Autoxidation in the Formation of Volatiles from Glucose−Lysine

Alessandra D'Agostina, Monica Negroni, and Anna Arnoldi*

Dipartimento di Scienze Molecolari Agroalimentari, Università di Milano, Via Celoria 2, 20133 Milano, Italy

The formation of volatile compounds from an aqueous glucose−lysine model system heated in the presence of either a free radical initiator (pro-oxidant) or an antioxidant was investigated. α-Tocopherol, 2,6-di-*tert*-butyl-4-methylphenol (BHT), and rosemary extract were used as antioxidants, and α,α′-azobis(isobutyronitrile) (AIBN) was used as a free radical initiator. The experiments were performed at pH 4 and 6, which were constantly maintained during the heating time by the addition of diluted NaOH. Principal component analysis was used to find similarities and differences among the model systems. Especially at pH 6 AIBN produces a depletion of pyrazine, 2-methylpyrazine, and 2,3-dimethylpyrazine, whereas the three antioxidants enhance the same compounds. This could be due to an autoxidation of the already formed pyrazines or of some intermediate: in particular, the sensitive material could be the C_2 fragment necessary for the formation of pyrazine, 2-methylpyrazine, and 2,3-dimethylpyrazine, but not of 2,5-dimethylpyrazine.

Keywords: *Maillard reaction; antioxidants; autoxidation; glucose−lysine; rosemary extract; BHT; tocopherol; AIBN*

INTRODUCTION

The reaction between amino acids and sugars, the Maillard reaction (Ledl and Schleicher, 1990), produces many volatile heterocyclic compounds, the structures, odor thresholds, and concentrations of which affect extensively the aroma of a food (Fors, 1983). The formation of the initial intermediates deriving from the interaction between an amino group and an α-hydroxycarbonyl moiety produces a cascade of complex reactions. Very recently, Yaylayan (1997) has proposed a new conceptual approach to the Maillard reaction, introducing the idea of a sugar fragmentation pool (S), an amino acid fragmentation pool (A), and an Amadori and Heyn's fragmentation pool (D), which react together to produce the compounds of the advanced and final stage of the Maillard reaction.

Food lipids are another important source of reactive intermediates: especially through autoxidation (Grosch, 1982, 1987) they produce many compounds that, extending the terminology suggested by Yaylayan (1997), could be named as a lipid fragmentation pool (L). Ho and co-workers (Chiu et al., 1990; Ho et al., 1989; Huang et al., 1987; Kim et al., 1996) and Mottram and co-workers (Farmer and Mottram, 1990, 1992; Farmer et al., 1989; Mottram and Whitfield, 1995) gave basic contributions in this field. An excellent review provides current information on the volatiles coming from the interaction of the Maillard reaction and lipids (Whitfield, 1992).

However, the relationship between the Maillard reaction and lipids is even more complex because some Maillard reaction products can exert an antioxidative effect and may be used to protect food lipids from oxidation (Namiki, 1988).

Much less effort has been devoted to the study of the direct effects on the Maillard reaction of the free radicals deriving from lipids through autoxidation. Most of the steps of the Maillard reaction are based on ionic mechanisms, but Namiki and co-workers showed by ESR that free radicals are formed in the very early stage of the sugar−amino acid interaction (Namiki, 1988). They observed clear ESR signals having hyperfine structure that disappears with heating time. The spectral characteristic permitted them to be assigned the structure of *N*,*N*′-disubstituted pyrazine radical cations. A possible pathway for their formation was proposed by Namiki and Hayashi (1983) and Hayashi et al. (1986). These intermediates would produce, besides other compounds, pyrazines that, owing to their high volatility and very low odor threshold, are very important constituents of food aroma. The presence of radicals has been confirmed recently by other techniques (Cämmerer and Kroh, 1995; Roberts and Lloyd, 1997).

To our knowledge, nobody has studied the relationship between this radical mechanism and lipid autoxidation and its subsequent effect on food aroma. As a first step in this direction, we decided to study the formation of volatile compounds from an aqueous glucose−lysine model system heated in the presence of either a free radical initiator (pro-oxidant) or an antioxidant. α-Tocopherol, 2,6-di-*tert*-butyl-4-methylphenol (BHT), and rosemary extract (Chang et al., 1977) were used as antioxidants, and α,α′-azobis(isobutyronitrile) (AIBN) was used as a free radical initiator. This compound is thermally decomposed to give gaseous nitrogen (N_2) and two alkyl radicals that can initiate a free radical chain (Figure 1).

A previous paper from our laboratory (Arnoldi and Corain, 1996) has presented some data on the same subject. However, recently the experimental methodology was changed because the Maillard reaction is very sensitive to pH. Previous model systems were heated at 120 and 100 °C in closed tubes after the pH had been set to the desired value: under these conditions, the pH slowly decreases to reach values below pH 4. To prevent

* Author to whom correspondence should be addressed (fax +39-2-70633062; e-mail Anna.Arnoldi@unimi.it).

S0021-8561(98)00053-3 CCC: $15.00
Published on Web 06/13/1998

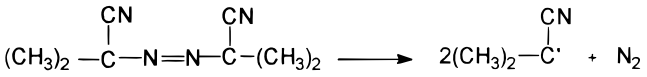

Figure 1. Thermal decomposition of AIBN.

the drop in pH, model systems were heated at 100 °C in a flask equipped with an autoclavable electrode; pH was monitored during all heating steps and maintained constant by the addition of dilute base using a procedure proposed by Ames and co-worker (Apriyantono and Ames, 1990).

MATERIALS AND METHODS

Materials. D-(+)-Glucose, L-lysine hydrochloride, BHT, AIBN, and α-tocopherol were of analytical grade. Water was prepared with a Milli-Q (Millipore), and dichloromethane was Ultra Resi-Analyzed (Baker, Deventer, The Netherlands). Rosemary extract was a gift of Soremartec, Belgium.

Model Systems. Mixtures containing equimolar amounts of glucose and lysine (70 mL of 0.5 M water solution) and a variable amount of additive (0 or 60 mg, and in some case 120 and 180 mg) were heated under reflux for 2 h in a flask equipped with an autoclavable electrode (ATI Russel). During this time the pH was monitored and kept constant at the desired value by the addition of diluted sodium hydroxide (NaOH). At the end of the heating time, the pH was adjusted to 8.0. Tetradecane was added as a first internal standard, and the volatile compounds were recovered by continuous extraction with dichloromethane (CH_2Cl_2). The solvent was carefully concentrated to 1 mL, and pentadecane was added as a second internal standard.

Analysis by GC and GC/MS. The volatiles were quantified by gas chromatography/flame ionization detection (GC/FID) on a DANI 86.10 gas chromatograph. Two internal standards, tetradecane and pentadecane, were used. Compound concentrations were calculated after determination of the correction factors and were expressed in milligrams per model system. Peaks were identified by GC/MS on a Shimadzu QP-5000 by comparison with the NIST62 spectra library and commercial standards as far as possible. Ions were generated by EI at 70 eV. A capillary column SPB-1701 (30 m × 0.2 mm, film 1 μm) was used in both cases. The temperature program was as follows: 37 °C for 10 min, raised at 4 °C/min to 200 °C, and then isothermal. Each experiment was repeated at least three times.

Analysis by Spectrophotometry. Drawings from the same model systems (one every 15 min) were analyzed with an UV−visible spectrophotometer (Jasco, model 7800) at 360 and 420 nm.

Principal Component Analysis (PCA). PCA was performed with the program SYSTAT 1992 (SYSTAT, Inc.) using the default procedure with standardization of the variables prior to analysis. Factors were extracted using the default principal component method and then rotated using EQUAMAX, QUARTIMAX, and VARIMAX equations. The rotated factor patterns were very similar: the discussed results were derived with VARIMAX.

RESULTS

pH is a very important parameter in the Maillard reaction. The common procedure adopted to maintain the pH constant is the use of buffers, but some preliminary experiments with continuous monitoring of the pH showed that, even with concentrated buffers, there is a slow decrease of the pH value (data not shown). We decided therefore to adopt a different procedure: all of the experiments were performed with the pH value kept constant during the heating time by the addition of diluted NaOH (the values indicated throughout the text refer to the actual pH read at 100 °C).

Standard glucose/lysine model systems were heated for 2 h at 100 °C at pH 3, 4, 5, 6, or 7. The volatile compounds were extracted continuously with dichloromethane to avoid progression of the Maillard reaction. Table 1 is a listing of the compounds detected and their Kovats indices (KI). The most interesting Maillard compounds were quantified by the internal standard method after determination of all the necessary response factors, except for 2,3-dihydro-2,3-dihydroxy-6-methyl-4*H*-pyran-4-one (DHMP). Table 2 collects the results of the model systems expressed as mean values and standard errors (obtained with Student *t* test).

2-Methylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, and Furaneol increase with the pH, and DHMP increases until pH 6 and then remains stable; pyrazine reaches a plateau between pH 5 and 6 and then decreases. 2-Furancarboxaldehyde, 5-(hydroxymethyl)-2-furancarboxaldehyde (HMF), and 2-acetylfuran were detected only at pH 3 and 4.

pH 4 and 6 were selected to study the effect of the anti- or pro-oxidants on the Maillard reaction. As a first step, to have an idea of their overall effect, the color development (at 360 and 420 nm) was monitored during heating in the presence of 60 mg of the additive. Experiments were performed with no attempt to exclude oxygen. The curves obtained in the presence of AIBN, α-tocopherol, and rosemary extract were very close to those of the standard model systems. BHT, on the contrary, produced a small increase in the absorbance at both pH values, which suggested that other concentrations should be investigated. Figure 2 shows the increase in the absorbance at 420 nm in model systems at pH 4 and 6 due to the addition of 60, 120, and 180 mg of this antioxidant. Increasing BHT concentrations appear to favor browning, especially at pH 4.

The discussion on the volatiles will be limited to selected compounds. Tables 3 and 4 compare the amounts of these volatiles extracted from the systems containing 60 mg of additive at pH 4 and 6, respectively. The differences in volatile formation between the standard model systems and those with additives are relatively small.

At pH 4 α-tocopherol and rosemary extract produce a decrease of most compounds, except for 2-methylpyrazine in the case of α-tocopherol. BHT, instead, increases 2-methylpyrazine, 2,5-dimethylpyrazine, and 2-acetylpyrrole, whereas it decreases pyrazine, 2-furanmethanol, Furaneol, and 3-methyl-1,2-cyclopentanedione. The free radical initiator AIBN increases 2-furanmethanol, DHMP, and 2-acetylpyrrole and decreases pyrazine.

At pH 6 all of the antioxidants increase pyrazine and 2-methylpyrazine, whereas 2,5-dimethylpyrazine and 2,3-dimethylpyrazine are only slightly influenced; 2-furanmethanol is increased by BHT and rosemary extract but is reduced by α-tocopherol. Furaneol is reduced by α-tocopherol and rosemary extract, and 2-acetylpyrrole is reduced by α-tocopherol and increased by BHT. AIBN decreases most of the compounds, except for 2-acetylpyrrole, 2-furanmethanol, and 3-methyl-1,2-cyclopentanedione.

DISCUSSION

The standard error is sometimes rather large: this is particularly true in the case of 2,3-dihydroxy-6-methyl-4*H*-pyran-4-one and Furaneol. The latter is an important naturally occurring food flavor with a caramel-like note; its quantitative analysis is known to be a

Table 1. Compounds Identified in the Glucose−Lysine Model Systems

peak	KI SPB-1701	standard KI SPB-1701	interpretation	label in the charts
1	785		1-hydroxypropan-2-one	
2	810	813	pyrazine	
3	837	831	3-hydroxybutan-2-one	
4	913	919	2-methylpyrazine	2-MP
5	963		ethyl acetate	
6	969	960	2-furancarboxaldehyde	2-furaldehyde
7	986	980	4-hydroxy-4-methylpentan-2-one	
8	1000	994	2,5-dimethylpyrazine	2,5-DMP
9	1011	1017	2,3-dimethylpyrazine	2,3-DMP
10	1013	1007	2-furanmethanol	2-FM
11	1041		2-acetylfuran	
12	1050		4-cyclopentene-1,3-dione	
13	1159	1157	3-methyl-1,2-cyclopentanedione	3-MCPD
14	1069		2,4-dimethyl-1,3-dioxane	
15	1084	1089	trimethylpyrazine	TMP
16	1199	1200	2,5-dimethyl-4-hydroxy-3(2*H*)-furanone	Furaneol
17	1207	1212	2-acetylpyrrole	2-AP
18	1279		2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	DHMP
19	1391	1383	5-(hydroxymethyl)furan-2-carboxaldehyde	HMF

Table 2. Volatile Compounds Formed from Glucose−Lysine after 2 h of Heating at 100 °C at Different pH Values[a]

compound	pH 3	pH 4	pH 5	pH 6	pH 7
pyrazine	0.019 ± 0.003	0.758 ± 0.113	2.375 ± 0.866	2.388 ± 0.379	1.167 ± 0.190
2-MP	0.110 ± 0.071	0.029 ± 0.016	0.435 ± 0.360	2.055 ± 0.200	5.051 ± 0.537
2,5-DMP	0.034 ± 0.003	0.016 ± 0.001	0.075 ± 0.008	0.165 ± 0.067	1.098 ± 0.173
2,3-DMP	nd[b]	nd	0.004 ± 0.001	0.025 ± 0.004	0.217 ± 0.036
2-FM	nd	0.009 ± 0.002	0.067 ± 0.043	0.067 ± 0.016	*c*
Furaneol	0.012 ± 0.009	0.024 ± 0.015	0.600 ± 0.224	2.672 ± 0.636	7.695 ± 3.769
2-AP	0.066 ± 0.013	0.018 ± 0.003	0.062 ± 0.007	0.056 ± 0.037	nd
DHMP	0.240 ± 0.125	0.758 ± 0.142	4.685 ± 0.908	9.095 ± 4.263	8.452 ± 5.698
3-MCPD	nd	0.006 ± 0.001	0.008 ± 0.003	0.028 ± 0.006	0.190 ± 0.053
2-furaldehyde	0.0012 ± 0.0003	0.0009 ± 0.0004	nd	nd	nd
HMF	2.950 ± 0.950	0.012 ± 0.008	nd	nd	nd
2-acetylfuran	0.007 ± 0.003	nd	nd	nd	nd

[a] Amounts expressed as milligrams per model system. Means and standard errors ($P < 0.5\%$) were obtained at least in triplicate experiments. [b] nd, not detected. [c] In the chromatogram the peak of interest was covered by other compounds.

challenging task because of its fragile nature and delicate gas chromatographic behavior (Pickenhagen et al., 1981; Blank et al., 1992). To solve this, very recently an isotope dilution assay was proposed by Blank et al. (1997).

An important feature of the methodology adopted in this work is that the pH is kept constant during the thermal treatment. This produces a depletion of the compounds formed because when the pH varies, the mechanism of the Maillard reaction changes and different compounds are more or less favored (Ledl and Schleicher, 1990); for example, the formation of 2-furancarboxaldehyde, HMF, and 2-acetylfuran takes place only at low pH values. The comparison with literature data is difficult, because we refer to the actual pH values read at high temperature.

Although a generic feature of the model system such as browning is favored by an increase of the pH, the different classes of volatile compounds are dependent on the more or less extensive formation of the intermediates belonging to the sugar fragmentation pool (S), the Amadori fragmentation pool (D), and the amino acid fragmentation pool (A). The Amadori degradation product 3-deoxyglucosone, favored at low pH, produces HMF, 2-furancarboxyaldehyde, and 2-furanmethanol, whereas 1-deoxyglucosone produces DHMP. At higher pH values the sugar fragmentation pool prevails. Hayashi and Namiki (1986) quantified C_2 and C_3 carbonyl products in a glucose/β-alanine reaction mixture and showed that the production of these compounds was greatly influenced by the pH: production was negligible at acidic pH and increased greatly at alkaline pH. The C_2 and C_3 fragments are assumed to be glycolaldehyde,

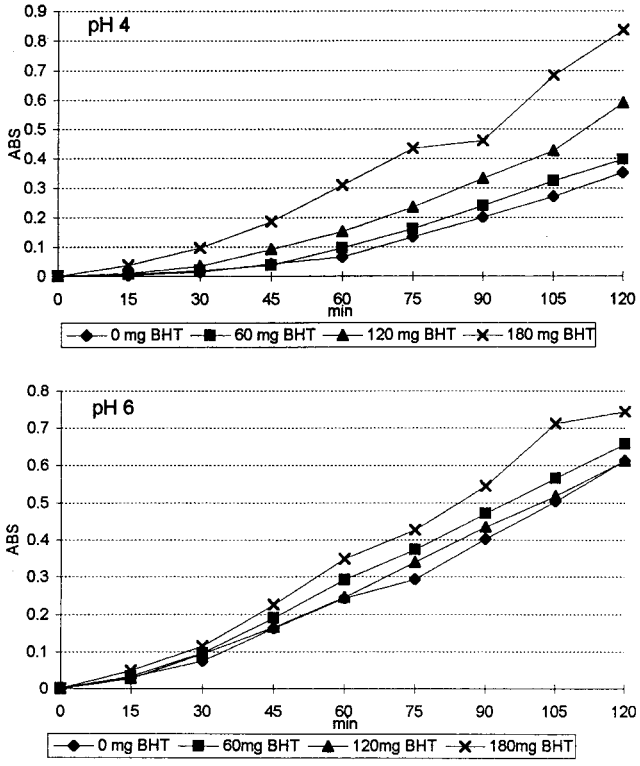

Figure 2. Absorbance at 420 nm of model systems with different amounts of BHT.

glyoxal, glyceraldehyde, methylglyoxal, or their imine derivatives, which are the key intermediates for the formation of pyrazines. These intermediates are pro-

Table 3. Volatile Compounds Formed at pH 4 in the Presence of AIBN or an Antioxidant (60 mg)[a]

compound	reference	BHT	tocopherol	rosemary extract	AIBN
pyrazine	0.758 ± 0.113	0.424 ± 0.215	0.266 ± 0.204	0.348 ± 0.065	0.290 ± 0.211
2-MP	0.029 ± 0.016	0.048 ± 0.034	0.036 ± 0.013	0.017 ± 0.011	0.034 ± 0.020
2,5-DMP	0.016 ± 0.001	0.024 ± 0.011	0.013 ± 0.006	0.007 ± 0.002	0.017 ± 0.010
2-FM	0.009 ± 0.002	0.006 ± 0.004	0.004 ± 0.003	0.003 ± 0.002	0.016 ± 0.009
Furaneol	0.024 ± 0.015	0.018 ± 0.013	0.012 ± 0.009	0.018 ± 0.001	*b*
2-AP	0.018 ± 0.003	0.044 ± 0.003	0.008 ± 0.006	0.007 ± 0.002	0.045 ± 0.001
DHMP	0.758 ± 0.142	0.804 ± 0.278	0.435 ± 0.260	0.500 ± 0.442	0.910 ± 0.018
3-MCPD	0.006 ± 0.001	0.004 ± 0.002	0.002 ± 0.001	nd[c]	b
2-furaldehyde	0.009 ± 0.004	0.0010 ± 0.0004	0.0010 ± 0.0005	nd	0.0010 ± 0.0004
HMF	0.012 ± 0.008	0.040 ± 0.015	0.008 ± 0.002	0.010 ± 0.009	0.054 ± 0.025

[a] Amounts expressed as milligrams per model system. Means and standard errors ($P < 0.5\%$) were obtained at least in triplicate experiments. [b] In the chromatogram the peak of interest was covered by other compounds. [c] nd, not detected.

Table 4. Volatile Compounds Formed at pH 6 in the Presence of AIBN or an Antioxidant (60 mg)[a]

compound	reference	BHT	tocopherol	rosemary extract	AIBN
pyrazine	2.388 ± 0.379	3.255 ± 1.047	3.282 ± 1.108	3.027 ± 0.222	1.960 ± 0.306
2-MP	2.055 ± 0.200	2.402 ± 0.832	2.422 ± 0.615	2.247 ± 0.514	1.595 ± 0.252
2,5-DMP	0.165 ± 0.067	0.156 ± 0.044	0.016 ± 0.001	0.167 ± 0.044	0.110 ± 0.025
2,3-DMP	0.025 ± 0.004	0.029 ± 0.009	0.025 ± 0.007	0.026 ± 0.021	0.017 ± 0.007
2-FM	0.067 ± 0.016	0.071 ± 0.023	0.033 ± 0.025	0.071 ± 0.012	0.073 ± 0.023
Furaneol	2.672 ± 0.636	2.735 ± 0.233	1.832 ± 0.629	1.802 ± 0.310	2.215 ± 0.328
2-AP	0.056 ± 0.037	0.065 ± 0.027	0.041 ± 0.010	0.053 ± 0.003	0.162 ± 0.045
DHMP	9.095 ± 4.263	10.935 ± 2.287	10.128 ± 4.052	3.917 ± 2.163	9.080 ± 0.935
3-MCPD	0.028 ± 0.006	0.019 ± 0.006	nd[b]	0.016 ± 0.006	0.055 ± 0.003

[a] Amounts expressed as milligrams per model system. Means and standard errors ($P < 0.5$) were obtained at least in triplicate experiments. [b] nd, not detected.

Table 5. Eigenvectors of Principal Components for the Volatiles Obtained from Model Systems at pH 4 (See Table 3)

descriptor	PC_1	PC_2	PC_3
pyrazine	0.011	0.066	0.985
2-MP	0.147	0.976	−0.156
2,5-DMP	0.397	0.890	0.110
2-FM	0.921	0.073	0.107
2-AP	0.806	0.536	−0.144
DHMP	0.901	0.336	0.243
2-furaldehyde	0.257	0.816	0.214
HMF	0.874	0.353	−0.317
% of total variance explained	41.481	36.807	15.560

duced very rapidly in the early stage of the reaction before the Amadori compound formation. Nevertheless, similar C_2 and C_3 fragments (such as enaminals of glyceraldehydee and hydroxyacetone) can derive also from the retro-aldol fragmentation of the Amadori compound (Yaylayan and Huyghues-Despointes, 1994).

With respect to pH, the presence of the pro- or antioxidants produces only minor variations: BHT at the concentration used in this work seems to be favorable for browning, but this does not happen with the other two antioxidants.

The effect on volatiles depends on the pH. The three antioxidants have different behaviors, especially at pH 4: the two natural antioxidants, α-tocopherol and rosemary extract, inhibit practically the formation of all the compounds, whereas BHT decreases pyrazine, 2-furanmethanol, Furaneol, and 3-methyl-1,2-cyclopentanedione but increases 2-acetylpyrrole and DHMP. At pH 6 their diversity is less evident because besides BHT also α-tocopherol and rosemary extract increase pyrazine and 2-methylpyrazine.

To find similarities and differences among the model systems, we tried to use PCA, a methodology which finds the underlying factors (principal components) that influence a chemical system (Dillon and Goldstein, 1984; Malinowski and Howery, 1980). These factors are linear combinations of a set of orthogonal vectors that are the eigenvectors of the variance−covariance matrix of the original data matrix. The procedure creates from the original ones a new set of variables, which are called principal components (PC) and are orthogonal to each other. PC_1 accounts for the largest proportion of the variation in the original set; the other PCs account for smaller and smaller proportions of the variation. If the first two (three) eigenvalues obtained from the data matrix are large enough that they account for a substantial fraction of the total variance in the data set, the overall structure of the data set may be revealed by generating a two-dimensional (or three-dimensional) plot of PC_1 vs PC_2 (vs PC_3).

Often the information contained in the data matrix contains redundancy (correlation between variables) that is easily identified by comparing the weighted contributions (loadings) of the original variables in the principal components. In our study redundancy means that two or more volatile compounds are influenced in a similar way by the presence of the additives.

At pH 4 PC_1 and PC_2 account for 78.29% of the original variance of the system, whereas by adding PC_3 93.83% of the variance is explained. Table 5 shows the eigenvectors for each PC. PC_1 is dominated mainly by 2-furanmethanol, 2-acetylpyrrole, DHMP, and HMF, whereas PC_2 is dominated by 2-methylpyrazine, 2,5-dimethylpyrazine, and 2-furancarboxaldehyde; PC_3 is particularly related to pyrazine. In general, it can be said that the very important PC_1 is related to the oxygenated compounds, whereas PC_2 and PC_3 are related to pyrazines, and that the unsubstituted pyrazine undergoes effects that are dissimilar from those of 2-methylpyrazine and 2,5-dimethylpyrazine. Figure 3 shows the loading plot for the first two PCs, useful to discuss the differences among the effects of the additives. AIBN (which has a positive value) and α-tocopherol (which has a negative value) are well separated from the standard system and those with BHT and rosemary extract by the very significant PC_1 (the oxygenated compounds component). BHT and rosemary

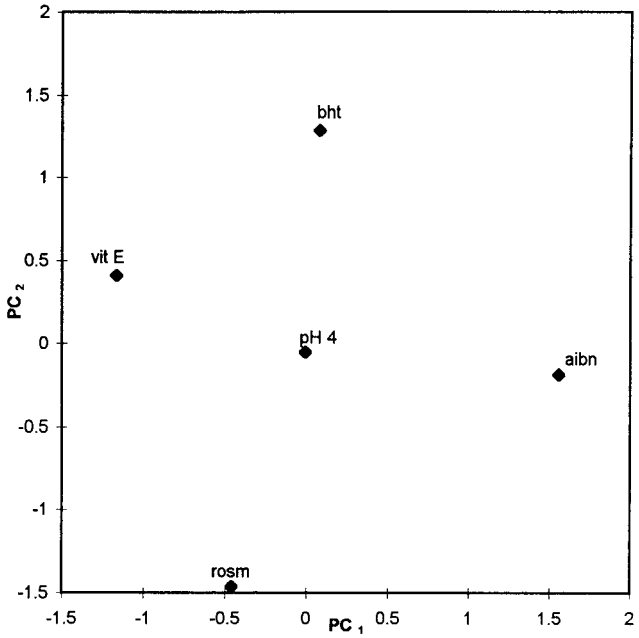

Figure 3. Loading plot of PC_1 vs PC_2 for all of the volatiles obtained from model systems at pH 4 (see Table 3).

Table 6. Eigenvectors of Principal Components for the Volatiles Obtained from Model Systems at pH 6 (See Table 4)

descriptor	PC_1	PC_2	PC_3
pyrazine	−0.924	−0.220	−0.053
2-MP	−0.981	−0.160	0.034
2,5-DMP	−0.015	0.997	−0.045
2,3-DMP	−0.960	0.240	0.139
2-FM	0.370	0.909	−0.020
Furaneol	0.020	0.553	0.825
2-AP	0.943	0.068	0.088
DHMP	0.032	−0.349	0.934
3-MPCD	0.916	0.374	0.133
% of total variance explained	51.161	28.054	17.825

extract have significant differences with respect to the standard system in PC_2 (the 2-methyl- and 2,5-dimethylpyrazine component). Taking into account together PC_1 and PC_2, the pro-oxidant AIBN appears to have an opposite behavior, especially with respect to the antioxidant α-tocopherol, except for their effect on pyrazine.

At pH 6 PC_1 and PC_2 account for 79.22% of the original variance, whereas by adding PC_3 97.4% of the variance is explained. Table 6 shows the eigenvectors for each PC. At this pH, PC_1 is dominated mainly by pyrazine, 2-methylpyrazine, 2,3-dimethylpyrazine, 2-acetylpyrrole, and 3-methyl-1,2-cyclopentanedione, even if the first three compounds have negative coefficients and the others positive; PC_2 is dominated by 2,5-dimethylpyrazine and 2-furanmethanol, and PC_3 is particularly related to DHMP and Furaneol. Figure 4 shows the loading plot for the first three PCs. AIBN is very well discriminated by PC_1 (high positive value); the three antioxidants have a very similar negative value (around −0.5), whereas the standard system has a value close to zero. PC_2 is useful to separate α-tocopherol from BHT and rosemary extract, whereas PC_3 separates BHT from rosemary extract.

Even if at the two pH values the variables contribute in different ways to the PCs, the model system with the pro-oxidant AIBN is well discriminated by PC_1 in both cases. The difference of the behavior of the three

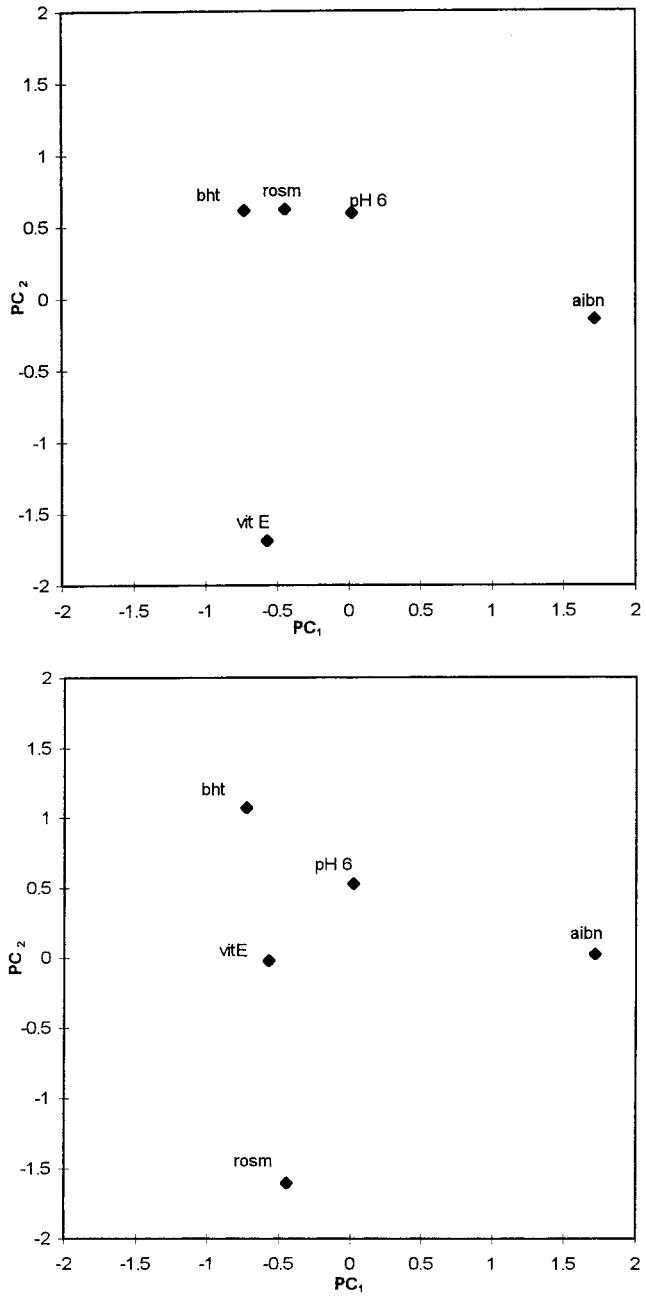

Figure 4. Loading plots of PC_2 vs PC_3 vs PC_1 for all of the volatiles obtained from model systems at pH 6 (see Table 4).

antioxidants may be due to their different solubility and reactivity.

It is always very difficult to discuss the mechanism of a reaction when the products are obtained in 0.01−0.1% yields. Unexpectedly sometimes even with the free radical initiator some compounds are increased (in particular 2-acetylpyrrole), whereas with the antioxidants some compounds decreased. These phenomena could be explained by the fact that the Maillard reaction is in reality a very complex network of competitive reactions and the actual yield of each compound derives from a complicated combination of many reaction constants. Sometimes even specific reactions could take place, such as alkylation by some electrophilic species of the phenolic nucleus of the antioxidants.

Perhaps the most interesting observation is that at pH 6 AIBN produces a significant depletion of pyrazine and 2-methylpyrazine (which are particularly abundant

at this pH) and of 2,3-dimethylpyrazine, whereas the three antioxidants enhance the same compounds. This could be due to an autoxidation of the already formed pyrazines or of some intermediate: in particular, the sensitive material could be the C_2 fragment necessary for the formation of pyrazine, 2-methylpyrazine, and 2,3-dimethylpyrazine, but not of 2,5-dimethylpyrazine. This autoxidation seems to be independent of the *N*,*N*-dialkylpyrazinium radical cations, which were observed by some groups by ESR in the reaction of primary (Namiki and Hayashi, 1983; Hayashi et al., 1986) and secondary (Roberts and Lloyd, 1997) amines with sugars, because in our experiments the pyrazines are enhanced when the free radical formation is inhibited.

In conclusion, autoxidation may have a role in volatile formation, but great caution must be used in considering results, because, depending on the concentration, antioxidants can become pro-oxidants (Pokorny, 1987), and in our model systems it is almost impossible to know the concentration and activity of the additives during the thermal treatment.

Nevertheless, in these kinds of systems in which gas chromatography furnishes a multivariate set of variables, PCA is a promising tool suitable to put in evidence the contribution of various parameters on product formation.

LITERATURE CITED

Apriyantono, A.; Ames, J. M. Volatile compounds produced on heating lysine and xylose. In *Flavour Science and Technology*; Bessiere, Y., Thomas, A. F., Eds.; Wiley: Chichester, U.K., 1990; pp 117−120.

Arnoldi, A.; Corain, E. Effects of free radicals on pyrazine formation in the Maillard reaction. In *Flavour Science. Recent Developments*; Taylor, A. J., Mottram, D. S., Eds.; The Royal Society of Chemistry: Cambridge, U.K., 1996; pp 217−220.

Blank, I.; Sen, A.; Grosch, W. Potent odorants of the roasted powder and brew of Arabica coffee. *Z. Lebensm. Unters. Forsch.* **1992**, *195*, 239−245.

Blank, I.; Fay, L. B.; Lakner, F. J.; Schlosser, M. Determination of 4-hydroxy-2,5-dimethyl-3(2H)-furanone and 2(or 5)-ethyl-4-hydroxy-5(or 2)-methyl-3(2H)-furanone in pentose sugar-based Maillard model systems by isotope dilution assays. *J. Agric. Food Chem.* **1997**, *45*, 2642−2648.

Cämmerer, B.; Kroh, L. W. Investigation of the contribution of radicals to the mechanism of the early stage of the Maillard reaction. *Food Chem.* **1996**, *57*, 217−221.

Chang, S. S.; Ostric-Matijasevic, B.; Hsieh, O. A. L.; Huang, C.-L. Natural antioxidants from rosemary and sage. *J. Food Sci.* **1977**, *42*, 1102−1106.

Chiu, E.-M.; Kuo, M.-C.; Bruechert, L. J.; Ho, C.-T. Substitution of pyrazines by aldehydes in model systems. *J. Agric. Food Chem.* **1990**, *38*, 58−61.

Dillon, W. R.; Goldstein, M. *Multivariate Analysis: Methods and Applications*; Wiley: New York, 1984.

Farmer, L. J.; Mottram, D. S. Interaction of lipid in the Maillard reactions between cysteine and ribose: the effect of a triglyceride and three phospholipids on the volatile compounds. *J. Sci. Food Agric.* **1990**, *53*, 505−525.

Farmer, L. J.; Mottram, D. S. Effect of cysteine and ribose on the volatile thermal degradation products of a triglyceride and three phospholipids. *J. Sci. Food Agric.* **1992**, *60*, 489−497.

Farmer, L. J.; Mottram, D. S.; Whitfield, F. B. Volatiles compounds produced in Maillard reactions involving cysteine, ribose and phospholipid. *J. Sci. Food Agric.* **1989**, *49*, 347−368.

Fors, S. Sensory properties of volatile Maillard reaction products and related compounds. In *The Maillard Reaction in Foods and Nutrition*; Waller, G. R., Feather, M. S., Eds.; ACS Symposium Series 215; American Chemical Society: Washington, DC, 1983; pp 185−286.

Grosch, W. Lipids degradation products and flavour. In *Food Flavours, Part A*; Morton, I. D., MacLeod, A. J., Eds.; Elsevier: Amsterdam, 1982; pp 325−398.

Grosch, W. Reaction of hydroperoxides—Products of low molecular weight. *Autoxidation of Unsaturated Lipids*; Chang, H. W.-S., Ed.; Academic Press: London, 1987; pp 95−140.

Hayashi, T.; Namiki, M. Role of sugar fragmentation in an early stage browning of amino-carbonyl reaction of sugar with amino acids. *Agric. Biol. Chem.* **1986**, *50*, 1965−1970.

Hayashi, T.; Mase, S.; Namiki, M. Formation of three-carbon sugar fragment at an early stage of the browning reaction of sugar with amines or amino acids. *Agric. Biol. Chem.* **1986**, *50*, 1959−1964.

Ho, C.-T.; Bruechert, L. J.; Zhang, Y.; Chiu, E.-M. Contribution of lipids to the formation of heterocyclic compounds in model systems. In *Thermal Generation of Aromas*; Parliment, T. H., McGorrin, R. J., Ho, C.-T., Eds.; American Chemical Society: Washington, DC, 1989; pp 105−113.

Huang, T. C.; Bruechert, L. J.; Hartman, T.-C.; Rosen, R. T.; Ho, C.-T. Effect of lipids and carbohydrates on thermal generation of volatiles from commercial zein. *J. Agric. Food Chem.* **1987**, *35*, 985−990.

Kim, Y. S.; Hartman, T. G.; Ho C.-T. Formation of 2-pentylpyridine from the thermal interaction of amino acids and 2,4-decadienal. *J. Agric. Food. Chem.* **1996**, *44*, 3906−3908.

Ledl, F.; Schleicher, E. New aspects of the Maillard reaction in foods and in the human body. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 565−706.

Malinowsky, E. R.; Howery, D. G. *Factor Analysis in Chemistry*; Wiley: New York, 1980.

Mottram, D. S.; Whitfield, F. B. Volatiles compounds from the reaction of cysteine, ribose and phospholipid in low moisture systems. *J. Agric. Food Sci.* **1995**, *43*, 984−988.

Namiki, M. Chemistry of the Maillard reactions: recent studies on the browning reaction mechanism and the development of antioxidants and mutagens. *Adv. Food Res.* **1988**, *32*, 115−184.

Namiki, M.; Hayashi, T. A new mechanism of the Maillard reaction involving sugar fragmentation and free radical formation. In *The Maillard Reaction in Foods and Nutrition*; Waller, G. R., Feather, M. S., Eds.; ACS Symposium Series 215; American Chemical Society: Washington, DC, 1983; pp 21−46.

Pickenhagen, W.; Velluz, A.; Passerat, J.-P.; Ohloff, G. Estimation of 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) in cultivate of wild strawberries, pineapples, and mangoes. *J. Sci. Food Agric.* **1981**, *32*, 1132−1134.

Pokorny, J. Major factors affecting autoxidation of lipids. In *Autoxidation of Unsaturated Lipids*; Chang, H. W.-S., Eds.; Academic Press: London, 1987; pp 141−206.

Roberts, R. L.; Lloyd, R. V. Free radical formation from secondary amines in the Maillard reaction. *J. Agric. Food Chem.* **1997**, *45*, 2413−2418.

Yaylayan, V. A. Classification of the Maillard reaction: a conceptual approach. *Trends Food Sci. Technol.* **1997**, *8*, 13−18.

Yaylayan, V. A.; Huyghues-Despointes, A. Chemistry of the Amadori rearrangement products: analysis, synthesis, kinetics, reactions, and spectroscopic properties. *Crit. Rev. Food Sci. Nutr.* **1994**, *34*, 321−369.

Whitfield, F. B. Volatiles from the interactions of the Maillard reaction and lipids. *Crit. Rev. Food Sci. Nutr.* **1992**, *31*, 1−58.

Received for review January 20, 1998. Revised manuscript received April 24, 1998. Accepted April 30, 1998. This study was carried out with financial support from the Commission of the European Communities, Agriculture and Fisheries (FAIR) specific RTD program, contract CT96-1080 "Optimisation of the Maillard reaction in Foods. A Way to improve food safety and quality".

JF980053O