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Impact of the N-Terminal Amino Acid on the Formation of Pyrazines from Peptides in Maillard Model Systems

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ABSTRACT: Only a minor part of Maillard reaction studies in the literature focused on the reaction between carbohydrates and peptides. Therefore, in continuation of a previous study in which the influence of the peptide *C*-terminal amino acid was investigated, this study focused on the influence of the peptide *N*-terminal amino acid on the production of pyrazines in model reactions of glucose, methylglyoxal, or glyoxal. Nine different dipeptides and three tripeptides were selected. It was shown that the structure of the *N*-terminal amino acid is determinative for the overall pyrazine production. Especially, the production of 2,5(6)-dimethylpyrazine and trimethylpyrazine was low in the case of proline, valine, or leucine at the *N*-terminus, whereas it was very high for glycine, alanine, or serine. In contrast to the alkyl-substituted pyrazines, unsubstituted pyrazine was always produced more in the case of experiments with free amino acids. It is clear that different mechanisms must be responsible for this observation. This study clearly illustrates the capability of peptides to produce flavor compounds such as pyrazines.

KEYWORDS: *peptides, Maillard, pyrazines, flavor, model reactions, SBSE*

INTRODUCTION

The most well-known reaction between amino acids, peptides, or proteins and carbohydrates comprises the condensation reaction between a free amino group of an amino acid, peptide, or protein and the carbonyl group of a reducing carbohydrate. This initial attack triggers a series of complex chemical reactions, known as the Maillard reaction or nonenzymatic browning reaction. The Maillard reaction strongly affects food quality, as it gives rise to modifications in color, taste, aroma, biological activity, and nutritional value.[1] Up to now, only a limited number of studies have investigated the role of peptides and proteins in the Maillard reaction. However, peptides and proteins are widespread in nature and occur naturally in traditional foods.[2,3] In addition, peptides are also added to food products to obtain the required properties because they influence the functional properties, affect the product taste, and exhibit biological activity.[4] A review presenting an overview of the chemical reactions of peptides in food systems, including the Maillard reaction, was published recently.[5] To extend the current knowledge on the reactivity of free amino acids, this study was undertaken to investigate the formation of flavor compounds from di- and tripeptides in the Maillard reaction.

With regard to flavor formation from peptides in Maillard model systems, mainly glutathione[6−9] or glycine-derived peptides such as diglycine, triglycine, and tetraglycine,[10,11] have been studied. These glycine-derived peptides are mostly used to represent di-, tri-, and tetrapeptides. However, peptides composed of other amino acids can exert different reactivities and produce different flavor compounds.

In our previous study, flavor formation from lysine-containing dipeptides was studied.[12] In that study, eight different dipeptides with lysine at the *N*-terminus (Lys-X) were reacted with glucose, methylglyoxal, and glyoxal at 130 °C for 2 h. The *C*-terminal amino acid was varied to study the influence of the neighboring amino acid on flavor production by the lysine residue because, theoretically, only the two amino groups of lysine are able to react. Pyrazines were the most important volatiles detected. Generally, the pyrazines were produced more in the case of dipeptides as compared to free amino acids. For reactions with glucose and methylglyoxal, this difference was mainly caused by the large amounts of 2,5(6)-dimethylpyrazine and trimethylpyrazine produced from the reactions with dipeptides. In contrast, unsubstituted pyrazine was produced more in the case of free amino acids. In addition, the production of amino acid specific pyrazines, resulting from the reaction between the dihydropyrazine intermediate and a Strecker aldehyde, was limited in the case of dipeptides, suggesting only minimal hydrolysis of the peptide bond. No clear influence of the neighboring amino acid on the reactivity of lysine could be distinguished.

In continuation of our previous study investigating the influence of the *C*-terminal amino acid, this study focuses on the influence of the *N*-terminal amino acid. For this purpose, three different dipeptides with lysine and six different dipeptides with glycine at the *C*-terminus were reacted with glucose, methylglyoxal, and glyoxal in this study. In addition, flavor formation from three tripeptides in Maillard model systems was studied. The flavor compounds produced by these reactions were compared with those obtained from the mixture of the corresponding free amino acids.

MATERIALS AND METHODS

Chemicals. Leucine (99%), valine (99%), and serine (99%) were purchased from Janssen Chimica (Geel, Belgium). Alanine (99%), lysine monohydrate (99%), and proline (99%) were purchased from Acros Organics (Geel, Belgium). Glycine (99%), Gly-Gly-Gly (99%), glucose (99.5%), glyoxal (40% in H_2O), and methylglyoxal (40% in

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Table 1. Pyrazines (GC-MS Peak Area × 10^8) Detected in the Model Reactions of Glucose with X-Lys Dipeptides and with the Corresponding Free Amino Acids (2 h, 130 °C)

LRI exptl[a]	LRI lit.[b]	compound	Gly + Lys	GlyLys	Ala + Lys	AlaLys	Val + Lys	ValLys	theor recovery (%)
759	760[c]	pyrazine	1.46	0.01	1.70	0.01	0.80	0.02	0.3
822	819[d]	methylpyrazine	1.30	0.03	1.00	0.06	0.32	0.01	0.8
908	908/909[d]	2,5(6)-dimethylpyrazine	1.53	4.79	0.92	4.94	0.41	0.07	2.0/1.6
911	912[d]	2-ethylpyrazine	0.01	–[h]	0.09	–	–	–	2.3
913	915[d]	2,3-dimethylpyrazine	0.19	0.32	0.14	0.07	0.07	0.01	1.6
995	997[c]	2-ethyl-6-methylpyrazine	–	0.01	0.04	0.01	–	–	
998	1000[c]	2-ethyl-5-methylpyrazine	0.06	3.03	0.17	0.99	0.10	–	
998	1000[d]	trimethylpyrazine	0.58	0.51	0.16	0.21	0.04	–	4.1
1015	1020[e]	2-ethenyl-6-methylpyrazine	0.01	0.01	–	0.01	–	–	
1018	1025[e]	2-ethenyl-5-methylpyrazine	0.01	0.36	0.01	0.30	0.01	–	
1064		2-(2-methylpropyl)pyrazine[g]	–	–	–	–	0.12	–	
1072	1078[f]	3-ethyl-2,5-dimethylpyrazine	0.09	0.13	1.73	0.25	0.04	–	
1080	1083[f]	2-ethyl-3,5-dimethylpyrazine	0.01	0.10	0.01	0.01	–	–	
1082	1083[d]	tetramethylpyrazine	0.02	–	–	–	–	–	8.4
1082	1084[f]	5-ethyl-2,3-dimethylpyrazine	0.02	0.41	0.02	0.02	0.01	–	
1087	1088[f]	2,5-diethylpyrazine	–	0.13	–	0.01	–	–	
1093	1095[f]	3-ethenyl-2,5-dimethylpyrazine	0.02	0.42	0.03	0.45	0.01	–	
1142	1139[f]	methyl-(2-methylpropyl)pyrazine	–	–	–	–	0.01	–	
1165	1155[f]	2,3-diethyl-5-methylpyrazine	–	0.01	0.06	–	–	–	30.0
1167	1157[f]	3,5-diethyl-2-methylpyrazine	trace	0.02	0.12	0.01	0.01	–	
1169	1159[f]	2,3,5-trimethyl-6-ethylpyrazine	0.03	0.02	0.27	–	–	–	
1178		acetylethylpyrazine[g]	–	0.01	–	–	–	–	
1197	1202[f]	2,5-dimethyl-3-(2-methylpropyl)pyrazine	–	–	–	–	0.85	–	
1255		2-(2′-furyl)pyrazine[g]	0.05	–	0.06	–	0.02	–	
1276		(2-methylpropyl)trimethylpyrazine[g]	–	–	–	–	0.12	–	
1382		1,4-dimethylpyrrole-(1,2a)-pyrazine[g]	0.10	–	0.18	–	–	–	
		total pyrazines	5.48	10.33	6.71	7.36	2.96	0.11	
		pyrazines (% of total GC-MS peak area)	40.1	86.7	26.3	79.8	18.1	3.2	

[a]LRI exptl = linear retention index determined experimentally on an HP5-MS stationary phase. [b]LRI lit. = linear retention index value from the literature. [c]Adams and De Kimpe.[18] [d]Adams.[19] [e]Van Loon et al.[20] [f]Wagner et al.[21] [g]Tentatively identified. [h]Not detected.

H_2O) were purchased from Sigma-Aldrich (Bornem, Belgium). The peptides Gly-Lys hydrochloride (98%), Ala-Lys hydrochloride (97%), Val-Lys hydrochloride (98%), Ala-Gly (99%), Val-Gly (99%), Leu-Gly (98%), Ser-Gly (99%), Pro-Gly (98%), Lys-Gly-Gly hydrochloride (97%), and Lys-Ala-Pro hydrochloride (96%) were purchased from Bachem (Bubendorf, Switzerland). The peptide Gly-Gly (99%) was purchased from Fluka (Bornem, Belgium).

Model Reactions. The model systems were prepared as in our previous paper on the formation of volatiles from lysine-containing dipeptides in Maillard model systems.[12]

Analysis of Flavor Compounds. The analysis of flavor compounds was performed as in our previous study on this topic.[12] Briefly, the reaction mixtures were extracted by means of Stir Bar Sorptive Extraction (SBSE) for 30 min at 35 °C and, afterward, the analytes were desorbed in a Gerstel Thermo Desorption System (TDS2). GC-MS analyses of the SBSE extracts were performed with an Agilent 6890 GC Plus coupled to a quadrupole mass spectrometer 5973 MSD (Agilent Technologies, Diegem, Belgium) and equipped with an HP5-MS capillary column (30 m length × 0.25 mm i.d.; 0.25 μm film thickness). Working conditions were as described in our previous study.[12]

In the case of the experiment that studied the influence of temperature on the volatiles produced in model systems containing glucose and glycine or diglycine, the volatiles were extracted by means of Solid-Phase Microextraction (PDMS/Car/DVB fiber) for 30 min at 35 °C. The extracted flavors were desorbed for 2 min at 250 °C. Analyses of the SPME extracts were performed as described above.

RESULTS

In line with our previous study in which flavor formation from dipeptides with lysine at the *N*-terminus was studied in Maillard model systems, nine dipeptides with various amino acids at the *N*-terminus, namely, glycine, alanine, serine, valine, leucine, and proline, were reacted with glucose, methylglyoxal, and glyoxal in this study. Lysine (X-Lys) or glycine (X-Gly) was always chosen as the *C*-terminal amino acid to limit the influence of the *C*-terminal amino acid on the reactivity of the *N*-terminal amino acid. In addition, flavor formation from three tripeptides (Gly-Gly-Gly, Lys-Gly-Gly, and Lys-Ala-Pro) in Maillard model systems was investigated. Model reactions and flavor analysis were conducted as in our previous study. Briefly, the investigated peptide or the mixture of the corresponding free amino acids was reacted with glucose, methylglyoxal, or glyoxal in unbuffered aqueous conditions at pH 8 at 130 °C for 2 h. It was decided to perform the experiments without buffer, because it has been shown that the anionic species of the buffer can exert a severe catalytic effect as has been extensively pointed out for the phosphate ion.[13,14] Afterward, the volatiles produced were sampled by means of SBSE-GC-MS. In our previous study it was shown that pyrazines were the most important volatiles detected in the case of Lys-X dipeptides. Therefore, the production of pyrazines was also the main focus in this study.

As a logical extension of our previous study in which dipeptides with lysine at the *N*-terminus were studied, this

Table 2. Pyrazines (GC-MS Peak Area × 10^8) Detected in the Model Reactions of Methylglyoxal with X-Lys Dipeptides and with the Corresponding Free Amino Acids (2 h, 130 °C)

LRI exptl[a]	LRI lit.[b]	compound	Gly + Lys	GlyLys	Ala + Lys	AlaLys	Val + Lys	ValLys	theor recovery (%)
822	819[c]	methylpyrazine	0.03	–[g]	0.05	0.04	0.03	–	0.8
908	908/909[c]	2,5(6)-dimethylpyrazine	2.21	8.31	3.32	16.81	2.79	3.86	2.0/1.6
913	915[c]	2,3-dimethylpyrazine	0.75	trace	0.07	0.33	0.04	trace	1.6
998	1000[d]	2-ethyl-5-methylpyrazine	0.03	0.13	0.01	0.21	0.01	0.03	
998	1000[c]	trimethylpyrazine	3.40	5.19	0.66	2.90	0.51	0.18	4.1
1072	1078[e]	3-ethyl-2,5-dimethylpyrazine	0.14	0.29	3.34	2.01	0.19	0.09	
1080	1083[e]	2-ethyl-3,5-dimethylpyrazine	0.03	0.07	–	–	0.05	–	
1082	1083[c]	tetramethylpyrazine	0.10	0.23	–	–	–	–	8.4
1082	1084[e]	5-ethyl-2,3-dimethylpyrazine	0.28	0.05	trace	0.03	–	–	
1093	1095[e]	3-ethenyl-2,5-dimethylpyrazine	–	–	–	0.05	–	–	
1123	1129[d]	2-acetyl-5-methylpyrazine	0.02	0.10	0.03	0.07	0.04	–	
1133	1134[d]	2-acetyl-6-methylpyrazine	0.03	trace	0.04	0.07	0.02	–	
1165	1155[e]	2,3-diethyl-5-methylpyrazine	–	trace	0.02	trace	–	–	30
1167	1157[e]	3,5-diethyl-2-methylpyrazine	–	–	0.02	trace	–	–	
1169	1159[e]	2,5-dimethyl-3-propylpyrazine	–	–	–	–	0.08	–	
1169	1159[e]	2,3,5-trimethyl-6-ethylpyrazine	0.02	–	0.08	–	–	–	
1180		acetyldimethylpyrazine[f]	–	0.01	0.01	0.08	0.01	–	
1211	1218[e]	3,5-dimethyl-2-(2-methylpropyl)pyrazine	–	–	–	–	1.46	0.01	
1219		acetyldimethylpyrazine[f]	0.05	0.02	0.04	0.14	0.01	–	
1229	1235[e]	2,3-dimethyl-5-(2-methylpropyl)pyrazine	–	0.06	0.02	0.21	0.02	–	
1241	1250[e]	2,5-dimethyl-3-(*E*-1-propenyl)pyrazine	–	0.08	–	1.13	–	–	
1276		trimethyl-(2-methylpropyl)pyrazine[f]	–	–	–	–	0.03	–	
		total pyrazines	7.13	14.53	7.75	24.09	5.31	4.17	
		pyrazines (% of total GC-MS peak area)	62.5	58.9	53.8	53.1	25.9	45.3	

[a]LRI exptl = linear retention index determined experimentally on an HP5-MS stationary phase. [b]LRI lit. = linear retention index value from the literature. [c]Adams.[19] [d]Adams and De Kimpe.[18] [e]Wagner et al.[21] [f]Tentatively identified. [g]Not detected.

Table 3. Pyrazines (GC-MS Peak Area × 10^8) Detected in the Model Reactions of Glyoxal with X-Lys Dipeptides and with the Corresponding Free Amino Acids (2 h, 130 °C)

LRI exptl[a]	LRI lit.[b]	compound	Gly + Lys	GlyLys	Ala + Lys	AlaLys	Val + Lys	ValLys	theor recovery (%)
759	760[c]	pyrazine	3.64	1.50	3.58	2.63	3.59	0.36	0.3
822	819[d]	methylpyrazine	0.06	0.04	0.04	0.09	0.11	–[f]	0.8
911	912[d]	2-ethylpyrazine	–	–	0.30	–	–	–	2.3
1064		2-(2-methylpropyl)pyrazine[e]	–	–	–	–	0.37	–	
		total pyrazines	3.70	1.54	3.92	2.71	4.07	0.36	
		pyrazines (% of total GC-MS peak area)	75.7	93.9	66.6	89.1	26.6	54.4	

[a]LRI exptl = linear retention index determined experimentally on an HP5-MS stationary phase. [b]LRI lit. = linear retention index value from the literature. [c]Adams and De Kimpe.[18] [d]Adams.[19] [e]Tentatively identified. [f] Not detected.

study was started by reacting three dipeptides with lysine at the *C*-terminus. The pyrazines produced during the reaction of the X-Lys dipeptides or the corresponding free amino acids with glucose are depicted in Table 1. The results obtained from the reactions with GlyLys and AlaLys were very similar to those obtained in our previous study in which Lys-X dipeptides were reacted. More specifically, it can be seen that pyrazines comprised a bigger portion of the total volatiles in the case of these dipeptides as compared to the corresponding free amino acids. This means that the diversity of volatile reaction products was lower in the case of these dipeptides. In addition, reactions with GlyLys and AlaLys produced higher and similar amounts of pyrazines, respectively. As for the Lys-X dipeptides, especially 2,5(6)-dimethylpyrazine was produced much more in the case of these dipeptides as compared to the corresponding free amino acids. In contrast, these results were not obtained from the reaction with ValLys. Very low amounts of pyrazines were found for this dipeptide. However, some observations, which were also found in the case of Lys-X dipeptides, were valid for all X-Lys dipeptides. For instance, unsubstituted pyrazine was produced more in the case of free amino acids as compared to dipeptides. In addition, the reactions with free amino acids produced much higher amounts of amino acid specific pyrazines, such as 3-ethyl-2,5-dimethylpyrazine and 6-ethyl-2,3,5-trimethylpyrazine from alanine and 2,5-dimethyl-3-(2-methylpropyl)pyrazine from valine.

Besides with glucose, model reactions were also performed with methylglyoxal and glyoxal, two common α-dicarbonyl compounds resulting from glucose degradation. In these cases, a 10-fold lower concentration of the dicarbonyl compound was used to avoid too many self-condensation reactions. Similar results were obtained for the model systems containing methylglyoxal and glyoxal.

Table 4. Pyrazines (GC-MS Peak Area × 10^8) Detected in the Model Reactions of Glucose with X-Gly Dipeptides and with the Corresponding Free Amino Acids (2 h, 130 °C)

LRI exptl[a]	LRI lit.[b]	compound	Gly (2 mmol)	GlyGly	Ala + Gly	AlaGly	Val + Gly	ValGly	Leu + Gly	LeuGly	Ser + Gly	SerGly	Pro + Gly	ProGly	theor recovery (%)
759	760[c]	pyrazine	0.02	–[h]	0.05	–	0.01	–	0.02	–	0.08	–	0.03	–	0.3
822	819[d]	methylpyrazine	0.02	0.02	0.05	0.03	0.02	–	0.04	0.03	0.09	0.09	0.06	–	0.8
908	908/909[d]	2,5(6)-dimethylpyrazine	0.11	2.38	0.57	3.29	0.16	–	0.51	0.57	0.18	7.19	0.03	–	2.0/1.6
913	915[d]	2,3-dimethylpyrazine	0.04	0.20	0.04	0.06	0.05	–	0.18	0.13	0.09	0.38	–	–	1.6
926	927[e]	ethenylpyrazine	0.02	–	0.02	0.01	–	–	0.02	trace	0.01	–	0.02	–	
995	997[c]	2-ethyl-6-methylpyrazine	trace	0.01	0.03	0.01	trace	–	0.01	trace	trace	0.01	–	–	
998	1000[d]	trimethylpyrazine	0.19	0.85	0.29	0.15	0.14	–	0.27	–	0.21	1.06	0.03	–	4.1
998	1000[c]	2-ethyl-5-methylpyrazine	0.02	1.68	0.13	1.63	0.05	–	0.17	0.23	0.03	2.55	–	–	
1015	1020[f]	2-ethenyl-6-methylpyrazine	–	–	–	0.01	–	–	trace	–	–	0.03	–	–	
1018	1017[d]	2-acetylpyrazine	–	–	–	–	–	–	–	–	0.02	–	–	–	0.8
1018	1025[f]	2-ethenyl-5-methylpyrazine	0.01	0.24	0.01	0.71	–	–	0.04	0.16	0.03	0.76	–	–	
1072	1078[e]	3-ethyl-2,5-dimethylpyrazine	trace	0.04	0.93	0.05	0.02	–	0.01	–	0.02	0.04	–	–	
1080	1083[e]	2-ethyl-3,5-dimethylpyrazine	–	0.08	0.02	0.03	trace	–	0.02	–	0.01	0.12	–	–	
1082	1083[d]	tetramethylpyrazine	0.12	–	0.04	–	0.01	–	0.04	–	0.03	–	0.01	–	8.4
1082	1084[e]	5-ethyl-2,3-dimethylpyrazine	0.01	0.24	0.06	0.05	0.03	–	0.05	0.01	0.03	0.24	–	–	
1087	1088[e]	2,5-diethylpyrazine	–	0.23	0.01	0.10	–	–	trace	0.01	–	0.08	–	–	
1093	1095[e]	3-ethenyl-2,5-dimethylpyrazine	trace	0.43	0.03	0.40	–	–	0.01	0.05	0.01	1.79	–	–	
1115	1109[e]	5-ethenyl-2,3-dimethylpyrazine	–	0.19	–	0.17	–	–	–	0.06	–	0.15	–	–	
1123	1129[c]	2-acetyl-5-methylpyrazine	–	–	–	–	–	–	–	–	0.01	0.03	–	–	
1165	1155[e]	2,3-diethyl-5-methylpyrazine	–	trace	0.04	trace	–	–	0.01	–	–	–	–	–	30.0
1167	1157[e]	3,5-diethyl-2-methylpyrazine	–	0.01	0.10	–	–	–	0.01	–	–	–	–	–	
1169	1159[e]	2,3,5-trimethyl-6-ethylpyrazine	0.02	–	0.31	–	0.01	–	–	–	0.01	–	–	–	
1184		2-(3-methylbutyl)pyrazine[g]	–	–	–	–	–	–	0.17	–	–	–	–	–	
1197	1202[e]	3-isobutyl-2,5-dimethylpyrazine	–	–	–	–	0.72	–	–	–	–	–	–	–	
1250		methyl-(3-methylbutyl)pyrazine[g]	–	–	–	–	–	–	0.08	–	–	–	–	–	
1253		methyl-(3-methylbutyl)pyrazine[g]	–	–	–	–	–	–	0.28	–	–	–	–	–	
1265		methyl-(3-methylbutyl)pyrazine[g]	–	–	–	–	–	–	0.22	trace	–	–	–	–	
1276		trimethyl-(2-methylpropyl)pyrazine[g]	–	–	–	–	0.26	–	–	–	–	–	–	–	
1315		dimethyl-(3-methylbutyl)pyrazine[g]	–	–	–	–	–	–	4.40	0.02	–	–	–	–	
1327		dimethyl-(3-methylbutyl)pyrazine[g]	–	–	–	–	–	–	0.03	–	–	–	–	–	
1337		dimethyl-(3-methylbutyl)pyrazine[g]	–	–	–	–	–	–	0.05	–	–	–	–	–	
1383		trimethyl-(2-methylbutyl)pyrazine[g]	–	–	–	–	–	–	0.70	–	–	–	–	–	
1387		trimethyl-(3-methylbutyl)pyrazine[g]	–	–	–	–	–	–	1.49	–	–	–	–	–	
		total pyrazines	0.57	6.59	2.72	6.70	1.48	0.00	8.83	1.27	0.84	14.52	0.18	0.00	
		pyrazines (% of total GC-MS peak area)	60.7	87.2	50.5	84.1	12.0	0.0	16.4	13.7	54.5	90.3	1.1	0.0	

[a]LRI exptl = linear retention index determined experimentally on an HP5-MS stationary phase. [b]LRI lit. = linear retention index value from the literature. [c]Adams and De Kimpe.[18] [d]Adams.[19] [e]Wagner et al.[21] [f]Van Loon et al.[20] [g]Tentatively identified. [h]Not detected.

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Table 5. Pyrazines (GC-MS Peak Area × 10^8) Detected in the Model Reactions of Methylglyoxal with X-Gly Dipeptides and with the Corresponding Free Amino Acids (2 h, 130 °C)

LRI exptl[a]	LRI lit.[b]	compound	Gly (2 mmol)	GlyGly	Ala + Gly	AlaGly	Val + Gly	ValGly	Leu + Gly	LeuGly	Ser + Gly	SerGly	Pro + Gly	ProGly	theor recovery (%)
759	760[c]	pyrazine	–[g]	–	–	–	–	–	–	–	0.06	–	–	–	0.3
822	819[d]	methylpyrazine	–	0.02	0.01	0.01	–	–	–	–	0.09	0.02	–	–	0.8
908	908/909[d]	2,5(6)-dimethylpyrazine	0.67	22.72	1.03	20.91	1.03	1.98	4.66	37.09	1.30	35.30	0.90	–	2.0/1.6
913	915[d]	2,3-dimethylpyrazine	0.05	–	0.05	–	–	–	0.08	–	0.06	–	–	–	1.6
998	1000[d]	trimethylpyrazine	2.94	23.01	4.12	6.25	5.20	0.23	15.13	7.13	2.83	51.02	4.72	–	4.1
1050	1049[e]	methyl-(1-methylethyl)pyrazine	–	–	–	–	–	–	0.19	–	–	–	–	–	
1072	1078[e]	3-ethyl-2,5-dimethylpyrazine	0.01	0.43	0.34	0.34	0.03	–	0.54	0.50	0.15	0.17	0.03	–	
1080	1083[e]	2-ethyl-3,5-dimethylpyrazine	0.09	0.23	0.09	0.04	0.14	–	0.38	0.05	0.09	0.45	0.09	–	
1082	1083[d]	tetramethylpyrazine	0.38	0.66	0.10	–	0.17	–	1.29	0.01	0.33	0.43	0.39	–	8.4
1082	1084[e]	5-ethyl-2,3-dimethylpyrazine	0.10	–	0.37	0.06	0.72	–	0.22	0.08	–	0.66	–	–	
1093	1095[e]	3-ethenyl-2,5-dimethylpyrazine	0.03	0.04	0.01	0.02	–	–	–	0.07	0.08	0.05	0.02	–	
1123	1129[c]	2-acetyl-5-methylpyrazine	0.03	0.06	0.04	0.04	0.03	–	0.15	0.09	0.08	0.11	0.09	–	
1133	1134[c]	2-acetyl-6-methylpyrazine	–	–	0.01	0.03	0.02	–	0.08	0.06	0.01	–	0.03	–	
1140	1134[e]	5-isopropyl-2,3-dimethylpyrazine	–	–	–	–	0.03	–	1.00	–	–	–	–	–	
1165	1155[e]	2,3-diethyl-5-methylpyrazine	–	–	0.01	–	–	–	–	–	0.01	–	–	–	30.0
1167	1157[e]	3,5-diethyl-2-methylpyrazine	–	–	0.03	–	–	–	–	–	0.01	–	–	–	
1169	1159[e]	2,3,5-trimethyl-6-ethylpyrazine	–	–	0.10	–	0.07	–	0.12	–	–	–	–	–	
1176	1166[e]	2,5-diethyl-3-methylpyrazine	0.03	–	0.01	–	0.04	–	0.04	–	–	–	–	–	
1197	1202[e]	2,5-dimethyl-3-(2-methylpropyl)pyrazine	–	–	–	–	0.04	–	0.04	0.15	–	–	–	–	
1211	1218[e]	3,5-dimethyl-2-(2-methylpropyl)pyrazine	–	–	–	–	–	–	0.16	–	–	–	–	–	
1219		acetyldimethylpyrazine[f]	0.03	0.03	0.07	0.03	0.05	–	0.24	0.05	0.08	0.25	0.04	–	
1220	1223[e]	2,3-dimethyl-5-(2-methylpropyl)pyrazine	–	–	–	–	–	–	0.33	–	–	–	–	–	
1229	1235[e]	2,5-dimethyl-3-(*E*-1-propenyl)pyrazine	–	0.10	–	0.12	–	–	–	0.12	–	0.08	0.01	–	
1241	1250[e]	2,3-dimethyl-5-(*E*-1-propenyl)pyrazine	–	0.04	–	0.10	–	–	0.02	0.13	–	0.03	–	–	
1244		2-isopropenyl-dimethylpyrazine[f]	–	–	–	0.13	–	–	0.02	0.26	–	0.05	–	–	
1250		methyl-(3-methylbutyl)pyrazine[f]	–	–	–	–	–	–	0.03	–	–	–	–	–	
1253		methyl-(3-methylbutyl)pyrazine[f]	–	–	–	–	–	–	0.10	0.01	–	–	–	–	
1276	1279[c]	(2-methylpropyl)-trimethylpyrazine	–	–	–	–	0.02	–	0.04	–	–	–	–	–	
1315		dimethyl-(3-methylbutyl)pyrazine[f]	–	–	–	–	–	–	5.15	1.46	–	–	–	–	
1327		dimethyl-(3-methylbutyl)pyrazine[f]	–	–	–	–	–	–	0.07	–	–	–	–	–	
1383		trimethyl-(2-methylbutyl)pyrazine[f]	–	–	–	–	–	–	0.24	–	–	–	–	–	
1387		trimethyl-(3-methylbutyl)pyrazine[f]	–	–	–	–	–	–	1.39	–	–	–	–	–	
		total pyrazines	4.36	47.33	6.39	28.09	7.61	2.22	31.72	47.26	5.18	88.63	6.32	0.00	
		pyrazines (% of total GC-MS peak area)	58.2	77.8	63.5	61.8	45.0	31.2	21.7	63.6	44.8	89.3	35.2	0.0	

[a]LRI exptl = linear retention index determined experimentally on an HP5-MS stationary phase. [b]LRI lit. = linear retention index value from the literature. [c]Adams and De Kimpe.[18] [d]Adams.[19] [e]Wagner et al.[21] [f]Tentatively identified. [g]Not detected.

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Table 6. Pyrazines (GC-MS Peak Area × 10^8) Detected in the Model Reactions of Glyoxal with X-Gly Dipeptides and with the Corresponding Free Amino Acids (2 h, 130 °C)

LRI exptl[a]	LRI lit.[b]	compound	Gly (2 mmol)	GlyGly	Ala + Gly	AlaGly	Val + Gly	ValGly	Leu + Gly	LeuGly	Ser + Gly	SerGly	Pro + Gly	ProGly	theor recovery (%)
759	760[c]	pyrazine	1.80	0.83	3.06	0.54	1.87	–[g]	3.39	0.07	2.68	0.14	1.62	–	0.30
822	819[d]	methylpyrazine	0.16	0.16	0.11	0.10	0.06	–	0.13	0.04	0.12	0.10	0.08	–	0.80
908	908/909[d]	2,5(6)-dimethylpyrazine	0.05	0.04	0.03	0.05	0.04	–	–	0.06	0.04	–	–	–	2.0/1.6
911	912[d]	2-ethylpyrazine	–	–	0.21	–	–	–	–	–	–	–	–	–	2.30
913	915[d]	2,3-dimethylpyrazine	0.10	0.02	0.10	0.03	0.03	–	–	0.01	0.06	–	–	–	1.60
977		2-(1-methylethyl)pyrazine[f]	–	–	–	–	0.03	–	0.03	–	–	–	–	–	
995	997[c]	2-ethyl-6-methylpyrazine	–	–	0.06	–	–	–	–	–	–	–	–	–	
998	1000[d]	trimethylpyrazine	0.18	–	–	–	0.09	–	0.04	–	0.09	–	–	–	4.10
998	1000[c]	2-ethyl-5-methylpyrazine	–	–	0.14	0.01	–	–	–	–	–	–	–	–	
1018	1017[d]	2-acetylpyrazine	–	–	0.12	–	0.02	–	0.02	–	0.02	–	0.02	–	0.80
1050	1049[e]	methyl-(1-methylethyl)pyrazine	–	–	–	–	0.06	–	–	–	–	–	–	–	
1057	1056[e]	methyl-(1-methylethyl)pyrazine	–	–	–	–	0.03	–	–	–	–	–	–	–	
1064		2-(2-methylpropyl)pyrazine[f]	–	–	–	–	0.28	–	0.04	–	–	–	–	–	
1072	1078[e]	3-ethyl-2,5-dimethylpyrazine	–	–	0.05	–	–	–	–	–	0.02	–	–	–	
1080	1083[e]	2-ethyl-3,5-dimethylpyrazine	–	–	0.04	–	–	–	–	–	–	–	–	–	
1082	1084[e]	5-ethyl-2,3-dimethylpyrazine	–	–	0.14	–	–	–	–	–	0.02	–	–	–	
1123	1129[c]	2-acetyl-5-methylpyrazine	0.04	–	–	–	–	–	–	–	–	–	–	–	
1144	1134[e]	2,3-dimethyl-5-(1-methylethyl)pyrazine	–	–	–	–	0.05	–	0.12	–	–	–	–	–	
1144	1136[e]	methyl-(2-methylpropyl)pyrazine	–	–	–	–	0.17	–	–	–	–	–	–	–	
1146	1139[e]	methyl-(2-methylpropyl)pyrazine	–	–	–	–	0.25	–	0.05	–	–	–	–	–	
1153	1146[e]	methyl-(2-methylpropyl)pyrazine	–	–	–	–	0.32	–	0.02	–	–	–	–	–	
1184		2-(3-methylbutyl)pyrazine[f]	–	–	–	–	–	–	3.02	0.14	–	–	–	–	
1197	1202[e]	2,5-dimethyl-3-(2-methylpropyl)pyrazine	–	–	–	–	0.04	–	–	–	–	–	–	–	
1211	1218[e]	3,5-dimethyl-2-(2-methylpropyl)pyrazine	–	–	–	–	0.16	–	–	–	–	–	–	–	
1220	1223[e]	2,3-dimethyl-5-(2-methylpropyl)pyrazine	–	–	–	–	0.27	–	0.01	–	–	–	–	–	
1253		methyl-(3-methylbutyl)pyrazine[f]	–	–	–	–	–	–	1.60	0.02	–	–	–	–	
1265		methyl-(3-methylbutyl)pyrazine[f]	–	–	–	–	–	–	1.18	0.09	–	–	–	–	
1266		1-(2-pyrazinyl)-3-methyl-1-butanone[f]	–	–	–	–	0.12	–	0.19	–	–	–	–	–	
1315		dimethyl-(3-methylbutyl)pyrazine[f]	–	–	–	–	–	–	0.34	–	–	–	–	–	
1327		dimethyl-(3-methylbutyl)pyrazine[f]	–	–	–	–	–	–	0.39	–	–	–	–	–	
1337		dimethyl-(3-methylbutyl)pyrazine[f]	–	–	–	–	–	–	0.83	–	–	–	–	–	
1383		trimethyl-(2-methylbutyl)pyrazine[f]	–	–	–	–	–	–	0.25	–	–	–	–	–	
		total pyrazines	2.32	1.06	4.09	0.73	3.97	0.00	11.67	0.43	3.04	0.24	1.72	0.00	
		pyrazines (% of total GC-MS peak area)	96.9	87.9	73.4	81.1	14.0	0.0	9.9	8.9	82.2	71.8	45.3	0.0	

[a]LRI exptl = linear retention index determined experimentally on an HP5-MS stationary phase. [b]LRI lit. = linear retention index value from the literature. [c]Adams and De Kimpe.[18] [d]Adams.[19] [e]Wagner et al.[21] [f]Tentatively identified. [g]Not detected.

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Table 7. Pyrazines (GC-MS Peak Area × 10^8) Detected in the Model Reactions of Glucose with Tripeptides and with the Corresponding Free Amino Acids (2 h, 130 °C)

LRI exptl[a]	LRI lit.[b]	compound	Gly (3 mmol)	GlyGlyGly	Lys + Gly + Gly	LysGlyGly	Lys + Ala + Pro	LysAlaPro	theor recovery (%)
759	760[c]	pyrazine	0.02	–[g]	0.47	–	1.18	–	0.3
822	819[d]	methylpyrazine	0.02	0.01	0.22	0.01	0.32	0.04	0.8
908	908/909[d]	2,5(6)-dimethylpyrazine	0.06	0.74	0.31	0.10	0.20	0.80	2.0/1.6
911	912[d]	ethylpyrazine	–	–	–	–	0.13	–	2.3
913	915[d]	2,3-dimethylpyrazine	0.04	0.08	0.06	–	–	0.20	1.6
995	997[c]	2-ethyl-6-methylpyrazine	–	0.42	0.03	–	0.01	0.05	
998	1000[d]	trimethylpyrazine	0.07	0.10	0.16	–	0.04	0.07	4.1
998	1000[c]	2-ethyl-5-methylpyrazine	–	–	–	0.03	–	–	
1018	1025[e]	2-ethenyl-5-methylpyrazine	–	0.09	–	–	–	0.06	
1072	1078[f]	3-ethyl-2,5-dimethylpyrazine	0.01	0.01	0.04	–	0.56	0.03	
1080	1083[f]	2-ethyl-3,5-dimethylpyrazine	–	0.02	–	–	–	–	
1082	1083[d]	tetramethylpyrazine	0.14	–	0.04	–	–	–	8.4
1082	1084[f]	5-ethyl-2,3-dimethylpyrazine	–	0.02	0.04	–	–	–	
1165	1155[f]	2,3-diethyl-5-methylpyrazine	–	–	–	–	0.02	–	30.0
1169	1159[f]	2,3,5-trimethyl-6-ethylpyrazine	0.02	–	0.03	–	0.12	–	
		total pyrazines	0.38	1.48	1.39	0.13	2.58	1.24	
		pyrazines (% of total GC-MS peak area)	30.8	60.2	38.8	7.5	11.8	37.2	

[a]LRI exptl = linear retention index determined experimentally on an HP5-MS stationary phase. [b]LRI lit. = linear retention index value from the literature. [c]Adams and De Kimpe.[18] [d]Adams.[19] [e]Van Loon et al.[20] [f]Wagner et al.[21] [g]Not detected.

Table 2 depicts the pyrazine formation of the reaction of the X-Lys dipeptides and the corresponding free amino acids with methylglyoxal. It can be seen that dipeptides GlyLys and AlaLys again behaved similarly, whereas the results obtained from ValLys were different again. In the case of reactions with GlyLys and AlaLys, pyrazines, especially 2,5(6)-dimethylpyrazine and trimethylpyrazine, were produced more as compared to the reactions with the corresponding free amino acids. In the case of ValLys, pyrazine production was comparable with the pyrazine production from the reaction with the mixture of valine and lysine. Also for these reactions, amino acid specific pyrazines, mainly 3-ethyl-2,5-dimethylpyrazine from alanine and 3,5-dimethyl-2-(2-methylpropyl)pyrazine from valine, were produced more in the case of reactions with free amino acids.

The pyrazines produced during the reaction of the X-Lys dipeptides or the corresponding free amino acids with glyoxal are depicted in Table 3. Only four pyrazines were detected in these model systems. The production of other volatiles was also very low, and these four pyrazines still comprised 54−94% of the total volatiles detected (as measured by GC-MS peak area) in the case of dipeptides and 27−76% in the case of the corresponding free amino acids. In the case of the reaction with the mixture of valine and lysine, the relatively high amounts of non-pyrazine volatiles were mainly caused by the substantial production of methylpropanal, the Strecker aldehyde of valine. As can be seen from Table 3, unsubstituted pyrazine was the main pyrazine detected. The lower amounts of unsubstituted pyrazine in reactions with dipeptides as compared to the corresponding free amino acids are in accordance with the results obtained from the glucose model systems and from the Lys-X dipeptides in our previous study.[12] For the reactions with glyoxal, the amino acid specific pyrazines ethylpyrazine and 2-(2-methylpropyl)pyrazine were exclusively detected in the model systems containing free amino acids and not in the model systems containing the corresponding dipeptides.

In a second series of experiments, again the influence of the *N*-terminal amino acid on the production of pyrazines was studied, but for these experiments glycine was chosen as the *C*-terminal amino acid. The *N*-terminal amino acids studied were glycine, alanine, valine, leucine, serine, and proline. The pyrazines produced during the reaction of the X-Gly dipeptides and the corresponding free amino acids with glucose are shown in Table 4. It can be seen that GlyGly, AlaGly, and SerGly behaved similarly as GlyLys and AlaLys and as the Lys-X dipeptides investigated in our previous study. For instance, also for these dipeptides relatively simple chromatograms were obtained: pyrazine production comprised 84−90% of the total volatile production (as measured by GC-MS peak area). This portion is much higher than in case of the free amino acids, for which pyrazine production comprised 50−61% of the total volatile production. Also in terms of absolute peak area, pyrazines were produced more by GlyGly, AlaGly, and SerGly dipeptides. Again, this difference was mainly caused by the large amounts of 2,5(6)-dimethylpyrazine produced from the reactions with dipeptides. However, these trends were not observed in model systems containing ProGly, ValGly, and LeuGly. In the case of ProGly and ValGly, no pyrazine production was found. It must be noted that pyrazine production was also very low in the case of the mixture of proline and glycine. This model system yielded mainly proline-specific pyrrolizines. These pyrrolizines were not found in the case of ProGly, which suggests that hydrolysis of the peptide bond did not occur. Also for LeuGly, the major trends in pyrazine production, which were found for Gly-X, Ala-X, Ser-X, and Lys-X from our previous study, were not observed. For instance, the total amount of pyrazines was low with respect to the total amount of volatiles produced for this dipeptide, whereas for most dipeptides this portion was very high. This difference was mainly caused by the production of 3-methylbutanal, the Strecker aldehyde of leucine. In addition, apart from the amino acid specific pyrazines, LeuGly produced similar amounts of pyrazines as the mixture of leucine and glycine. For most other dipeptides tested, this production was much higher than in the case of reactions with their

Table 8. Pyrazines (GC-MS Peak Area × 10^8) Detected in the Model Reactions of Methylglyoxal with Tripeptides and with the Corresponding Free Amino Acids (2 h, 130 °C)

LRI exptl[a]	LRI lit.[b]	compound	Gly (3 mmol)	GlyGlyGly	Lys + Gly + Gly	LysGlyGly	Lys + Ala + Pro	LysAlaPro	theor recovery (%)
822	819[c]	methylpyrazine	–[g]	–	trace	–	trace	trace	0.8
908	908/909[c]	2,5(6)-dimethylpyrazine	0.83	8.75	1.42	2.60	2.07	5.38	2.0/1.6
998	1000[d]	2-ethyl-5-methylpyrazine	–	0.11	trace	trace	–	0.02	
998	1000[c]	trimethylpyrazine	6.18	5.78	3.45	3.40	0.46	3.64	4.1
1072	1078[e]	3-ethyl-2,5-dimethylpyrazine	0.03	0.25	0.34	0.04	1.36	0.30	
1080	1083[e]	2-ethyl-3,5-dimethylpyrazine	0.20	0.04	0.12	0.02	trace	0.04	
1082	1083[c]	tetramethylpyrazine	1.01	0.12	0.22	0.04	–	0.05	8.4
1123	1129[d]	2-acetyl-5-methylpyrazine	0.09	trace	0.02	0.33	trace	0.24	
1133	1134[d]	2-acetyl-6-methylpyrazine	0.05	–	0.02	–	–	0.06	
1219		acetyldimethylpyrazine[f]	0.06	0.01	0.05	0.23	0.01	0.37	
1269		2-acetyl-3,5,6-trimethylpyrazine[f]	0.02	–	trace	trace	–	–	
		total pyrazines	8.47	15.05	5.64	6.67	3.90	10.09	
		pyrazines (% of total GC-MS peak area)	66.9	87.2	54.8	62.8	27.9	51.3	

[a]LRI exptl = linear retention index determined experimentally on an HP5-MS stationary phase. [b]LRI lit. = linear retention index value from the literature. [c]Adams.[19] [d]Adams and De Kimpe.[18] [e]Wagner et al.[21] [f]Tentatively identified. [g]Not detected.

Table 9. Pyrazines (GC-MS Peak Area × 10^8) Detected in the Model Reactions of Glyoxal with Tripeptides and with the Corresponding Free Amino Acids (2 h, 130 °C)

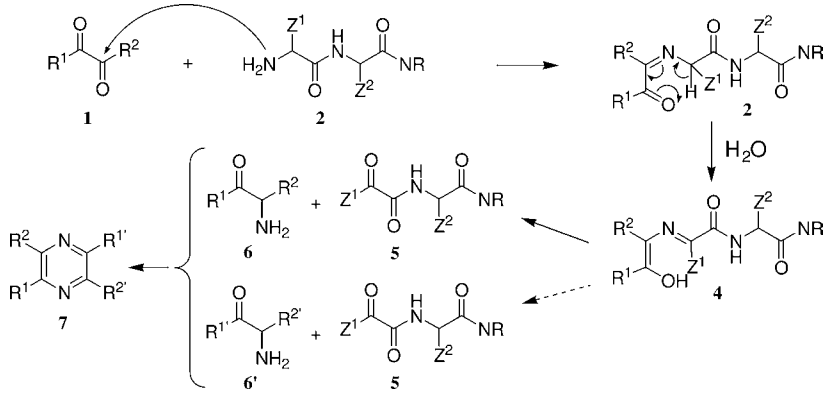
LRI exptl[a]	LRI lit.[b]	compound	Gly (3 mmol)	GlyGlyGly	Lys + Gly + Gly	LysGlyGly	Lys + Ala + Pro	LysAlaPro	theor recovery (%)
759	760[c]	pyrazine	2.39	0.05	2.76	0.13	4.88	1.33	0.3
822	819[d]	methylpyrazine	0.11	0.02	0.13	–[f]	0.08	0.03	0.8
908	908/909[d]	2,5(6)-dimethylpyrazine	0.10	–	0.02	–	–	–	2.0/1.6
911	912[d]	2-ethylpyrazine	–	–	–	–	0.75	–	2.3
913	915[d]	2,3-dimethylpyrazine	0.10	–	0.01	–	–	–	1.6
998	1000[d]	trimethylpyrazine	0.83	–	0.01	–	–	–	4.1
1018	1017[d]	acetylpyrazine	–	–	–	–	0.03	–	
1072	1078[e]	2,6-diethylpyrazine	–	–	–	–	0.03	–	
1080	1083[e]	2-ethyl-3,5-dimethylpyrazine	0.01	–	0.01	–	–	–	
1082	1083[d]	tetramethylpyrazine	0.04	–	–	–	–	–	8.4
1082	1084[e]	5-ethyl-2,3-dimethylpyrazine	0.08	–	–	–	–	–	
1093	1095[e]	3-ethenyl-2,5-dimethylpyrazine	0.01	–	–	–	–	–	
1123	1129[c]	2-acetyl-5-methylpyrazine	0.02	–	–	–	–	–	
		total pyrazines	3.70	0.07	2.95	0.13	5.77	1.36	
		pyrazines (% of total GC-MS peak area)	98.4	38.7	84.7	17.0	68.5	92.1	

[a]LRI exptl = linear retention index determined experimentally on an HP5-MS stationary phase. [b]LRI lit. = linear retention index value from the literature. [c]Adams and De Kimpe.[18] [d]Adams.[19] [e]Wagner et al.[21] [f]Not detected.

corresponding free amino acids. However, some observations, which were also found in the case of Lys-X and X-Lys dipeptides, were valid for all X-Gly dipeptides. For instance, in all model reactions, amino acid specific pyrazines were produced more in the case of reactions with free amino acids as compared to the reactions with dipeptides. In addition, unsubstituted pyrazine was produced more in the case of free amino acids, as it was not detected in model systems containing dipeptides.

Table 5 depicts the pyrazine formation of the reaction of the X-Gly dipeptides and the corresponding free amino acids with methylglyoxal. Similar to the reactions with glucose, a clear distinction could be made between dipeptides GlyGly, AlaGly, and SerGly, on the one hand, and ProGly and ValGly, on the other hand. A borderline behavior was found for LeuGly. Again, reactions with GlyGly, AlaGly, and SerGly yielded high amounts of pyrazines, especially 2,5(6)-dimethylpyrazine and trimethylpyrazine. The total pyrazine production of the reactions with these dipeptides was higher than the total pyrazine production of the reactions with the corresponding free amino acids. For ProGly and ValGly, this total pyrazine production was lower. In fact, no pyrazine production was found in the case of ProGly. In the case of the reaction of LeuGly with methylglyoxal, the results were slightly different from the reaction with glucose. With methylglyoxal, LeuGly produced much higher amounts of 2,5(6)-dimethylpyrazine, but lower amounts of trimethylpyrazine than Leu/Gly. It must be noted that with methylglyoxal, ValGly also produced slightly higher amounts of 2,5(6)-dimethylpyrazine than Val/Gly. In addition, for all reactions with methylglyoxal, amino acid specific pyrazines were again produced more in the case of reactions with free amino acids.

Scheme 1. Hypothetical Formation Mechanism of α-Aminoketones 6 and 6′ from the Reaction between a Peptide 2 and a Dicarbonyl Compound 1, Which Finally Results in the Formation of Pyrazines 7 (Adapted from Van Lancker et al.[12])



The pyrazines produced during the reaction of the X-Gly dipeptides or the corresponding free amino acids with glyoxal are depicted in Table 6. It can be seen that also for these reactions, unsubstituted pyrazine was produced more in the case of reactions with free amino acids as compared to reactions with dipeptides. Again, this was the main pyrazine detected in model reactions with glyoxal. However, a greater diversity of pyrazines was found than in the case of model reactions containing lysine. Also for these reactions, amino acid specific pyrazines were produced more in the case of reactions with free amino acids.

In a last series of experiments, flavor formation from three tripeptides in Maillard model systems was determined. Table 7 depicts the pyrazine formation of the reaction of tripeptides GlyGlyGly, LysGlyGly, and LysAlaPro and the corresponding free amino acids with glucose. Comparison of the results from this table with the results obtained for dipeptides shows that pyrazine production from tripeptides was much lower than from dipeptides. Also, the trends concerning pyrazine production that were found for Lys-X dipeptides were not found for Lys-X-X tripeptides. For LysAlaPro, the production of 2,5(6)-dimethylpyrazine was higher than in the case of the corresponding free amino acids, but for LysGlyGly it was lower. Also, the total pyrazine production was not higher in the case of Lys-X-X tripeptides as compared to the corresponding free amino acids, but lower. For GlyGlyGly, on the other hand, the trends in pyrazine production were similar as in the case of GlyGly. In addition, similar to all experiments with dipeptides, unsubstituted pyrazine was produced more in the case of free amino acids as compared to tripeptides. The amino acid specific pyrazines were also produced more in the case of reactions with free amino acids as compared to tripeptides.

The pyrazines produced during the reaction of the investigated tripeptides and the corresponding free amino acids with methylglyoxal and glyoxal are shown in Tables 8 and 9, respectively. In contrast to the results obtained from reactions with glucose, methylglyoxal yielded more pyrazines, especially 2,5(6)-dimethylpyrazine, in the case of all tripeptides as compared to the corresponding free amino acids (Table 8). However, it must be noted that the difference was lower than in the case of the dipeptides with glycine or lysine at the *N*-terminus. As can be seen from Table 9, unsubstituted pyrazine was also produced more in reactions with free amino acids and glyoxal as compared to the reactions with tripeptides and glyoxal. Although the production of amino acid specific pyrazines was generally very low, it was higher in the case of reactions with free amino acids.

DISCUSSION

In our previous study on the formation of flavor compounds in Maillard model systems of lysine-containing dipeptides,[12] it was found that pyrazine production from those dipeptides was much higher than from the corresponding free amino acids. Because typical Strecker degradation involving decarboxylation followed by hydrolysis of the imine is not possible due to the absence of the free carboxyl group in the case of dipeptides, the formation of α-aminoketones, which finally leads to the formation of pyrazines, must occur through a different mechanism. A hypothesized reaction mechanism for the formation of α-aminoketones from a peptide and an α-dicarbonyl compound was proposed in our previous study (Scheme 1). In accordance to the reaction with free amino acids, the reaction of the α-dicarbonyl compound with the dipeptide starts with the formation of an imine. Deprotonation followed by a 1,5-H-shift leads to enolization of the carbonyl of the α-aminoketone and formation of a 4-hydroxy-2-azadiene. Hydrolysis of the imino moiety of this 2-azadiene produces the α-aminoketone, which eventually leads to the production of pyrazines. This reaction mechanism will also be used to explain the results obtained in this study.

The results obtained in our previous study and in this study show that, in reactions with glucose, most dipeptides produced very high amounts of pyrazines, especially 2,5(6)-dimethylpyrazine. The pyrazine production was higher than in the case of reactions of glucose with free amino acids or tripeptides. This could be due to the catalysis of the Amadori rearrangement in the dipeptide/sugar adduct, which has been proposed by de Kok and Rosing.[14] These authors suggested that due to the conformation of the dipeptide/sugar adduct, the imino nitrogen of the imine is protonated intramolecularly by the carboxy terminus. In the case of free amino acids or tripeptides, the possibility of direct interaction between the COOH group and the amino terminus is much lower. However, different results were found for dipeptides ProGly, LeuGly, ValGly, and ValLys. The reaction of glucose with these dipeptides yielded no or only small amounts of pyrazines. In the case of ProGly, the low reactivity could be due to the presence of the secondary amino group instead of the primary amino group in the other dipeptides tested. In addition, catalysis of the Amadori rearrangement in the dipeptide/sugar adduct is structurally impossible in the case of Pro-X dipeptides. Because dipeptides

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Table 10. Volatiles (GC-MS Peak Area × 10^6) Detected in the Model Reactions of Glucose with Glycine (2 mmol) or Diglycine (1 mmol) for 2 h at Different Temperatures (pH 8)

			100 °C		130 °C		150 °C		180 °C	
LRI exptl[a]	LRI lit.[b]	compound	Gly	GlyGly	Gly	GlyGly	Gly	GlyGly	Gly	GlyGly
		2-methylfuran[f]	–[h]	–	–	–	6.8	29.2	10.0	99.8
		2-ethylfuran[f]	–	–	–	–	–	8.5	1.3	4.1
		2,5-dimethylfuran[f]	–	–	–	–	2.0	10.1	8.8	26.1
		2-vinylfuran[f]	–	–	–	–	3.2	1.8	9.9	6.3
754	760[c]	pyrazine	-	-	0.9	-	8.3	trace	40.9	2.3
798		dihydro-2-methyl-3(2*H*)-furanone[g]	–	–	–	–	4.0	3.1	3.0	7.7
817	819[d]	2-methylpyrazine	–	–	–	–	2.7	trace	60.8	8.5
822	828[d]	furfural	–	–	–	–	20.6	19.6	25.4	102.9
904		2-acetylfuran[g]	–	–	0.1	–	7.7	3.4	32.4	18.0
906	908/909[d]	2,5(6)-dimethylpyrazine	–	–	0.7	15.2	14.4	16.6	210.6	54.2
911	915[d]	2,3-dimethylpyrazine	–	–	–	–	–	–	53.2	–
961	957[d]	5-methylfurfural	–	–	–	–	5.5	6.3	161.3	290.9
992	997[c]	2-ethyl-6-methylpyrazine	–	–	–	–	–	–	20.5	–
998	1000[d]	trimethylpyrazine	–	–	0.4	1.3	10.2	1.8	405.9	9.9
998	1000[c]	2-ethyl-5-methylpyrazine	–	–	–	6.9	1.9	4.7	113.4	17.4
1075	1078[e]	3-ethyl-2,5-dimethylpyrazine	–	–	–	–	–	–	22.4	trace
1080	1083[e]	2-ethyl-3,5-dimethylpyrazine	–	–	–	–	0.8	–	73.8	trace
1082	1084[e]	5-ethyl-2,3-dimethylpyrazine	–	–	–	–	–	–	157.5	trace
1089	1088[e]	2,5-diethylpyrazine	–	–	–	0.5	1.8	trace	33.6	trace
1188		2-(2-furanylmethyl)-5-methylfuran[g]	–	–	–	–	–	–	–	17.4

[a]LRI exptl = linear retention index determined experimentally on an HP5-MS stationary phase. [b]LRI lit. = linear retention index value from the literature. [c]Adams and De Kimpe.[18] [d]Adams.[19] [e]Wagner et al.[21] [f]Identified by comparison with standard. [g]Tentatively identified. [h]Not detected.

LeuGly, ValGly, and ValLys, which are very similar in structure, behaved similarly, it seems that the structure of the *N*-terminal amino acid is determinative for the overall pyrazine production. This is in accordance with our previous study in which all Lys-X dipeptides behaved similarly, whereas no clear influence of the neighboring amino acid could be distinguished. It is not known what causes the lower reactivity of peptides with valine or leucine at the *N*-terminus, but because LeuGly, ValGly, and ValLys also produced more pyrazines as compared to the corresponding free amino acids in reactions with methylglyoxal, it seems that the early phase of the Maillard reaction is slower. Within the early phase of the Maillard reaction, the amino compound reacts with the intact sugar skeleton because degradation products are not present yet. However, with methylglyoxal, pyrazine formation from LeuGly, ValGly, and ValLys was still lower than from the other dipeptides. Therefore, it is assumed that also pyrazine formation itself is slower. Possibly, deprotonation at the α-position of the amide moiety is hindered by the bulkier side chain of valine or leucine.

In contrast to the high production of 2,5(6)-dimethylpyrazine in the case of most dipeptides, unsubstituted pyrazine was produced more in the case of all reactions with free amino acids. At first, it was believed that this difference was caused by the difference between free and bound lysine, because it has been reported that the ε-amino function of lysine produces mainly unsubstituted pyrazine and methylpyrazine.[15] In this respect, it would be understandable that in the case when lysine is bound to another amino acid, the ε-amino group becomes less available and thus less reactive. However, similar results were found for the X-Gly and GlyGlyGly experiments, in which lysine was absent. It must be noted that the amounts of unsubstituted pyrazine produced in these experiments with glucose were much lower than in the case of the Lys-X, X-Lys, and Lys-X-X experiments, but the difference in unsubstituted pyrazine production between reactions with peptides as compared to free amino acids was also observed. No satisfying explanation could be found for this observation, but it is clear that different mechanisms must be responsible for the formation of unsubstituted pyrazine, on the one hand, and substituted pyrazines, on the other hand. A different behavior for unsubstituted pyrazine and 2,5-dimethylpyrazine was also reported by Negroni et al.[16] These authors studied the effect of some important edible oils, for example, olive oil, canola oil, and sunflower oil, on the formation of volatiles from the Maillard reaction of lysine with xylose and glucose. A decreased production of unsubstituted pyrazine was always accompanied with an increased production of 2,5-dimethylpyrazine for these model reactions. In addition, as discussed in our previous paper,[12] an intermediate behavior between unsubstituted pyrazine and dimethylpyrazine was found for methylpyrazine.

As mentioned previously in the Introduction, glycine-derived peptides such as diglycine, triglycine, and tetraglycine are often used to represent di-, tri- and tetrapeptides, respectively. In this respect, also the formation of flavor compounds from Maillard model systems of diglycine and glucose have been studied.[10,11] However, the results obtained in these studies differed from the results obtained from the GlyGly model systems in our study. According to Oh et al.[10] and Lu et al.,[11] pyrazine production from diglycine was much lower than from glycine or triglycine. On the other hand, furfurals were produced more in the case of diglycine. The main difference with our study is that higher reaction temperatures were used. Whereas in the present study 130 °C was used as the reaction temperature, Oh et al.[10] and Lu et al.[11] performed their reactions at 180 and 160 °C, respectively. To study whether the different reaction temperatures induce the conflicting results, glycine (2 mmol) and diglycine (1 mmol) were reacted with glucose at different temperatures (2 h, pH 8). The flavor compounds produced during these experiments are depicted in Table 10. It must be noted that for these experiments the sampling of the volatiles

was performed by means of SPME instead of SBSE. Therefore, the results obtained at 130 °C in this experiment are not exactly the same as those presented in Table 4. At 100 °C, no flavor compounds were detected. At 130 °C, it was confirmed that pyrazines were produced more in the case of diglycine as compared to glycine. However, increasing the reaction temperature indeed changed the flavor profiles. At 150 °C, pyrazine production from the reaction with glycine was already higher than from the reaction with diglycine. As was reported by Oh et al.[10] and Lu et al.,[11] the production of furans and furfurals became more important when diglycine was reacted at this temperature. The difference between pyrazine production, on the one hand, and furan and furfural production, on the other hand, became even more pronounced at 180 °C. These experiments clearly explain the origin of the differences between literature data and our results and illustrate the importance of choosing an appropriate reaction temperature.

With regard to the tripeptides tested, it was already mentioned before that the production of pyrazines was lower than from dipeptides. In addition, no clear trend could be found for the differences in pyrazine production from tripeptides and glucose, on the one hand, and from free amino acids and glucose, on the other hand. However, with methylglyoxal, all tripeptides produced higher amounts of pyrazines than the corresponding free amino acids. As in the case of LeuGly, ValGly, and ValLys, this suggests that especially the early phase of the Maillard reaction is slower for tripeptide LysGlyGly.

For all reactions with dipeptides or tripeptides, the production of amino acid specific pyrazines was low. The mechanism that leads to the production of the amino acid specific pyrazines involves the reaction between the intermediate dihydropyrazine, which is formed by the condensation reaction of two α-aminocarbonyl compounds, and the Strecker aldehyde of the specific amino acid.[17] However, in the case of peptides, typical Strecker degradation involving decarboxylation followed by hydrolysis of the imine is not possible due to the absence of the free carboxyl group. Hydrolysis of the peptide bond, resulting in the liberation of the free amino acids, should occur to produce the Strecker aldehyde. Therefore, the limited amounts of amino acid specific pyrazines in the case of the dipeptides and tripeptides studied suggest only minimal hydrolysis of the peptide bond during these model reactions.

In conclusion, the formation of pyrazines from Maillard model systems containing di- and tripeptides was studied. Pyrazines are known to contribute significantly to the unique roasted aroma of many heated food products. It was shown that most dipeptides produced very high amounts of pyrazines, especially 2,5(6)-dimethylpyrazine and trimethylpyrazine. This pyrazine production was higher than in the case of reactions of glucose with free amino acids or tripeptides. Probably, catalysis of the Amadori rearrangement in the dipeptide/sugar adduct causes this observation. However, peptides with valine, leucine, or proline at the *N*-terminus behaved differently. Therefore, it seems that the structure of the *N*-terminal amino acid is determinative for the overall pyrazine production. In contrast to the production of substituted pyrazines, unsubstituted pyrazine was always produced more in the case of free amino acids. No satisfying explanation could be found for this observation, but it is clear that different mechanisms must be responsible for the formation of unsubstituted pyrazine, on the one hand, and substituted pyrazines, on the other hand. These results indicate that for heat-treated food, also the production of flavor compounds from peptides should be taken into account. In addition, hydrolysis of the peptide bond of both di- and tripeptides was minimal during thermal treatment of 2 h at 130 °C.

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