4H-pyran-4-one,
1.48	0.86	0.0	3,5-Dihydroxy-2-methyl-4H-pyran-4-one
34.5	35.59	33.57	5-(hydroxymethyl)-2-furancarboxaldehyde
0.07	0.00	0.0	[2,2′-Bifuran]-3-carboxylic acid
0.48	0.00	0.30	5-[(5-Methyl-2-furanylmethyl)1-2-furancarboxaldehyde

assigned to glucosone and/or to 3- or 1-deoxyglucosones; common non-nitrogen containing reactive intermediates during Maillard reaction. GC/MS analysis of the dialyzable ftaction (see Table 3) indicated the formation of Amadori product during the reaction since Amadori specific products such as 5-hydroxy-1,3-dimethyl-2[1H]quinoxalinone was identified in this fraction (Keyhani and Yaylayan, 1997).

Table 5. Pyrolysis products of polymer A

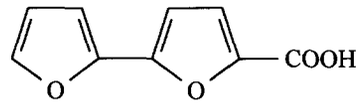
% Area	Compound
3.52	Acetic acid, methyl ester
25.23	Acetic acid
6.04	1-Hydroxy-2-propanone
2.77	1-Methyl-1H-pyrrole
2.69	2,3-Butanediol
1.96	Pyridine
0.43	4-Methyl-pyridine
3.43	Methy-pyrazine
2.02	2-Furancarboxaldehyde
0.96	1H-Pyrrole-2,4-dimethyl
2.87	Cyclopent-2-en-1,4-dione
0.76	1-(2-Furanyl)-ethanone
3.88	2,6-Dimethyl-pyrazine
0.88	2,3-Dimethyl-pyrazine
1.72	5-Methyl-2-furancarboxaldehyde
2.16	Trimethyl-pyrazine
0.67	1-(1H-pyrrol-2-yl)-ethanone
1.53	3-Hydroxy-2-methyl-4H-pyran-4-one (maltol)
1.89	5-Methyl-1H-pyrrole-2-carboxaldehyde
2.43	2,3-Dihydro-3,5-dihydroxy-2-methyl-4H-pyran-4-one



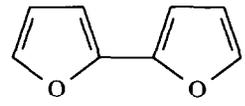
Scheme 3. The origin of nitrogenous (A) and non-nitrogenous (B1 and B2) polymers.

Proposed structure of non-nitrogen containing polymers

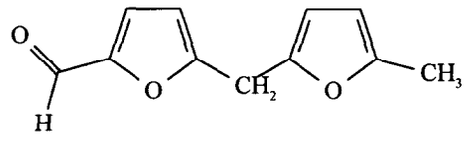
The elemental composition of the non-nitrogenous polymers B1 and B2, indicates the presence of high oxygen content and a carbon, oxygen, hydrogen ratio similar to glucose as indicated in Table 1. FTIR data (Table 2) confirms the presence of CH_3, CH_2, C–O–H, C–O–C and COO^- moieties. Pyrolysis of the both polymers predominantly produces furanoid species similar to sucrose (Table 4 and Scheme 4) indicating the presence of glycosidically linked sugar derivatives. Polymers, with elemental composition similar to glucose, could be envisaged to be formed, as indicated in Scheme 3, from the known deoxyglucosones or glucosone intermediates formed during the Maillard reaction. As depicted in Scheme 5, the monomeric units could be generated from furanose forms of glucosone or 3-deoxyglucosone (structure I in Scheme 5) which after oxidation can form carboxylic acid derivatives IIa and IIb. On the other hand, 1-deoxyglucosone can exist in furanose



2,2'-bifuran-5-carboxylic acid

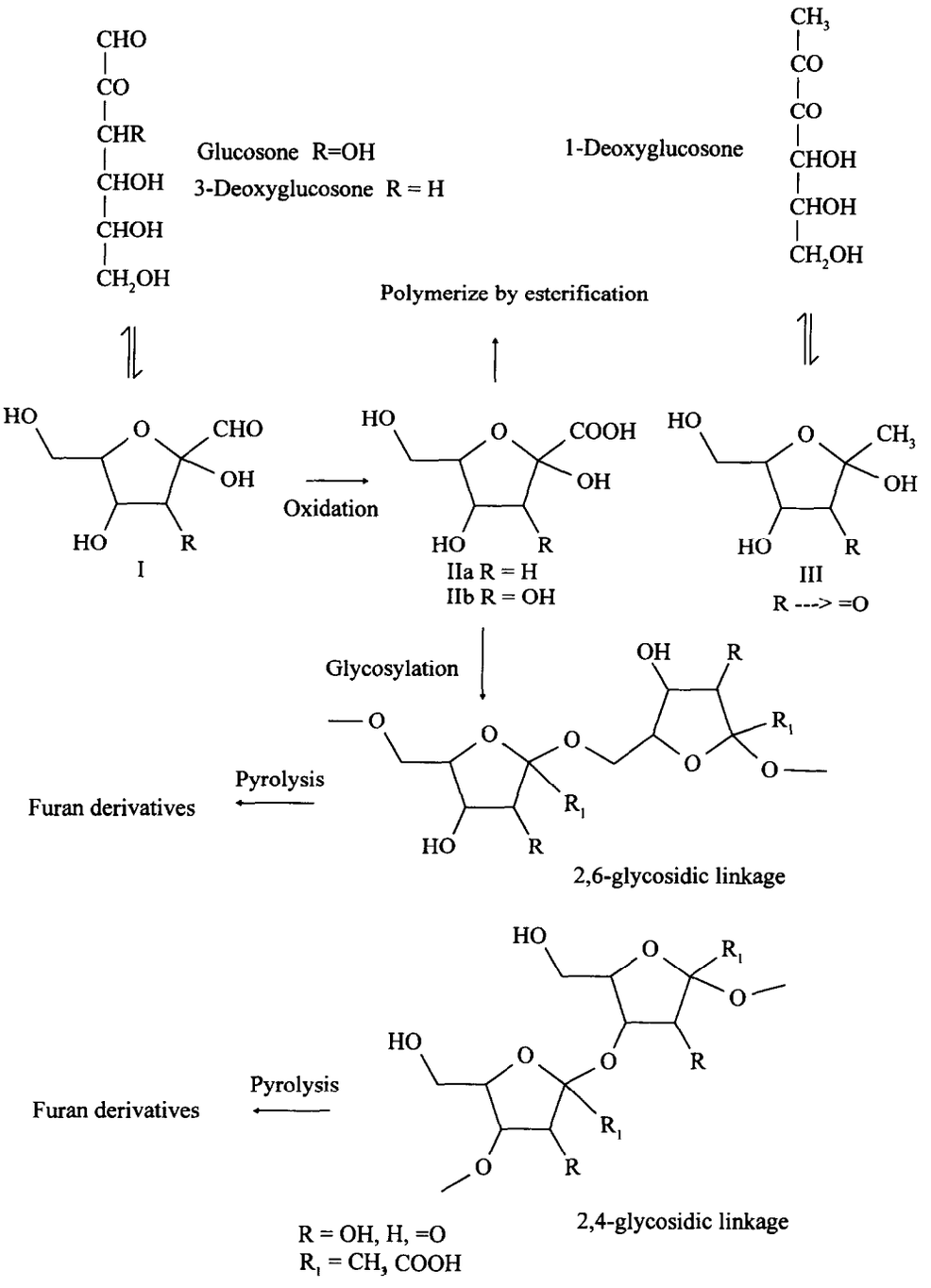


2,2'-bifuran



2-furancarboxaldehyde-5-[(5'-methyl-2'-furanylmethyl)]

Scheme 4. Selected bi-furan derivatives identified by Py/GC/MS of non-nitrogenous polymers.



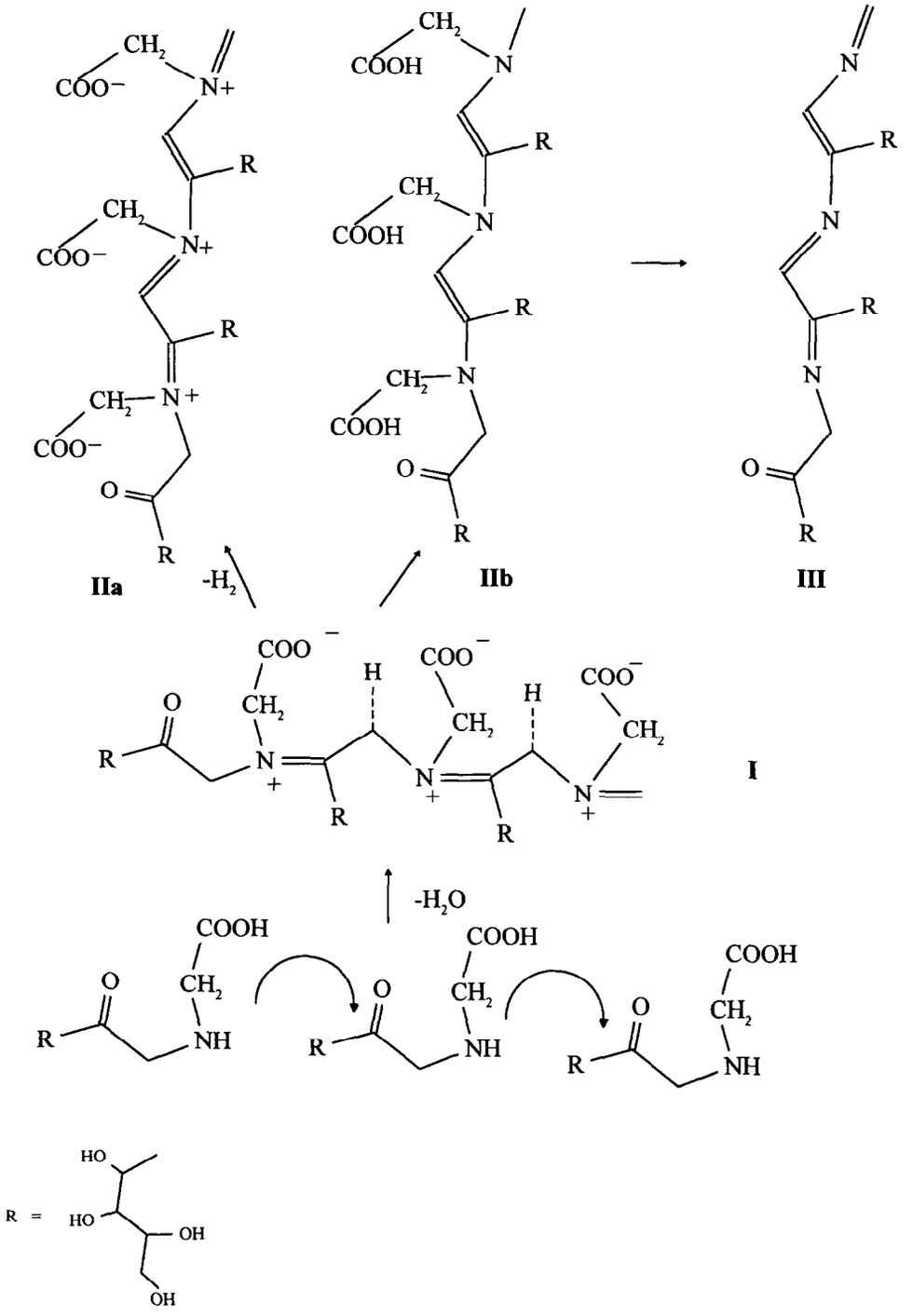
Scheme 5. Proposed structure and mechanism of formation of non-nitrogenous polymers (B1 and B2).

form III. Polymerization of IIa, IIb and III can generate structures that are consistent with the experimental data. Polymerization could be effected by 2,6-, 2,3- and 2,4-glycosylation reactions, the commonly observed polymerization process in nature. Any permutations of IIa-IIb-III could be glycosidically linked to generate the B1 and B2 polymers.

Proposed structure of nitrogen-containing polymer

Elemental analysis of the polymer A (Table 1) indicates that the empirical formula ($C_7H_{11}N_1O_4$) differs from the molecular formula of the hypothetical glycine Amadori monomer ($C_8H_{15}N_1O_7$) by a CH_4O_3 unit. This implies certain oligomeric units that make up the polymer have lost molecules of CO_2, H_2O and H_2 at certain positions. Loss of water is a mandatory process for polymerization to occur. FTIR analysis confirms the presence of CH_2, C-O-H and COO^- and/or conjugated moieties. Due to the dark brown color of this polymer (Table 1), the strong absorption band at 1607 cm^{-1} in the FTIR spectrum is mainly attributed to extensive conjugation and partially to the presence of carboxylate moiety. Pyrolysis of polymer A (Table 5) generates components

typical of Amadori products such as pyrazines, pyrroles, pyridines, furans, etc. In addition, the presence of 2,3-dihydro-3,5-dihydroxy-2-methyl-4H-pyran-4-one, in the pyrolysis products of polymer A, strongly suggests that the certain polymeric units are formed by minimal deydrations at the sugar moiety. Based on the experimental observations, a mechanism is proposed for the formation of polymer A as depicted in Scheme 6. According to this scheme, Amadori products can polymerize through nucleophilic addition reactions of amino groups to the carbonyl moieties of a second molecule, followed by dehydration to form the zwitterionic polymer I. The polymer I can either lose a hydrogen molecule to form conjugated zwitterionic polymer IIa or undergo an intramolecular hydrogen transfer to form a neutral derivative IIb which can be converted into the conjugated derivative III, through the loss of a succinic acid moiety as depicted in Scheme 6. The structure of the isolated polymer could incorporate, in different percentages, the above mentioned polymeric moieties I, IIa,



Scheme 6. Proposed structure and mechanism of formation of nitrogenous polymer (A).

Table 6. Comparison of microanalysis data of polymer A with those reported in literature[a]

Glycine/Glucose	%Carbon	%Hydrogen	%Nitrogen	%Oxygen	a	y/a[b]	Reference
Water, 100°C, 10 h, 1:1, pH 7	49.05	5.25	6.12	39.57	1.20	3.10	Cämmerer and Kroh, 1994
Solvent free, 170°C, 20 min, 1:1 pH not controlled	53.42	5.38	4.26	37.61	2.19	3.05	Cämmerer and Kroh, 1994
Water, 60°C, 160 h, 1:1, pH 5	43.02	4.78	6.94	45.25	0.75	2.89	Cämmerer and Kroh, 1994
Water, 100°C, 8 h, 1:1, pH 3·5	51.2	5.28	6.24	37.28			Feather and Nelson, 1984
Water, 90°C, 22 h, 1:1 pH not controlled[c]					1.06	2.79	Wedzicha and Kaputo, 1992
H_2O/CH_3OH, 65°C 7 h, 1:1 pH not controlled	46.10	6.43	8.08	37.43	0.91	2.79	Present study

[a]a number of moles of sugar incorporated into the polymer per mole of amino acid.
[b]y/a number of moles of water liberated per mole of sugar (Cämmerer and Kroh, 1994).
[c]Empirical formula $C_8H_{12}NO_5$.

IIb and III. The calculated empirical formula reflects an average of empirical formulas of different moieties. The proposed structure is consistent with the general structure suggested by Cämmerer and Kroh (1994). Table 6 compares the microanalysts data of polymer A to that of other polymers generated from glycine/D-glucose mixtures, reported in literature.

ACKNOWLEDGEMENT

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REFERENCES

Cämmerer, B. and Kroh, L. W. (1994) Investigation of the influence of reaction conditions on the elementary composition of melanoidins. *Food Chem.* **23**, 55–59.

Feather, M. S. and Nelson, D. (1985) Maillard polymers derived from D-glucose, D-fructose, 5-(hydroxymethyl)-2-furaldehyde, and glycine and methionine. *J. Agric. Food Chem.* **32**, 1428–1432.

Keyhani, A. and Yaylayan, V. (1997) Glycine specific novel Maillard reaction products: 5-hydroxy-1,3-dimethyl-2-[1H] quinoxalinone and related compounds. *J. Agric. Food Chem.* **45**, 697–702.

Wedzicha, B. L. and Kaputo, M. T. (1992) Melanoidins from glucose and glycine: Composition, characteristics and reactivity towards sulphite ion. *Food Chem.* **43**, 359–367.