Effects of Olive, Canola, and Sunflower Oils on the Formation of Volatiles from the Maillard Reaction of Lysine with Xylose and Glucose

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Some important edible oils (extra virgin olive oil, canola oil, and sunflower oil) were added to aqueous glucose−lysine or xylose−lysine model systems to investigate their effect on the formation of volatiles from the Maillard reaction (MR). The volatile compounds were extracted by a Likens−Nickerson apparatus and quantified. Pyrazines, Maillard reaction products with an important impact on food flavor, appeared to be particularly sensitive to the presence of the oils in both the xylose−lysine and glucose−lysine model systems. The unsubstituted pyrazine was formed more with olive oil, less with canola oil, and even less with sunflower oil, whereas 2-methylpyrazine, 2,5-methylpyrazine, and 2,3-dimethylpyrazine were formed less with olive oil, more with canola oil, and even more with sunflower. The oxidative states of the oils and their fatty acid fingerprints were determined: the results indicated that the relative amounts of the pyrazines are sensitive to the degree of unsaturation of the oil. The autoxidation of the volatile compounds generated from the MR, investigated by the addition of free radical modulators (antioxidants α-tocopherol, 2,6-di-*tert*-butyl-4-methylphenol, and rosemary extract; or pro-oxidant α,α′-azobis-isobutyronitrile, a free radical initiator), was limited in respect to aqueous model systems.

Keywords: *Maillard reaction; olive oil; canola oil; sunflower oil; xylose−lysine; glucose−lysine; pyrazines*

INTRODUCTION

Most foods that are thermally processed, such as meat, fish, soups, sauces, cereals, snacks, fried potatoes, nuts, seeds, and coffee, contain lipids in the raw materials or as ingredients. The typical aroma of these foods derives from a very complex mixture of volatile compounds and depends on the relative concentrations of the different components. These volatiles derive in part from the Maillard reaction (*1*), and in part from lipids, another important source of reactive intermediates in foods. The structures, odor thresholds, and concentrations of Maillard reaction products (MRPs) and of lipid autoxidation products (LAPs) extensively affect the aroma of foods (*2*).

In the Maillard reaction, the formation of the initial intermediates deriving from the interaction between an amino group and an α-hydroxycarbonyl moiety produces a cascade of very complex reactions. Yaylayan (*3*) 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}, that react together to produce the compounds of the advanced and final stage of the Maillard reaction. Recently (*4*), we have proposed to extend the approach suggested by Yaylayan by introducing a lipid fragmentation pool {L}; in fact, lipids are another important source of reactive intermediates in foods (*5* and *6*).

An excellent review provides current information on the volatiles coming from the interaction of the Maillard reaction and lipids (*2*). Ho and co-workers (*7*−*10*) and Mottram and co-workers (*11*−*13*) have given major contributions to research in this field.

The target of this work was to elucidate the effects of some important edible oils on the formation of volatiles from the MR. The oils were chosen taking into account either their specific composition or their economic importance in the European Union; in particular, it was decided to select oils derived from the major oilseed crops in West Europe, such as: (a) olive oil, the most important oil produced in the Mediterranean area, especially in Italy, Spain, and Greece; (b) canola oil, obtained from "zero" erucic acid rape cultivars, the main crop for the production of oil in some cold European countries, such as France and Germany; and (c) sunflower oil, the main European polyunsaturated oil that has the advantage to be produced in marginal agricultural areas, because sunflower is not very demanding from the viewpoint of soil and water.

MATERIALS AND METHODS

The oils used were olive oil by San Giorgio (Val di Pesa, Firenze, Italy); "zero" erucic acid rape-seed oil (canola oil) by Zucchi (Cremona, Italy); and sunflower oil by Carapelli (Firenze, Italy).

Determination of the Peroxide Index. The peroxide index was determined by following the official method of the European Union (GU EC 5.9.1991, N. L. 248/8, annex III). Fresh samples of olive oil contained about 1.5 mequiv O_2/Kg oil, sunflower oil contained 0.9 mequiv O_2/Kg oil, and canola oil contained 1.4 mequiv O_2/Kg oil. Each bottle was checked at least every 3 weeks, and when this parameter exceeded 10 mequiv O_2/Kg oil, the bottle was discarded.

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Fatty Acid Composition. The fatty acid methyl esters were prepared by the sodium methylate method, following the official procedure of the European Union (GU EC 5.9.1991, N. L. 248/44, annex XB). The analysis was conducted by GC on a SP-2340 column (60 m × 0.25 mm); film 0.20 μm; temperature from 170 °C to 210 °C, rate 2 °C/min. A commercial standard mixture (Supelco, Bellefonte, PA) was used for peak identification. The values that were found are regular: olive oil containing 18.0% palmitic acid, 1.7% palmitoleic acid, 3.0% stearic acid, 85.0% oleic acid, 9.0% linoleic acid, and 1.3% linolenic acid; sunflower oil containing 18.7% palmitic acid, 0.36% palmitoleic acid, 3.1% stearic acid, 19.3% oleic acid, 58.2% linoleic acid, and 0.34% linolenic acid; and canola oil containing 12.9% palmitic acid, 0.86% palmitoleic acid, 1.6% stearic acid, 56% oleic acid, 19.7% linoleic acid, and 8.9% linolenic acid (*14*).

Tocopherol Determination. The oil (3 g) was diluted with hexane (7 mL), and a solution of 2-methoxynaphthalene as internal standard was added (100 μL, concentrated = 0.5 mg/mL). After filtration, the solution was injected on a Lichrosorb SI60 column (5 μm); flow 1 mL/min; eluent hexane/2-propanol, 98:2); detection by fluorimetry: excitation 295 nm, emission 310 nm (*15*). The quantitation was performed by the internal standard method after having checked that the response was proportional to the concentration in the range of interest. The total amount of tocopherols in olive oil was 15.7 mg/100 mL (α-tocopherol 14.7, γ-tocopherol 0.85, and δ-tocopherol 0.16 mg/100 mL). The total amount of tocopherols in sunflower oil was 54.7 mg/mL (α-tocopherol 51.3, β-tocopherol 1.9, γ-tocopherol 1.7, and δ-tocopherol 0.32 mg/100 mL). The total amount of tocopherols in canola oil was 34.9 mg/mL (α-tocopherol 12.5, β-tocopherol 4.5, and γ-tocopherol 17.9 mg/100 mL).

Maillard Model Systems. Aqueous mixtures containing equimolar amounts of xylose or glucose and lysine (70 mL of 0.5 M water solution) were added with the oil (25 g), an additive [BHT, tocopherol, rosemary extract (0.30 mmol), or AIBN (0.36 mmol)], and 0.381 mg of tetradecane (the first internal standard). The mixtures were heated and extracted for 3 h at 100 °C in a flask equipped with a Likens−Nickerson apparatus; the solvent used to extract the volatiles was CH_2Cl_2 (200 mL). During this time the pH was monitored and kept constant by adding diluted sodium hydroxide (NaOH). After careful concentration of the solvent to 1 mL, pentadecane was added as a second internal standard, and the peaks were identified and quantified by GC−mass spectrometry (GC−MS) on a Shimadzu QP-5000 by comparison with the NIST 62 spectra library and commercial standards. A capillary SPB-1701 column (30 m × 0.2 mm, film 1 μm) was used; temperature program: 37 °C × 10 min, 4 °C to 200 °C, then isothermal.

Principal Components Analysis. Principal components analysis (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 components 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

As the Maillard reaction is very sensitive to the pH value, in our preceding experiments without the addition of oils (*4, 16*), its value was controlled and kept constant by the addition of a dilute base during heating. The same approach was chosen also for the present work and the value was set to 6.

Xylose−lysine and glucose−lysine model systems (70 mL of 0.5 M solutions) were added with 25 g of sunflower, canola, or olive oil. The presence of large amounts of oil in our model systems made the recovery of the volatile compounds very complex, but after some attempts using different techniques, enclosing SPME,

Table 1. Compounds Quantified in the Model Systems

compounds	Ki SPB-1701	standard Ki SPB-1701	label in the charts and tables
pyrazine	810	813	pyrazine
2-methylpyrazine	913	919	2-MP
2-furancarboxaldehyde	969	960	2-FA
2, 5-dimethylpyrazine	1000	994	2, 5-DMP
2, 3-dimethylpyrazine	1011	1017	2, 3-DMP
2-furanmethanol	1013	1007	2-FM
octanal	577	584	octanal
nonanal	1197	1161	nonanal
Z-2-decenal	1333	1298	Z-2-decenal

Likens−Nickerson extraction was chosen as the best. A positive feature of this procedure is that it was possible to carry it out directly during the heating of the model systems in order to avoid a further progress of the Maillard reaction after the time selected. Therefore, in these experiments the heating time and the extraction time coincide.

The quantification was performed by the internal standard method, after determination of the correction factors in respect to the internal standard (tetradecane), and was limited to some important Maillard reaction products such as pyrazine, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, 2-furanmethanol, 2-furancarboxaldehyde, and the main long chain aldehydes, such as octanal, nonanal and *Z*-2-decenal (Table 1). All the experiments were repeated at least 4 times: the standard deviation was much larger than that of the aqueous model systems that were analyzed in our previous work (*4, 16*). The differences could be due to the large amount of out-of-phase lipid, which can influence the Maillard reaction itself or the Likens−Nickerson extraction despite the good stirring applied.

Starting from xylose−lysine model systems and comparing the three oils (Figure 1), it appears that they have a clear effect on the formation of the main pyrazines: the unsubstituted one is formed more with olive oil, less with canola oil, and even less with sunflower oil, whereas 2-methylpyrazine, 2,5-methylpyrazine, and 2,3-dimethylpyrazine are formed less with olive oil, more with canola oil, and even more with sunflower. The results indicate that the relative amounts of the pyrazines are sensitive to the degree of unsaturation of the oil that has an opposite effect on the unsubstituted and the mono or disubstituted pyrazines. A very similar relative reactivity was observed in the glucose−lysine model systems (Figure 2), the only difference being that levels formed with olive oil and canola oil are almost equivalent.

To get a deeper insight into this phenomenon, it was decided to decrease the formation of free radicals by the addition of some antioxidants or to increase it by adding a free radical initiator. α-Tocopherol, 2,6-di-*tert*-butyl-4-methylphenol (BHT), and rosemary extract (*17*) were chosen as antioxidants, and α,α′-azobis-isobutyronitrile (AIBN) was chosen as a free radical initiator.

Starting from xylose−lysine model systems containing olive oil (Table 2), the addition of BHT increased mostly pyrazine, 2,5-dimethylpyrazine, 2-furancarboxaldehyde, and 2-furanmethanol; the addition of tocopherol increased pyrazine, 2-methylpyrazine, and 2,5-dimethylpyrazine; whereas the third antioxidant, rosemary extract, increased 2,5-dimethylpyrazine; AIBN had a very scarce effect.

With canola oil (Table 3), BHT and rosemary extract decreased most compounds, and tocopherol increased pyrazine, 2,5-dimethylpyrazine, 2,3-dimethylpyra-

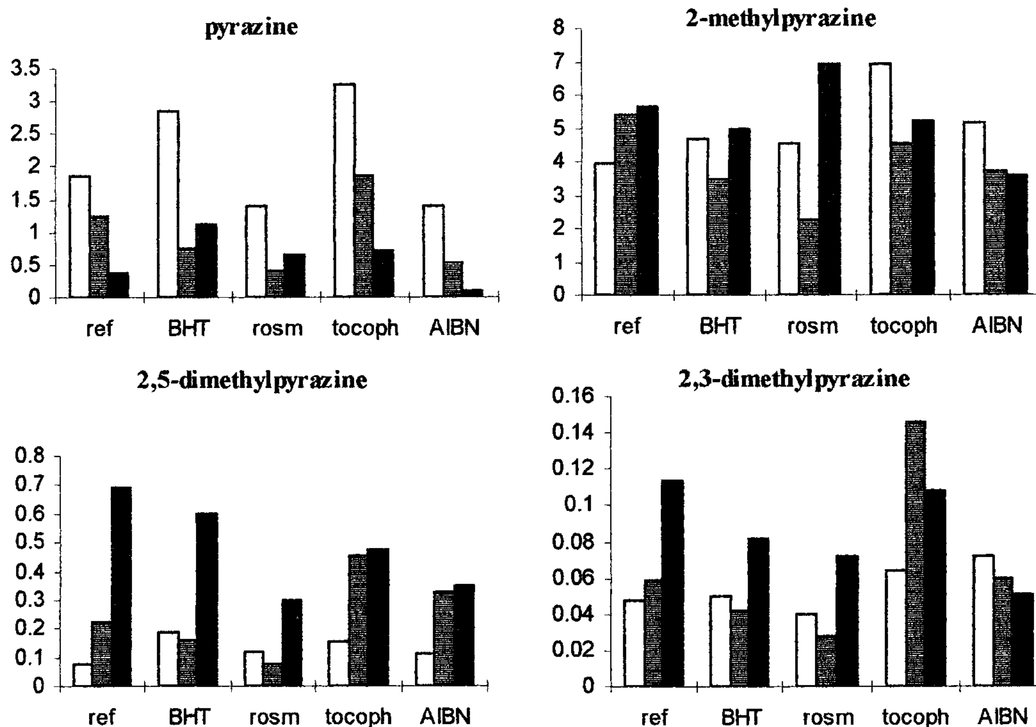

Figure 1. Pyrazines formed in xylose–lysine model systems with added olive (white bar), canola (grey bar), or sunflower oil (black bar).

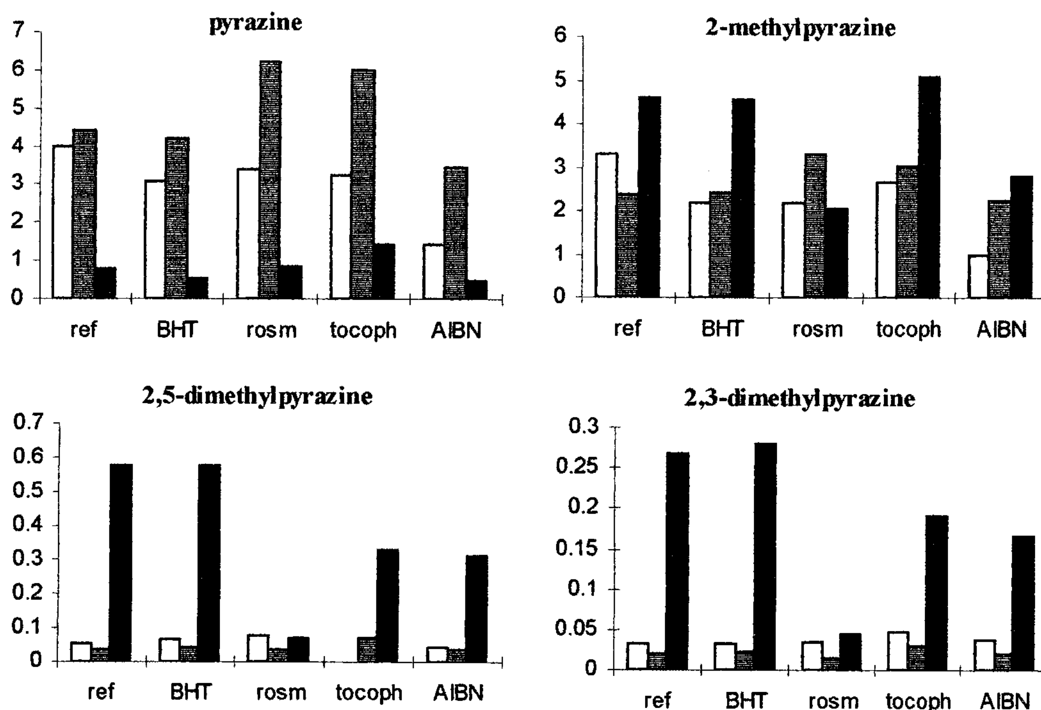

Figure 2. Pyrazines formed in glucose–lysine model systems with added olive (white bar), canola (grey bar), or sunflower oil (black bar).

zine, trimethylpyrazine, and 2-furanmethanol. AIBN decreased pyrazine and 2-methylpyrazine, whereas it increased 2,5-dimethylpyrazine and trimethylpyrazine.

With sunflower oil (Table 4), the addition of BHT increased pyrazine and 2-furanmethanol; rosemary extract decreased 2,5-dimethylpyrazine, 2,3-dimethylpyrazine, and trimethylpyrazine, whereas it slightly increased pyrazine and 2-methylpyrazine; tocopherol slightly decreased 2,5-dimethylpyrazine and increased pyrazine; and AIBN produced a significant decrease of all pyrazines and 2-furanmethanol.

In Figure 1, that permits comparison of the three oils, it appears clearly that the trends of 2,5-dimethylpyrazine and 2,3-dimethylpyrazine are very similar, because they were produced in a lower amount with rape-seed oil and olive oil than with sunflower oil. The addition of the additives was less effective than the changing of the oil: with the exception of the combination tocopherol/canola oil, they were ineffective or decreased these two pyrazines. The decrease was particularly evident with sunflower oil and in the model systems containing rosemary extract. Pyrazine, on the contrary,

Table 2. Volatile Compounds Formed in Xylose−Lysine Model Systems with Olive Oil at pH 6 in the presence of AIBN (0.36 mmol) or an Antioxidant (0.3 mmol)[a]

compounds (mg)	reference	BHT	tocopherol	rosemary extract	AIBN
pyrazine	1.85 ± 0.39	2.84 ± 1.03	3.26 ± 1.207	1.39 ± 0.89	1.41 ± 0.74
2-MP	3.92 ± 1.522	4.64 ± 2.331	6.92 ± 2.024	4.57 ± 2.04	5.17 ± 2.73
2, 3-DMP	0.047 ± 0.016	0.050 ± 0.020	0.064 ± 0.056	0.040 ± 0.014	0.072 ± 0.059
2, 5-DMP	0.075 ± 0.056	0.190 ± 0.080	0.156 ± 0.084	0.117 ± 0.046	0.114 ± 0.063
2-FA	0.036 ± 0.021	0.068 ± 0.042	0.050 ± 0.048	0.059 ± 0.042	0.032 ± 0.025
2-FM	0.144 ± 0.116	0.356 ± 0.008	0.371 ± 0.332	0.324 ± 0.286	0.195 ± 0.129
octanal	0.033 ± 0.023	0.034 ± 0.018	0.014 ± 0.009	0.041 ± 0.032	0.023 ± 0.007
nonanale	0.119 ± 0.041	0.075 ± 0.061	0.108 ± 0.014	0.083 ± 0.038	0.109 ± 0.009
Z-2-decenale	0.120 ± 0.021	0.260 ± 0.150	0.155 ± 0.032	0.525 ± 0.186.	0.193 ± 0.047

[a] Amounts expressed as mg/model system. Means and standard errors ($P < 0.5\%$) were obtained in at least four experiments.

Table 3. Volatile Compounds Formed in Xylose−Lysine Model Systems with Canola Oil at pH 6 in the Presence of AIBN (0.36 mmol) or an Antioxidant (0.3 mmol)[a]

compounds (mg)	reference	BHT	tocopherol	rosemary extract	AIBN
pyrazine	1.23 ± 0.76	0.737 ± 0.257	1.86 ± 0.906	0.411 ± 0.263	0.521 ± 0.400
2-MP	5.41 ± 0.96	3.44 ± 1.34	4.52 ± 1.74	2.26 ± 0.68	3.67 ± 1.22
2, 5-DMP	0.224 ± 0.115	0.159 ± 0.090	0.451 ± 0.220	0.078 ± 0.054	0.33 ± 0.300
2, 3-DMP	0.058 ± 0.020	0.042 ± 0.015	0.146 ± 0.032	0.028 ± 0.022	0.060 ± 0.027
2-FM	0.036 ± 0.0045	0.025 ± 0.018	0.062 ± 0.051	0.017 ± 0.010	0.060 ± 0.044
TMP	0.043 ± 0.037	0.024 ± 0.009	0.145 ± 0.079	0.0095 ± 0.0078	0.087 ± 0.080
nonanal	nd	nd	nd	nd	0.0025 ± 0.0015

[a] Amounts expressed as mg/model systems. Means and standard errors ($P < 0.5\%$) were obtained in at least four experiments. nd, not detected.

Table 4. Volatile Compounds Formed in Xylose−Lysine Model Systems with Sunflower Oil at pH 6 in the Presence of AIBN (0.36 mmol) or an Antioxidant (0.3 mmol)[a]

compounds (mg)	reference	BHT	tocopherol	rosemary extract	AIBN
pyrazine	0.284 ± 0.182	1.10 ± 0.71	0.703 ± 0.201	0.659 ± 0.354	0.101 ± 0.058
2-MP	5.66 ± 2.148	4.96 ± 0.834	5.24 ± 1.610	6.93 ± 3.365	3.60 ± 0.822
2, 5-DMP	0.690 ± 0.306	0.600 ± 0.446	0.474 ± 0.376	0.302 ± 0.150	0.347 ± 0.276
2, 3-DMP	0.114 ± 0.044	0.082 ± 0.027	0.108 ± 0.088	0.072 ± 0.046	0.055 ± 0.028
2-FM	0.024 ± 0.020	0.095 ± 0.031	0.050 ± 0.020	0.034 ± 0.020	0.008 ± 0.004
TMP	0.163 ± 0.055	0.136 ± 0.072	0.100 ± 0.075	0.054 ± 0.032	0.078 ± 0.041

[a] Amounts expressed as mg/model system. Means and standard errors ($P < 0.5\%$) were obtained in at least four experiments.

Table 5. Volatile Compounds Formed in Glucose−Lysine Model Systems with Olive Oil at pH 6 in the Presence of AIBN (0.36 mmol) or an Antioxidant (0.3 mmol)[a]

compound (mg)	reference	BHT	tocopherol	rosemary extract	AIBN
pyrazine	3.99 ± 1.38	3.07 ± 0.90	3.23 ± 1.02	3.39 ± 0.76	1.42 ± 0.62
2-MP	3.30 ± 1.74	2.20 ± 0.42	2.63 ± 0.85	2.20 ± 1.193	0.975 ± 0.555
2, 5-DMP	0.052 ± 0.020	0.066 ± 0.020	0.076 ± 0.029	0.077 ± 0.063	0.040 ± 0.024
2, 3-DMP	0.031 ± 0.014	0.033 ± 0.012	0.048 ± 0.021	0.034 ± 0.029	0.037 ± 0.017
2-FM	0.006 ± 0.006	0.024 ± 0.018	0.055 ± 0.026	0.048 ± 0.057	0.005 ± 0.003
TMP	0.007 ± 0.005	0.007 ± 0.004	0.006 ± 0.001	0.004 ± 0.002	0.002 ± 0.001
nonanal	0.01 ± 0.001	0.023 ± 0.012	0.018 ± 0.008	0.011 ± 0.003	0.017 ± 0.006
Z-2-decenal	0.136 ± 0.013	0.155 ± 0.073	0.074 ± 0.023	0.084 ± 0.042	0.038 ± 0.019

[a] Amounts expressed as mg/model systems. Means and standard errors ($P < 0.5\%$) were obtained in at least four experiments.

was particularly abundant in the model systems with olive oil and increased with the two antioxidants, BHT and tocopherol, whereas it decreased with rosemary extract and AIBN. 2-Methylpyrazine had a borderline behavior because the differences among the oils was less evident. A reduction of all the pyrazines was observed with AIBN and was particularly significant with sunflower oil.

Considering now glucose−lysine model systems (Table 5), with olive oil, BHT decreased pyrazine and 2-methylpyrazine; tocopherol decreased pyrazine, whereas it increased 2,5-dimethylpyrazine and 2,3-dimethylpyrazine; rosemary extract decreased pyrazine, 2-methylpyrazine, and trimethylpyrazine; and AIBN decreased all the pyrazines.

With canola oil (Table 6), BHT had a minimum effect; rosemary extract increased all the pyrazines; tocopherol increased pyrazine and 2-methylpyrazine, whereas it decreased 2,3-dimethylpyrazine. Trimethylpyrazine was very difficult to detect.

With sunflower oil (Table 7), BHT decreased pyrazine, whereas it increased trimethylpyrazine; tocopherol increased pyrazine and 2-methylpyrazine, whereas it decreased 2,5-dimethylpyrazine and 2,3-dimethylpyrazine; rosemary extract increased pyrazine; and AIBN decreased all the compounds.

Figure 2 offers a global overview of all glucose−lysine model systems: the general trend was rather similar to that obtained with xylose−lysine; in fact, 2,5-dimethylpyrazine and 2,3-dimethylpyrazine also with this sugar were formed more with sunflower oil, whereas pyrazine was formed more with olive oil and canola oil, and 2-methylpyrazine had an intermediate trend. The main difference was the inversion between olive oil and canola oil which was observed with pyrazine. BHT and tocopherol had a very small effect, and rosemary extract

Table 6. Volatile Compounds Formed in Glucose−Lysine Model Systems with Canola Oil at pH 6 in the Presence of AIBN (0.36 mmol) or an Antioxidant (0.3 mmol)[a]

compound (mg)	reference	BHT	tocopherol	rosemary extract	AIBN
pyrazine	4.39 ± 1.76	4.20 ± 1.38	6.21 ± 2.08	5.99 ± 1.56	3.42 ± 0.058
2-MP	2.37 ± 0.99	2.44 ± 1.07	3.30 ± 0.60	3.030 ± 1.41	2.23 ± 0.61
2, 5-DMP	0.036 ± 0.023	0.041 ± 0.033	0.037 ± 0.011	0.073 ± 0.047	0.035 ± 0.006
2, 3-DMP	0.021 ± 0.013	0.022 ± 0.020	0.014 ± 0.005	0.030 ± 0.019	0.021 ± 0.004

[a] Amounts expressed as mg/model systems. Means and standard errors ($P < 0.5\%$) were obtained in at least four experiments.

Table 7. Volatile Compounds Formed in Glucose−Lysine Model Systems with Sunflower Oil at pH 6 in the Presence of AIBN (0.36 mmol) or an Antioxidant (0.3 mmol)[a]

compound (mg)	reference	BHT	tocopherol	rosemary extract	AIBN
pyrazine	0.774 ± 0.296	0.547 ± 0.394	1.43 ± 0.79	0.827 ± 0.203	0.459 ± 0.153
2-MP	4.61 ± 0.24	4.56 ± 1.96	5.09 ± 1.18	2.05 ± 0.33	2.80 ± 0.93
2, 5-DMP	0.574 ± 0.251	0.578 ± 0.254	0.332 ± 0.119	0.070 ± 0.021	0.311 ± 0.196
2, 3-DMP	0.268 ± 0.082	0.280 ± 0.116	0.190 ± 0.069	0.044 ± 0.012	0.165 ± 0.089
2-FM	0.005 ± 0.016	nd	nd	nd	nd
TMP	0.053 ± 0.009	0.137 ± 0.060	0.015 ± 0.005	0.005 ± 0.003	0.041 ± 0.026

[a] Amounts expressed as mg/model system. Means and standard errors ($P < 0.5\%$) were obtained in at least four experiments.

Table 8. Data Matrix for the PCA on Both Xylose−Lysine and Glucose−Lysine Model Systems

		xylose − lysine					glucose − lysine					
	oil	refer.	BHT	rosem.	tocoph.	AIBN	refer.	BHT	rosem.	tocoph.	AIBN	label
pyrazine	olive	1.85	2.84	1.39	3.26	1.41	3.99	3.073	3.39	3.23	1.42	OP
	canola	1.23	0.737	0.411	1.86	0.521	4.39	4.20	6.21	5.99	3.42	CP
	sunflower	0.384	1.10	0.659	0.703	0.101	0.774	0.547	0.827	1.43	0.459	SP
2-Me-pyrazine	olive	3.92	4.64	4.57	6.92	5.17	3.30	2.20	2.20	2.63	0.975	O2MP
	canola	5.41	3.44	2.26	4.52	3.67	2.37	2.44	3.30	3.03	2.23	C2MP
	sunflower	5.66	4.96	6.93	5.24	3.60	4.60	4.56	2.05	5.09	2.80	S2MP
2,5-Me_2-pyrazine	olive	0.075	0.19	0.117	0.156	0.114	0.052	0.066	0.076	0.077	0.040	O25DM
	canola	0.224	0.159	0.078	0.451	0.330	0.036	0.041	0.037	0.073	0.035	C25DM
	sunflower	0.69	0.60	0.302	0.474	0.347	0.574	0.578	0.070	0.332	0.311	S25DM
2,3-Me_2-pyrazine	olive	0.047	0.050	0.040	0.064	0.072	0.031	0.033	0.034	0.048	0.037	O23DM
	canola	0.058	0.042	0.028	0.146	0.060	0.021	0.022	0.014	0.030	0.021	C23DM
	sunflower	0.114	0.082	0.072	0.108	0.051	0.268	0.280	0.044	0.190	0.165	S23DM

and AIBN mostly produced a generalized decrease of all the pyrazines.

To better evaluate the effects of the free radical scavengers or initiators, principal component analysis (PCA) was applied to these data. This technique finds the underlying factors (principal components) that influence a chemical system (*18* and *19*). 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 PC's account for smaller and smaller proportions of the variation. If the first two (or three) eigenvalues obtained from the data matrix are large enough that they account for a substantial fraction of the total variance, the overall structure of the data set may be revealed by generating a two- (or three-) dimensional plot of PC_1 vs PC_2 (and possibly vs PC_3).

Often the information 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.

At the beginning, the xylose−lysine and glucose−lysine model systems were analyzed separately, then they were enclosed in the same statistical model. Only the latter results will be discussed here. The data matrix was prepared by putting in each column the data of the pyrazines obtained by adding the three oils on each

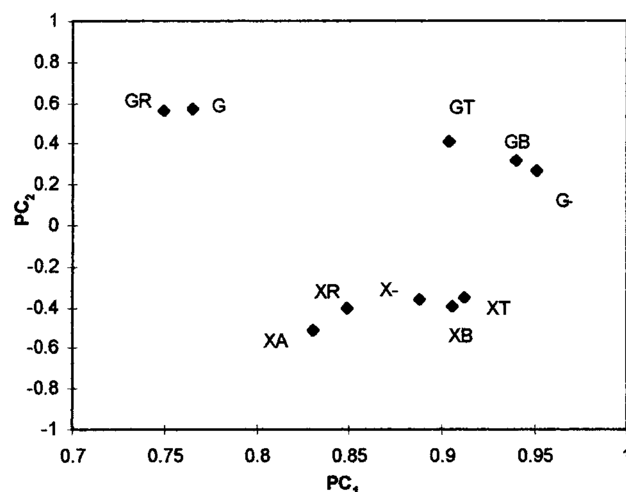

Figure 3. PCA of all the xylose−lysine and glucose−lysine model systems with the different oils and the additives. Loading plot of PC_1 vs PC_2. Labeling: the first letter indicates the sugar (G, glucose; X, xylose), the second one indicates the additive (-, none; A, AIBN; B, BHT; R, rosemary extract; T, tocopherol).

sugar/additive combination (Table 8). PC_1 accounted for 76.0% of the total variance, PC_2 for 18.3%, and PC_3 for only 3.7%. The loading plot (Figure 3) shows very clearly that PC_1, the most statistically significant, can separate very well the model systems without additives or containing BHT or tocoperol, from those containing rosemary extract or AIBN. PC_2, instead, was useful to distinguish the glucose from the xylose model systems.

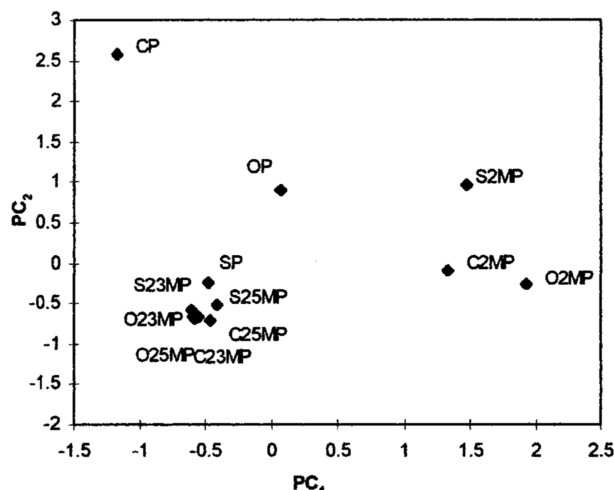

Figure 4. PCA of all the xylose–lysine and glucose–lysine model systems added with the different oils and the additives. Score plot of PC_1 vs PC_2. Labelings: the first letter indicates the oil, the second one indicates the pyrazine (see the last column of Table 10).

This confirmed what was proposed above: that is, that the systems without any additive and those with BHT or tocopherol behave in a similar way, whereas those with rosemary extract resemble those with AIBN.

Figure 4 shows the scores plot that permits comparison of the sensitivity of the volatiles to the presence of the additives. In all the model systems 2,5-dimethylpyrazine and 2,3-dimethylpyrazine are grouped in a very small area, indicating that they have the same behavior in all the oils, whereas pyrazine and 2-methylpyrazine are relatively scattered.

CONCLUSION

In aqueous model systems (*4, 16*), the antioxidants determined an increase, and AIBN determined a decrease, of the formation of the pyrazines. This supports the occurrence of a limited process of autoxidation during the Maillard reaction that could involve the pyrazines themselves or some intermediates necessary for their formation. In the presence of oils, however, the effect of the free radical modulators is much lower, probably because their activity takes place more on the radicals deriving from fatty acids than on those deriving from MRP's. However, as in water, a generalized reduction of the pyrazines was observed with AIBN (highest in the presence of the highly unsaturated sunflower oil), despite the presence of natural antioxidants in these oils.

The most important result of this work was that, in both the xylose and glucose model systems, the oil has a direct effect on the pyrazine profile. In fact, starting from xylose, pyrazine was particularly abundant with olive oil, less with canola oil, and even less in sunflower oil, and starting from glucose it was abundant in canola and olive oil. On the contrary, 2,5-dimethylpyrazine and 2,3-dimethylpyrazine (which have very similar behaviors) with canola and olive oil were produced in lower amounts than with sunflower oil. 2-Methylpyrazine had a border-line behavior, with the differences between the oils being less evident.

It is not very easy to find an explanation for these experimental data. Although during heating the mixtures were kept under good stirring, certainly water and oil are not miscible and all the solutes are partitioned between the two phases depending on their physicochemical characteristics. This phenomenon involves both the end products and the intermediates, and possibly can modify the relative rates of some important steps of the Maillard reaction. The main differences of these oils are their degree of unsaturation and their composition in minor compounds such as natural antioxidants. Nevertheless, taking into account that the external addition of antioxidants or pro-oxidants has a very limited effect, it does not seem probable that minor compounds are responsible for the differences that were observed. Thus, it seems more feasible that the degree of unsaturation is more relevant as it changes, for example, the viscosity and therefore the mass transfer between the two phases and probably influences also the partition of the intermediates or the end products.

Even if it is not easy to explain how the oils influence the pyrazine profile, it appears important to have demonstrated that the choice of the oil influences the aroma of foods not only through its specific volatile compounds but also through a modification of the Maillard reaction.

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