Effect of Pressure on the Maillard Reaction between Ribose and Cysteine in Supercritical Carbon Dioxide

Honggao XU, Wenhao HE, Xuan LIU and Yanxiang GAO

College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, P. R. China

Abstract

Xu H., He W., Liu K., Gao Y. (2010): **Effect of pressure on the Maillard reaction between ribose and cysteine in supercritical carbon dioxide**. Czech J. Food Sci., **28**: 192–201.

An aqueous ribose-cysteine model system, heated at 140°C under supercritical carbon dioxide (SC-CO_2) and supercritical nitrogen (SC-N_2), was investigated with emphasis on the formation of volatile compounds. In general, SC-CO_2 facilitated the overall intermediates accumulation while suppressing the advanced stage of browning. 3-Methyl-1, 2-dithian-4-one increased with increasing SC-CO_2 pressure, and was always more concentrated than in the case of SC-N_2-treatment. The formation of thiols, disulfides, and formyl substituted thiophenes was also promoted in SC-CO_2-treated reaction products, while the effect of high pressure on the individual components followed different patterns. The reversible pH decrease and reinforced acid-base catalysis of 2, 3-enolisation by SC-CO_2 could attribute to the decreased browning and higher amounts of most intense meaty aromatic compounds.

Keywords: supercritical carbon dioxide (SC-CO_2); pressure; Maillard reaction; ribose; cysteine; volatiles

Supercritical carbon dioxide (SC-CO_2), an excellent alternative to conventional organic chemical solvents, has found wide applications in organic synthesis, essential extraction, dyeing, particle formation, etc (Sihvonen *et al.* 1999; Oakes *et al.* 2001; Beckman 2004; Maeda *et al.* 2004; Vemavarapu *et al.* 2005; Herrero *et al.* 2006). Over the past 10 years, the SC-CO_2 processing, one of the novel non-thermal food-processing techniques, has been the subject of intense scrutiny in view of the sterilisation of microorganisms and inactivation of enzymes, providing alternative functional properties and/or conservation of freshness (Damar & Balaban 2006). SC-CO_2 processing, involving pressure below 50 MPa, is now commercially viable for selected foods or food ingredients. For this reason, interest has grown in the chemical and biochemical changes occurring in foods or food ingredients subjected to SC-CO_2, but only a few such studies have been reported concerning the Maillard reaction. Yalpani (1993) demonstrated a high degree of water soluble imine-linked, branched chitosan derivatives conversion in the SC-CO_2-treated mixtures of chitosan and glucose or malto-oligosaccharides. Casal *et al.* (2006) treated lactose-ovine caseinmacropeptide (CMP) and lactose-β-lactoglobulin (β-lg) in static SC-CO_2 conditions at 30 MPa and 50°C for up to 5 h and found that SC-CO_2 did not favour lactosylation of CMP or β-lg, or the formation of the intermediate product, furosine. Xu *et al.* (2008a,b) showed that the amounts of thiols and

Supported by the Natural Science Foundation Committee of China, Project No. 20436020.

furfural in ribose-cysteine system treated with SC-CO_2 at 140°C and 40 MPa were enhanced in comparison with those generated in conventional reaction systems.

Pressure is an important parameter of supercritical fluids. The Maillard reaction under high pressure, especially high hydrostatic pressure (HHP), has been investigated to a certain extent. However, there was not a consistent pattern of pressure on the initial and advanced stages of the Maillard reaction. Hill *et al.* (1996) found that HHP retarded the browning taking place with glucose and lysine in the acidic system, while it accelerated the advanced reaction in alkaline systems with pH values > 8.0. The reduced reaction rate of buffered system was accounted to the decrease of pH with pressure-induced ionisation of the carboxylate group. Moreno *et al.* (2003) also observed a retarded initial stage of the Maillard reaction in pH ≤ 8.0 buffered systems and attributed it to the decrease of pH. Bristow and Isaacs (1999) demonstrated that the formation of Amadori rearrangement product was accelerated by high pressure while its degradation was greatly suppressed, and the maximum concentration of 4-hydroxy-5-methyl-3(2H)-furanone was much diminished. The effect of pressure on the Maillard reaction in SC-CO_2 has to be investigated well before applying such technique in the process of flavours manufacturing, which is based mainly on the Maillard reaction.

To achieve the understanding of high pressure effects on the Maillard reaction, ribose and cysteine, the two most important precursors of meat flavours, were used as a model system and treated in SC-CO_2 at 140°C under high pressure (ranged from 10 MPa to 40 MPa) for one hour. The Maillard reaction of the same model system in supercritical nitrogen (SC-N_2) was also investigated for exploring the possible mechanisms.

MATERIALS AND METHODS

Chemicals. L-Cysteine mono hydrochloride hydrate, sodium pyrophosphate tetrabasic, *n*-hexane, and *n*-heptane were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Sodium pyrophosphate dibasic, alkane standard solution C_8–C_{20}, and tridecane were purchased from Sigma-Aldrich Chemical Co. (Shanghai, China). D-Ribose was purchased from Amresco Inc. (Ohio, USA). The authentic aromatic chemicals used to determine linear retention indices (LRI) were purchased from Sigma-Aldrich (Shanghai, China), and were of the highest purity grade available.

Reactions. D-Ribose and L-cysteine mono hydrochloride (0.1M) in 0.2M pyrophosphate buffer at pH 5.6 were prepared separately. All the Maillard reactions in SC-CO_2 were carried out using a CWYF-2 apparatus (Hua'an Petroleum Scientific Instrument Co., Nantong, China) following the procedures described previously (Xu *et al.* 2008a). To detect the volatiles formed by the Maillard reaction and attributed to SC-CO_2 or high pressure, ribose-cysteine reaction mixtures, with the introducing nitrogen, were heated at 140°C for the same period of time using the same reaction apparatus and procedures. The conventional control experiments were also conducted on the same apparatus without the pressure media. All the samples of the reaction products were stored below –18°C until the analysis.

Analysis. The overall intermediate and final products in the reaction mixtures were measured by a UV-VIS spectrophotometer (UV757CRT, Shanghai Precision & Scientific Instrument Co., China) at 280 nm and 420 nm, respectively (Carabasa-Giribet & Ibarz-Ribas 2000; Komthong *et al.* 2003). Appropriate dilutions were made before the measurements.

The volatile compounds in the reaction mixture as well as the three absorption buffers were analysed by headspace solid-phase microextraction in tandem with gas chromatography coupled to mass spectrometry (HS-SPME-GC-MS). The absorption using DVB/CAR/PDMS fibre (50/30 μm, 1 cm, Supelco Co., Bellefonte, USA) was carried out at 60°C for 20 minutes. The oven temperature was initially set at 40°C, then raised to 60°C at 20°C/min, and held for 5 min, then raised to 250°C at 4°C/min and kept at this temperature for 10 minutes. Approximate quantities of the volatiles from each sample adsorbed with the SPME fibre were estimated by comparing their peak areas, integrated using the TIC signals, with that of the tridecane internal standard (15.13 ng) using a response factor of 1. The concentration and type of the volatile compounds reported were the combined results obtained with the reaction mixture and the three absorption buffers. All the results were expressed as mean values of independent experiments. The mean coefficient of variance (*CV*) for the quantities

of the individual components was < 20%; with the exception of some compounds that were present in relatively small amounts, no compound showed a CV > 47%.

Statistical analysis. The whole experiment was conducted in duplicate and all analyses were done at least in triplicates. The data were analysed by one way analysis of variance (ANOVA) using the OriginPro 7.5 software (OriginLab Corporation, Northampton, USA). Significant differences of means were determined by the Tukey test.

RESULTS AND DISCUSSION

Overall intermediates and final browning products

The trends of the overall intermediate and final products accumulation in the ribose-cysteine model system, treated with different pressure media, were different (Figure 1). In general, SC-CO_2 favoured the formation of intermediates but suppressed the final browning when compared with those generated in SC-N_2 system. In the SC-CO_2-treated samples, the absorbance values at 280 nm showed maximum in the reaction mixtures treated under 10 MPa. When the pressure was elevated to 20 MPa, 30 MPa, and 40 MPa, the concentration of the overall intermediates decreased to not significantly different levels (P > 0.05) as compared with that of the control, and was in a good agreement with the results found by Casal *et al.* (2006). The overall intermediates showed a gradient decrease in SC-N_2-treated reaction mixtures, although the decrease was so small that the slope of the linear fit curve was only –0.002 (where: Abs_{280nm} = –0.002 P + 0.6906, R^2 = 0.9979, Abs_{280nm} = Absorbance at 280 nm, P = pressure). The higher amount of the overall intermediates in the SC-CO_2-treated reaction mixture under 10 MPa could be due to the predominant effect of pH decrease in the reaction matrix with the presence of carbon dioxide (Gevaudan *et al.* 1996). As can be seen from the figure reported by Gevaudan *et al.* (1996), a saturated carbon dioxide system would take place beyond 20 MPa and no further pH decline would happen. The differences between the overall intermediates concentrations in the mixtures treated under 20 MPa, 30 MPa, and 40 MPa, could be overlooked (P > 0.05), while a high pressure (< 40 MPa) showed a less important effect on the overall intermediates accumulation than did carbon dioxide (Figure 1a).

SC-CO_2 had a significant suppressing effect on the nonenzymatic browning over the pressure range studied (Figure 1b). There was a gradient decrease of the absorbance at 420 nm from the conventional control (0.3 MPa) to 20 MPa. The general final browning revealed no significant differences (P > 0.05) between the reaction mixtures treated at 20 MPa, 30 MPa, or 40 MPa. There was

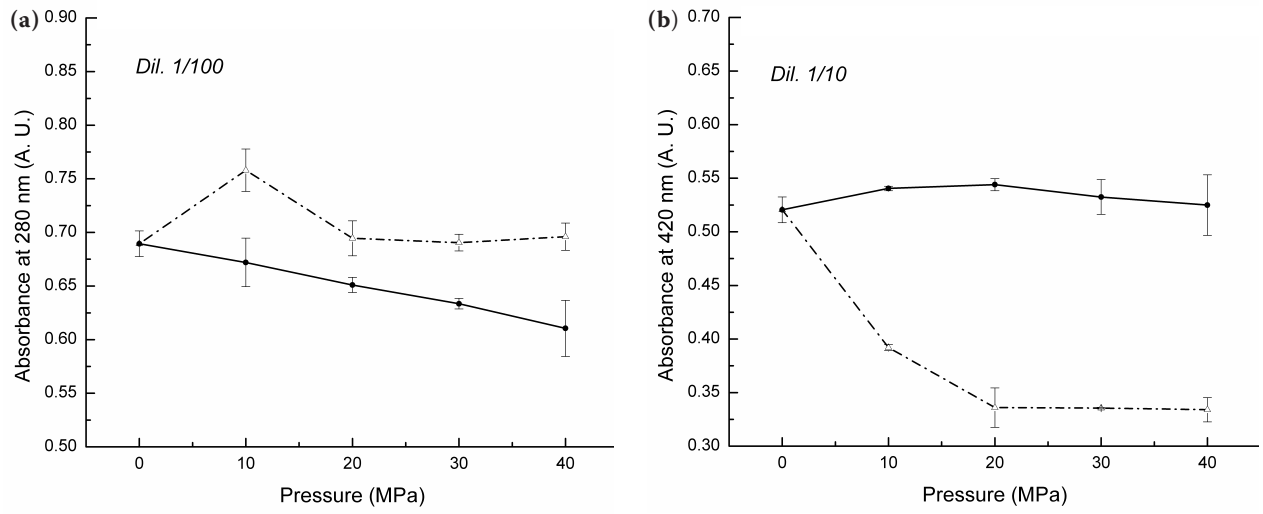

Figure 1. Relative overall intermediate (a) and final (b) products of ribose-cysteine model system in SC-N_2 (solid line) and SC-CO_2 (dash dot line) system under different pressure values. Treatments were carried out in duplicate, each sample underwent triplicate analyses at 280 nm and 420 nm with different dilutions. Error bars represent ± 1 standard deviation, n = 2

no significant effect either ($P > 0.05$) of high pressure generated with SC-N_2 on the browning of the given model system. These results indicated that high pressure, below 40 MPa, had a small effect on the browning of the ribose-cysteine model system buffered with 0.2M pyrophosphate (pH 5.6). Both Hill *et al.* (1996) and Komthong *et al.* (2003) found that Maillard browning, occurring under the treatment with HHP, increased with the elevation of pH and vice versa. The decrease of pH due to carbon dioxide dissolving in the matrix could account for the decrease of the absorbance at 420 nm of the SC-CO_2-treated samples. With the pressure above 20 MPa, a similar browning extent, occurring in the SC-CO_2-treated mixtures, was probably due to a saturated carbon dioxide solution as described above.

Volatile compounds

The volatile compounds generated in ribose-cysteine model system can be classified as thiols, thiophenes, disulfides, furans, pyrazines, and other sulfur-containing compounds. Typical chromatograms obtained by HS-SPME-GC-MS are shown in Figure 2. All kinds of total volatiles, generated in SC-CO_2 and SC-N_2 at different pressure levels, are shown in Figure 3. The differences between total volatiles at different pressure levels cannot be expressed in a simple way while the differences between different pressure media are easy to figure out, except total thiophenones. The relative amounts of total thiols, total thiophenes, total disulfides, and total polysulfur heterocyclic compounds were higher in SC-CO_2-treated samples than those in the SC-N_2-treated

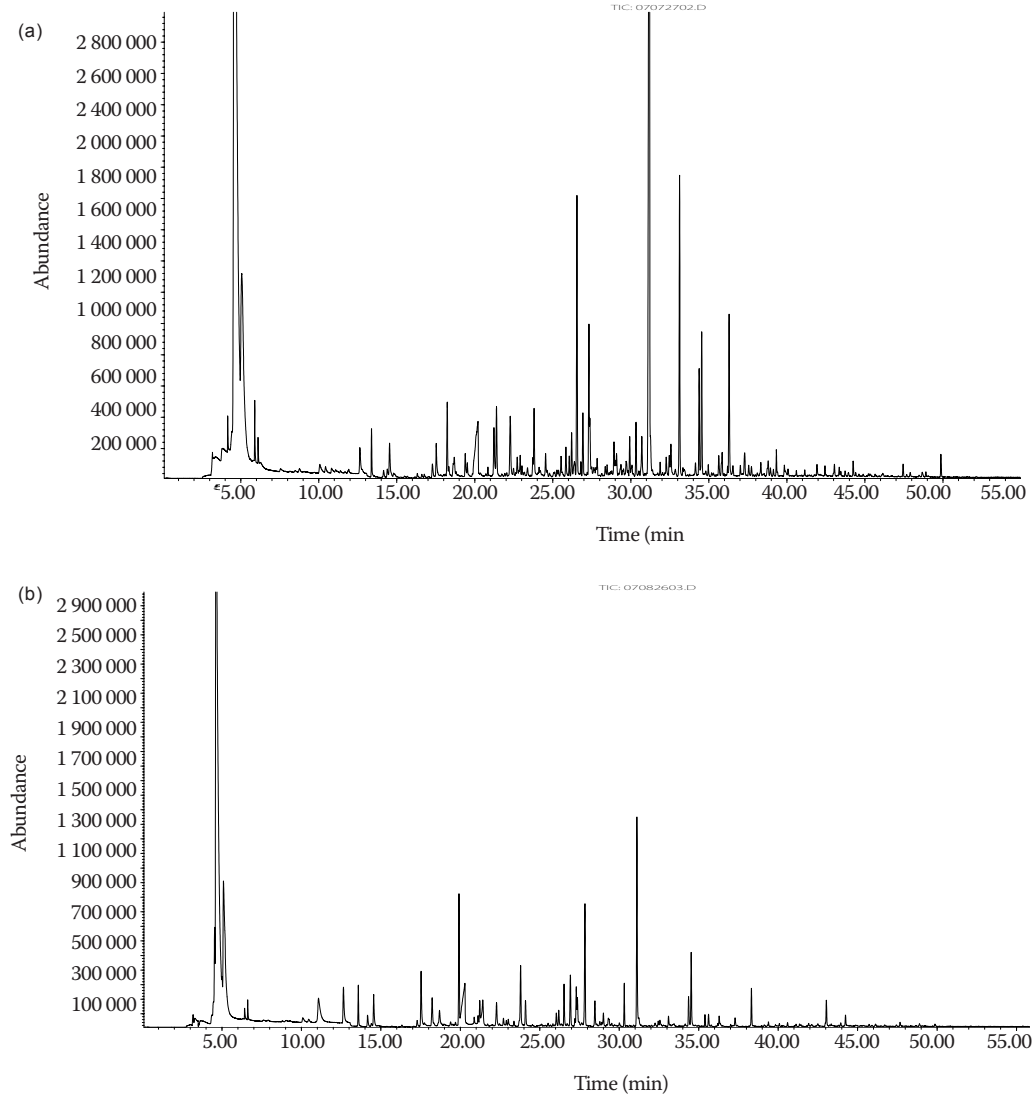

Figure 2. Typical total ion chromatograms of volatiles from ribose-cysteine model system reacted in SC-N_2 (a) and SC-CO_2 (b) system (40 MPa, 140°C, 60 min)

Table 1. Approximate quantities[a] of volatiles identified in the headspace of ribose-cysteine model system[b] under different pressure media

LRI[c]	Compounds	Control	10 MPa		20 MPa		30 MPa		40 MPa		I.D[d]
			SC-CO_2	SC-N_2	SC-CO_2	SC-N_2	SC-CO_2	SC-N_2	SC-CO_2	SC-N_2	
Thiol											
818	3-Mercapto-2-butanone	4.0 ± 0.7	3.4 ± 1.2	4.1 ± 0.2	6.8 ± 2.7	4.9 ± 0.6	6.6 ± 0.4	2.7 ± 0.3	4.5 ± 1.1	3.6 ± 0.0	MS+LRI
869	2-Methyl-3-furanthiol	7.2 ± 2.0	35.8 ± 9.5	7.2 ± 0.2	58.1 ± 9.1	7.0 ± 0.6	46.4 ± 3.5	7.4 ± 1.5	34.6 ± 15.8	7.3 ± 0.3	MS+LRI
904	3-Mercapto-2-pentanone	1.9 ± 0.3	5.9 ± 2.4	2.3 ± 0.8	14.4 ± 5.4	1.8 ± 0.2	13.8 ± 0.5	1.6 ± 0.0	9.8 ± 3.2	1.8 ± 0.1	MS+LRI
909	2-Mercapto-3-pentanone	2.3 ± 0.2	4.7 ± 0.9	2.5 ± 0.4	6.0 ± 2.2	2.4 ± 0.2	4.3 ± 0.1	2.3 ± 0.3	2.6 ± 0.8	2.2 ± 0.1	ms+LRI[1]
913	2-Furanmethanethiol	7.3 ± 1.9	32.9 ± 12.8	10.0 ± 1.4	61.5 ± 18.9	9.6 ± 1.3	43.4 ± 3.6	8.4 ± 1.1	23.9 ± 9.7	7.8 ± 0.1	MS+LRI
980	3-Thiophenethiol	8.0 ± 1.6	24.7 ± 12.5	8.6 ± 0.5	40.1 ± 10.5	9.0 ± 2.4	45.5 ± 3.1	8.6 ± 1.8	41.1 ± 20.0	9.4 ± 0.4	ms+LRI[1]
1066	2-Methyl-3-thiophenethiol	12.3 ± 2.5	25.5 ± 9.7	14.9 ± 1.0	31.5 ± 0.6	17.3 ± 0.8	20.6 ± 1.6	16.2 ± 0.1	15.8 ± 5.6	14.8 ± 0.2	ms+LRI[2]
Total thiols		43.0 ± 8.8	132.8 ± 49.1	49.7 ± 2.2	218.4 ± 49.4	51.9 ± 4.8	180.7 ± 10.8	47.1 ± 4.8	132.2 ± 56.1	46.8 ± 0.0	
Thiophene											
773	2-Methylthiophene	6.9 ± 1.1	2.3 ± 0.2	3.6 ± 1.5	2.0 ± 0.0	1.7 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	0.7 ± 0.1	1.1 ± 0.1	ms+LRI[2]
873	2,5-Dimethylthiophene	7.0 ± 2.7	–	2.3 ± 0.7	–	1.8 ± 0.0	–	1.6 ± 0.2	–	+	ms+LRI[2]
1006	2-Thiophenecarboxaldehyde	3.7 ± 0.2	10.9 ± 1.8	4.3 ± 0.9	11.1 ± 0.2	4.1 ± 0.7	11.3 ± 0.1	4.7 ± 0.3	11.9 ± 1.6	4.4 ± 0.2	ms
1070	2-Formyl-2,3-dihydrothiophene	12.6 ± 0.1	31.6 ± 0.4	14.9 ± 1.8	26.5 ± 1.0	13.2 ± 0.9	23.0 ± 0.9	15.8 ± 0.4	24.3 ± 5.3	15.7 ± 1.0	ms
1095	2-Acetylthiophene	2.5 ± 0.4	1.2 ± 0.1	2.7 ± 0.8	0.8 ± 0.1	2.6 ± 0.6	0.7 ± 0.1	2.6 ± 0.2	0.6 ± 0.2	2.4 ± 0.3	ms+LRI[1]
1100	2-Formyl-5-methyl-2,5-dihydrothiophene	3.0 ± 0.5	5.6 ± 1.3	3.4 ± 0.3	2.9 ± 0.4	3.4 ± 0.7	3.7 ± 0.8	3.5 ± 0.3	4.5 ± 2.0	3.8 ± 0.2	ms
1128	5-Methyl-2-fromylthiophene	15.6 ± 0.0	30.3 ± 3.2	16.1 ± 5.2	29.4 ± 0.7	16.3 ± 2.0	29.8 ± 1.1	18.3 ± 0.1	31.3 ± 2.7	17.4 ± 1.3	ms+LRI[3]
1189	2-Propionylthiophene	9.9 ± 0.3	15.4 ± 0.5	11.3 ± 1.9	9.9 ± 1.0	10.7 ± 1.8	8.5 ± 1.2	11.8 ± 0.4	8.0 ± 1.8	12.1 ± 0.7	ms+LRI[4]
1194	2-Acetyl-3-methylthiophene	6.9 ± 1.1	5.6 ± 1.1	7.8 ± 2.0	5.8 ± 0.8	7.2 ± 1.5	3.0 ± 0.5	6.7 ± 0.5	–	6.5 ± 0.1	MS+LRI
1208	3-ethyl-2-formylthiophene	8.9 ± 2.2	28.1 ± 1.6	13.2 ± 0.4	30.2 ± 1.3	18.0 ± 1.9	26.7 ± 7.0	17.3 ± 1.7	26.3 ± 5.1	14.2 ± 0.4	MS+LRI
1289	3-Acetyl-2,5-dimethylthiophene	6.3 ± 0.7	4.2 ± 1.8	7.4 ± 0.0	1.1 ± 0.0	11.7 ± 1.0	0.5 ± 0.1	10.0 ± 0.2	–	9.1 ± 0.1	ms
Total thiophenes		83.3 ± 7.5	135.2 ± 1.9	87.0 ± 15.5	119.7 ± 0.2	90.8 ± 11.2	108.0 ± 9.9	93.4 ± 0.2	107.7 ± 18.9	86.7 ± 3.8	
Thiophenone											
959	Dihydro-3(2H)-thiophenone	1.1 ± 0.0	0.6 ± 0.1	1.1 ± 0.2	0.6 ± 0.0	1.4 ± 0.2	0.4 ± 0.1	1.3 ± 0.3	tr	1.3 ± 0.3	MS+LRI
996	Dihydro-2-methyl-3(2H)-thiophenone	15.4 ± 1.9	23.6 ± 0.5	17.8 ± 3.5	19.3 ± 1.3	22.2 ± 1.0	16.4 ± 0.6	22.7 ± 0.3	14.0 ± 3.0	22.7 ± 1.1	MS+LRI
1026	4,5-Dihydro-2,4-dimethyl-3(2 H)-thiophenone (*E* or *Z*)	4.4 ± 0.3	2.3 ± 1.2	4.1 ± 1.0	–	5.1 ± 0.4	–	5.1 ± 0.5	–	5.2 ± 0.4	ms+LRI[1]
Total thiophenones		20.9 ± 2.2	26.5 ± 0.7	23.0 ± 4.7	19.9 ± 1.3	28.8 ± 1.5	16.9 ± 0.6	29.1 ± 1.1	14.0 ± 3.0	29.2 ± 1.3	
Fused bicyclic compound											
1197	2,3-Dihydro-6-methylthieno[2,3-*c*]furan	36.1 ± 2.7	55.4 ± 9.3	44.4 ± 0.8	47.3 ± 10.0	72.7 ± 4.2	26.7 ± 2.0	75.9 ± 2.0	18.4 ± 2.9	73.6 ± 2.1	ms+LRI[1]
1215	a-Thienothiophene	2.6 ± 0.1	–	3.5 ± 0.1	–	2.5 ± 0.4	1.9 ± 0.1	2.4 ± 0.4	3.4 ± 1.4	2.6 ± 0.3	ms
1220	Thieno[2,3-*b*]thiophene	19.6 ± 0.4	16.9 ± 5.3	17.9 ± 2.9	16.2 ± 2.0	21.1 ± 2.0	12.3 ± 0.8	16.8 ± 0.2	15.9 ± 1.4	14.4 ± 0.4	ms+LRI[1]
1262	Thieno[3,2-*b*]thiophene	10.4 ± 0.3	2.1 ± 0.4	9.1 ± 2.2	2.5 ± 0.3	10.2 ± 1.5	1.1 ± 0.5	8.9 ± 0.3	1.1 ± 0.5	8.2 ± 0.4	ms
1306	2-Methylthieno[2,3-*b*]thiopene	1.7 ± 0.0	1.1 ± 0.1	2.1 ± 0.1	0.9 ± 0.1	5.4 ± 2.1	0.6 ± 0.1	1.4 ± 0.1	0.6 ± 0.1	1.4 ± 0.1	ms
1311	a-Dihydrothienothiophene	11.8 ± 0.2	0.7 ± 0.0	14.3 ± 1.0	–	16.5 ± 2.5	–	14.7 ± 0.2	–	13.6 ± 0.5	ms+LRI[1]
1325	a-Dihydrothienothiophene	487.8 ± 17.3	217.9 ± 4.2	444.0 ± 66.8	147.8 ± 0.4	520.0 ± 62.3	107.5 ± 17.8	505.2 ± 0.4	97.2 ± 17.2	463.6 ± 17.7	ms
1364	2-Methylthieno[3,2-*b*]thiopene	21.2 ± 0.4	13.6 ± 0.2	15.0 ± 2.6	10.7 ± 1.7	17.3 ± 2.0	7.3 ± 0.4	14.6 ± 0.1	6.3 ± 1.0	13.8 ± 0.1	ms
1381	a-Methyldihydrothienothiophene	85.9 ± 3.4	28.9 ± 5.4	72.4 ± 4.5	15.6 ± 1.2	98.6 ± 8.0	7.3 ± 1.6	82.6 ± 1.2	3.8 ± 0.1	74.8 ± 3.1	ms+LRI[1]

1418	a-Methyldihydrothienothiophene	31.4 ± 1.1	27.0 ± 5.5	28.8 ± 4.6	18.2 ± 0.2	34.3 ± 3.9	15.1 ± 4.9	32.0 ± 0.2	13.5 ± 3.2	29.6 ± 1.0	ms+LRI[1]
1423	a-Methyldihydrothienothiophene	43.1 ± 1.2	46.5 ± 6.4	36.3 ± 5.8	43.2 ± 0.1	44.0 ± 5.3	35.0 ± 11.1	41.5 ± 0.1	32.3 ± 5.9	38.9 ± 0.4	ms+LRI[1]
1477	a-Dimethyldihydrothienothiophene	44.0 ± 0.5	28.8 ± 6.3	34.6 ± 5.4	16.6 ± 1.3	51.2 ± 4.1	8.4 ± 2.3	41.6 ± 0.4	5.3 ± 0.5	37.7 ± 3.2	ms
	Total fused bicyclic compounds	795.7 ± 20.6	438.9 ± 43.8	722.4 ± 96.5	319.0 ± 4.6	893.8 ± 89.1	223.3 ± 42.6	837.6 ± 0.4	197.8 ± 35.0	772.3 ± 12.3	
Polysulfur heterocyclic compounds											
1153	3,5-Dimethyl-1,2,4-trithiolane (*E* or *Z*)	7.8 ± 2.0	–	4.4 ± 2.2	–	3.0 ± 0.1	–	1.8 ± 0.6	–	1.8 ± 0.0	MS[5]
1160	3,5-Dimethyl-1,2,4-trithiolane (*E* or *Z*)	9.5 ± 2.1	–	6.1 ± 2.5	–	4.6 ± 0.3	–	1.8 ± 0.4	–	1.3 ± 0.5	MS[5]
1185	1,2-Dithian-4-one	4.6 ± 0.3	8.7 ± 0.8	5.1 ± 0.5	7.1 ± 0.3	4.9 ± 0.7	5.7 ± 0.2	5.8 ± 0.2	6.9 ± 0.5	6.1 ± 0.3	ms
1232	3-Methyl-1,2-dithian-4-one	4.2 ± 0.4	16.4 ± 0.3	4.6 ± 1.0	45.2 ± 1.4	4.1 ± 0.6	50.8 ± 3.5	4.8 ± 0.1	55.0 ± 8.9	5.4 ± 0.4	MS[5]
1264	3,(5 or 6)-Dimethyl-1,2-dithian-4-one (*E* or *Z*)	3.0 ± 0.2	9.1 ± 1.3	2.6 ± 0.4	8.7 ± 0.2	2.5 ± 0.2	6.7 ± 0.8	3.1 ± 0.1	6.1 ± 1.1	3.4 ± 0.1	ms
1266	3-Methyl-1,2,4-trithiane	5.7 ± 0.0	–	8.2 ± 2.9	–	9.0 ± 2.2	–	7.1 ± 1.0	–	6.2 ± 0.9	MS[5]
1274	3, (5 or 6)-Dimethyl-1,2-dithian-4-one (*E* or *Z*)	3.2 ± 0.7	4.8 ± 2.4	4.0 ± 1.4	3.3 ± 0.2	3.1 ± 1.1	2.5 ± 0.4	4.4 ± 0.7	2.3 ± 0.2	4.6 ± 1.1	ms
Total polysulfur heterocyclics		37.9 ± 2.4	39.0 ± 4.8	35.0 ± 12.8	64.3 ± 1.5	31.2 ± 5.1	65.7 ± 4.1	28.8 ± 3.4	70.2 ± 13.7	28.8 ± 1.3	
Disulfide											
1542	bis(2-Methyl-3-furyl)disulfide	3.0 ± 0.4	20.9 ± 0.2	2.9 ± 0.3	30.6 ± 2.2	3.2 ± 0.1	23.2 ± 1.0	4.0 ± 0.4	18.6 ± 8.6	3.6 ± 0.1	MS+LRI
1578	3-[(2-Methyl-3-furyl)dithio]-2-pentanone	–	2.6 ± 0.4	–	4.2 ± 0.8	–	3.6 ± 0.9	–	3.3 ± 1.0	–	LRI[6]
1643	2-Methyl-3-[(2-furfuryl)dithio]furan	–	1.6 ± 0.1	–	1.8 ± 0.2	–	2.5 ± 0.8	–	2.3 ± 1.2	–	LRI[6]
1668	3-(2-furfuryldithio)-2-pentanone	–	–	–	tr	–	tr	–	tr	–	LRI[6]
1702	2,3-Dihydro-5-methyl-4-[(2-methyl-3-furyl)dithio]furan	2.5 ± 0.2	10.0 ± 1.1	2.6 ± 0.2	14.6 ± 1.9	3.1 ± 0.4	16.1 ± 0.8	3.2 ± 0.5	16.0 ± 7.6	3.6 ± 0.3	ms
1745	2-Methyl-3-[(2-methyl-3-thienyl)dithio]furan	2.9 ± 0.7	11.8 ± 1.6	3.3 ± 0.4	14.1 ± 2.8	3.5 ± 0.5	7.8 ± 0.6	3.3 ± 1.0	6.2 ± 2.7	3.3 ± 0.3	LRI[1]
Total disulfides		8.4 ± 1.2	47.0 ± 2.0	8.9 ± 0.5	65.4 ± 7.9	9.7 ± 1.1	53.3 ± 0.7	10.5 ± 1.8	46.5 ± 21.0	10.5 ± 0.1	
Miscellaneous											
825	Methylpyrazine	3.0 ± 0.3	–	2.2 ± 0.3	–	2.0 ± 0.5	–	1.9 ± 0.1	–	1.4 ± 0.1	MS+LRI
832	Frufural	2.0 ± 0.3	9.1 ± 0.1	1.9 ± 0.3	23.0 ± 1.1	1.6 ± 0.3	27.9 ± 2.4	1.7 ± 0.1	34.5 ± 3.0	1.3 ± 0.2	MS+LRI
850	2-Furanmethanol	2.1 ± 0.1	1.5 ± 0.5	1.9 ± 0.3	1.6 ± 0.1	1.7 ± 0.0	–	2.1 ± 0.2	–	1.9 ± 0.1	MS+LRI
952	1-(2-Furyl)-2-propanone	1.5 ± 0.1	3.1 ± 0.5	1.0 ± 0.1	3.4 ± 0.0	0.9 ± 0.1	2.1 ± 0.1	+	1.6 ± 0.5	+	ms
1022	2-Acetylthiazole	6.5 ± 0.1	3.1 ± 0.3	6.8 ± 1.5	1.8 ± 0.1	7.3 ± 1.2	2.0 ± 0.2	6.8 ± 0.5	1.6 ± 0.2	5.9 ± 0.4	MS+LRI
1075	4-hydroxy-5-methyl-3(2H)-furanone	38.7 ± 2.6	56.0 ± 6.9	45.4 ± 10.2	39.1 ± 14.3	35.1 ± 15.7	52.9 ± 10.1	51.4 ± 20.7	71.8 ± 23.1	33.5 ± 3.9	MS+LRI
Total miscellaneous		53.7 ± 2.9	72.8 ± 5.7	59.1 ± 10.6	68.8 ± 13.5	48.6 ± 17.9	84.9 ± 12.2	63.9 ± 21.4	109.5 ± 26.8	44.1 ± 3.1	
Grand total		1042.9 ± 37.9	892.2 ± 93.2	984.9 ± 140.4	875.4 ± 59.5	1154.9 ± 130.8	732.7 ± 56.5	1110.5 ± 19.6	677.9 ± 174.6	1018.4 ± 12.8	

[a]Approximate quantities in headspace (ng/ml of mixture) given as means of independent experiments; tr = trace (< 0.3 ng/ml of mixture); – = below detection limit (~ 0.07 ng/ml of mixture); += present in small amounts and quantification confounded by adjacent peak; [b]Each model system consisted of 5 mmol ribose and 5 mmol cysteine in 100 ml 0.2M pyrophosphate buffer; [c]Linear retention index; [d]MS+LRI = mass spectrum and LRI agree with those of authentic compound analysed in our laboratory; ms+LRI = mass spectrum agrees with the standard spectra in NIST 98 or Wiley 7n Mass Spectral Databases and the LRI near to the literature cited; MS = mass spectrum agrees with literature spectrum; ms = mass spectrum agrees with the standard spectra in NIST 98 or Wiley 7n Mass Spectral Databases. Compounds identified with references are indicated by superscript numbers: (1) Whitfield and Mottram (1999), (2) Ames *et al.* (2001), (3) Parker *et al.* (2000), (4) Mottram and Whitfield (1995); (5) Zhang *et al.* (1988); (6) Mottram *et al.* (1995)

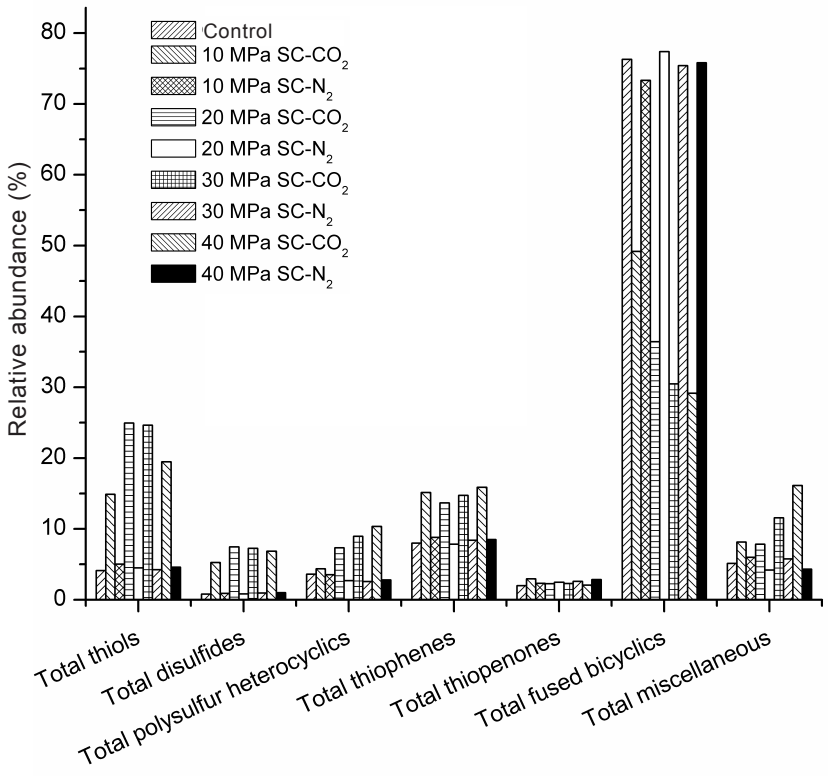

Figure 3. Relative abundances of each class volatiles for ribose-cysteine reaction products at different pressures

ones. However, total fused bicyclic compounds were favoured in SC-N_2 and conventional control systems. The characteristics of the volatiles given in Table 1, as formed at various pressure levels and in different media, are discussed below.

Key intermediates. Furfural and 4-hydroxy-5-methyl-3(2H)-furanone are the two key intermediates in the Maillard reaction where pentoses are involved. Furfural increased with the pressure increasing in the SC-CO_2-treated reaction mixtures. By comparison, furfural in the SC-N_2-treated samples was present at such a low level as was that detected in the control. Based on the lower degradation rate of ribose in SC-CO_2 (data not shown), the decrease of pH in the SC-CO_2-treated matrices during the reaction could inhibit the advanced stage of the Maillard reaction and furfural accumulation. 4-Hydroxy-5-methyl-3(2H)-furanone is derived from the Amadori compound via 2, 3-enolisation pathway favoured in high pH systems (Mottram & Nobrega 2002). At first sight, a slight discrepancy occurred between the decreasing pH in the SC-CO_2 system and the high 4-hydroxy-5-methyl-3(2H)-furanone accumulation. Following the conclusion of Mottram and Nobrega (2002), 2,3-enolisation of Amadori compound was catalysed by buffers and the effect of catalysis was greater than any effect of pH. The reinforced catalysis by carbon dioxide favoured the accumulation of 4-hydroxy-5-methyl-3(2H)-furanone. The high amounts of the two key intermediates indicated that they were produced simultaneously, or the Amadori compound degradation underwent the enolisation pathways via 1, 2-enol and 2, 3-enol in parallel.

Thiols. The mercapto group containing volatiles, including 2-furanmethanethiol, 2-methyl-3-furanthiol, 3-thiophenethiol, 2-methyl-3-thiophenethiol, 3-mercapto-2-pentanone, and 3-mercapto-2-butanone, are always characterised by extremely low thresholds and meat-like, sulphury, roast odour quality. As shown in Table 1, there are more thiols generated in the SC-CO_2-treated samples than in SC-N_2-treated ones. With the increasing pressure, the quantities of thiols in the SC-CO_2-treated mixtures increased to a peak and then dropped, the pressure corresponding to the maximum point of the thiols being at 20 MPa except for 3-thiophenethiol (30 MPa). There were no significant differences ($P > 0.05$) between the quantities of 2-furanmethanethiol and 2-methyl-3-furanthiol in the SC-CO_2-treated ribose-cysteine mixtures. 2-Furanmethanethiol was produced from hydrogen sulfide with furfural, and 2-methyl-3-furanthiol was produced from 4-hydroxy-5-methyl-3(2H)-furanone above 140°C (Van Den Ouweland &

Peer 1975; Hofmann & Schieberle 1998; Whitfield & Mottram 1999). The higher amounts of the two key intermediates in the SC-CO_2-treated mixtures, as described above, could account for these results. The pressure caused by SC-N_2 showed a small effect on the accumulation of thiols in comparison with the data detected in the conventional control reaction mixtures.

Thiophenes. Thiophene and its substituted derivatives were an important category of compounds found in aqueous ribose-cysteine reaction products. High pressure did not show a consistent pattern in their formation. An interesting phenomenon was that nearly all the formyl group substituted thiophenes found in the SC-CO_2-treated samples were larger than those found in the SC-N_2-treated and control samples, such as 2-thiophenecarboxaldehyde, 3-methyl-2-formylthiophene, 2-formyl-2,3-dihydrothiophene, 3-ethyl-2-formylthiophene, etc. With the increasing pressure, the increasing densities of carbon dioxide and nitrogen in each system seemed to have no apparent effects on their formation. The probable explanation for such different results may be the higher concentrations of 4-hydroxy-5-methyl-3(2H)-furanone, a precursor of formyl thiophenes (Whitfield & Mottram 1999), detected in the SC-CO_2-treated mixtures.

Fused bicyclic compounds. Thienothiophenes and thienofurans are two main fused bicyclic compounds detected in ribose-cysteine reaction mixtures. Different pressure media showed different patterns of the individual fused bicyclic components. Quantitatively, one dihydrothienothiophene (LRI = 1325), contained in the highest concentration of the volatiles identified in all the systems, decreased with the pressure increasing in SC-CO_2 system, while in SC-N_2, a peak value (520 ng/ml) was reached under 20 MPa and then decreased with the pressure increasing. The introduction of SC-CO_2 during the Maillard reaction alleviated the polymerisation of thiophene and its derivatives in comparison with those generated in the conventional and SC-N_2-treated systems. The decrease of pH in the SC-CO_2-treated matrices may be the dominant reason for this phenomenon considering the acceleration of polymerisation at high pressure. In the SC-N_2-treated samples, the amount of 2,3-dihydro-6-methylthieno[2,3-*c*]furan increased with the pressure of up to 30 MPa and then decreased. As concerns the samples treated with SC-CO_2, a gradual decrease of the amount of 2,3-dihydro-6-methylthieno[2,3-*c*]furan occurred and then declined even below that detected in the conventional control. The gradient decrease of the amount of 2,3-dihydro-6-methylthieno[2,3-*c*]furan, which is favoured at higher pH (Xu *et al.* 2008a), could be a combined effect of the pH decrease and homogeneous phase reaction.

Disulfides. Disulfides are dimers of thiols, their extremely low thresholds impart them a high potency in the odour profile of flavourings. The covalent bond formation of disulfides was more ready in SC-CO_2 than in SC-N_2, the largest amount of disulfides being presented by bis(2-methyl-3-furyl)disulfide which had a peak value of 30.60 ng/ml at 20 MPa and then declined with the pressure further increasing. The data suggest that the high concentration of disulfides in the SC-CO_2-treated samples can be ascribed to their high quantity of monomers.

Polysulfur heterocyclics. Polysulfur heterocyclics, such as 3,5-dimethyl-1,2,4-trithiolane (*E* or *Z*), are the characteristic identifiers of the condensation of the thermal breakdown products of cysteine (Chen & Ho 2002). In SC-CO_2-treated mixtures, large amount of carbon dioxide could terminate the Strecker degradation of cysteine (Xu *et al.* 2008b), no 3,5-dimethyl-1,2,4-trithiolane (*E* or *Z*) having been detected. A decline of 3,5-dimethyl-1,2,4-trithiolane (*E* or *Z*) in SC-N_2-treated samples also occurred due to high pressure inhibiting the cleavage of cysteine. 3-Methyl-1,2-dithian-4-one, one of the different volatiles detected in the reaction mixtures on the treatment with SC-N_2 and SC-CO_2, drastically increased in the SC-CO_2-treated samples and increased progressively with the elevating of carbon dioxide pressure. It could be detected in model systems containing thiamine (Hartman *et al.* 1984; Güntert *et al.* 1990; Cerny & Briffod 2007) at different pH values and temperatures, and also be found in "ribose-cysteine" model system, which was heated above 140°C (Farmer *et al.* 1989; Meynier & Mottram 1995). The mechanism of 3-methyl-1,2-dithian-4-one formation was suggested to be similar to that of 2-methyl-4,5-dihydro-3(2H)-thiophenone (Hartman *et al.* 1984), while the low pH values inhibited its formation (Farmer *et al.* 1989). In the present study, the higher concentrations of 4-hydroxy-5-methyl-3(2H)-furanone under different pressure levels in SC-CO_2 could account for the high levels of 3-methyl-1,2-dithian-4-one. In the SC-N_2-treated mixtures, 3-methyl-1,2, 4-trithiane amount first increased up to 20 MPa,

and then declined. While in the SC-CO_2 system no 3-methyl-1,2,4-trithiane was detected.

In summary, SC-CO_2 treatment could result in fewer browning reaction products. The level of the overall intermediates reached the maximum at 10 MPa and then decreased to that usual in the conventional control. There was no significant effect of high pressure on the overall intermediate and final products amounts in the saturated carbon dioxide system within the pressure range studied. Except thiols, the quantities of furfural, 4-hydroxy-5-methyl-3(2H)-furanone, 3-methyl-1,2-thian-4-one, and formyl substituted thiophenes, as determined in SC-CO_2, were larger than those obtained in SC-N_2 treatment. This could be due to the reinforced 2,3-enolisation for Amadori compound degradation by carbon dioxide dissolving in the matrices. The high pressure within the range studied showed no significant effect on the accumulation of most volatiles, while the high quantity of high potency aromatic compounds and fewer browning products, generated in the SC-CO_2 treatment, indicate that SC-CO_2 has a potential application in manufacturing of process flavours.

References

Ames J.M., Guy R.C.E., Kipping G.J. (2001): Effect of pH and temperature on the formation of volatile compounds in cysteine/reducing sugar/starch mixtures during extrusion cooking. Journal of Agricultural and Food Chemistry, **49**: 1885–1894.

Beckman E.J. (2004): Supercritical and near-critical CO_2 in green chemical synthesis and processing. The Journal of Supercritical Fluids, **28**: 121–191.

Bristow M., Isaacs N.S. (1999): The effect of high pressure on the formation of volatile products in a model Maillard reaction. Journal of the Chemical Society, Perkin Transactions, **2**: 2213–2218.

Carabasa-Giribet M., Ibarz-Ribas A. (2000): Kinetics of colour development in aqueous glucose systems at high temperatures. Journal of Food Engineering, **44**: 181–189.

Casal E., Ramírez P., Ibañez E., Corzo N., Olano A. (2006): Effect of supercritical carbon dioxide treatment on the Maillard reaction in model food systems. Food Chemistry, **97**: 272–276.

Cerny C., Briffod M. (2007): Effect of pH on the Maillard reaction of [$^{13}C_5$] xylose, cysteine, and thiamin. Journal of Agricultural and Food Chemistry, **55**: 1552–1556.

Chen Y., Ho C.T. (2002): Effect of carnosine on volatile generation from Maillard reaction of ribose and cysteine. Journal of Agricultural and Food Chemistry, **50**: 2372–2376.

Damar S., Balaban M.O. (2006): Review of dense phase CO_2 technology: microbial and enzyme inactivation, and effects on food quality. Journal of Food Science, **71**: R1–R11.

Farmer L.J., Mottram D.S., Whitfield F.B. (1989): Volatile compounds produced in Maillard reactions involving cysteine, ribose and phospholipid. Journal of the Science of Food and Agriculture, **49**: 347–368.

Gevaudan S., Lagaude A., Tarodo De La Fuente B., Cuq J.L. (1996): Effect of treatment by gaseous carbon dioxide on the colloidal phase of skim milk. Journal of Dairy Science, **79**: 1713–1721.

Güntert M., Brüning J., Emberger R., Köpsel M., Kuhn W., Thielmann T., Werkhoff P. (1990): Identification and formation of some selected sulfur-containing flavor compounds in various meat model systems. Journal of Agricultural and Food Chemistry, **38**: 2027–2041.

Hartman G.J., Scheide J.D., Ho C.T. (1984): Effect of water activity on the major volatiles produced in a model system approximating cooked meat. Journal of Food Science, **49**: 607–613.

Herrero M., Cifuentes A., Ibañez E. (2006): Sub- and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae and microalgae a review. Food Chemistry, **98**: 136–148.

Hill V.M., Ledward D.A., Ames J.M. (1996): Influence of high hydrostatic pressure and pH on the rate of Maillard browning in a glucose-lysine system. Journal of Agricultural and Food Chemistry, **44**: 594–598.

Hofmann T., Schieberle P. (1998): Quantitative model studies on the effectiveness of different precursor systems in the formation of the intense food odorants 2-furfurylthiol and 2-methyl-3-furanthiol. Journal of Agricultural and Food Chemistry, **46**: 235–241.

Komthong P., Katoh T., Igura N., Shimoda M., Hayakawa I. (2003): Effect of high hydrostatic pressure combined with pH and temperature on glucose/fructose-leucine/lysine/glutamate browning reactions. Journal of the Faculty of Agriculture, Kyushu University, **48**: 135–142.

Maeda S., Kunitou K., Hihara T., Mishima K. (2004): One-bath dyeing of polyester/cotton blends with reactive disperse dyes in supercritical carbon dioxide. Textile Research Journal, **74**: 989–994.

Meynier A., Mottram D.S. (1995): The effect of pH on the formation of volatile compounds in meat-related model systems. Food Chemistry, **52**: 361–366.

Moreno F.J., Molina E., Olano A., López-Fandiño R. (2003): High-pressure effects on Maillard reaction between glucose and lysine. Journal of Agricultral and Food Chemistry, **51**: 394–400.

Mottram D.S., Nobrega I.C.C. (2002): Formation of sulfur aroma compounds in reaction mixtures containing cysteine and three different forms of ribose. Journal of Agricultural and Food Chemistry, **50**: 4080–4086.

Mottram D.S., Whitfield F.B. (1995): Maillard-lipid interactions in nonaqueous systems: volatiles from the reaction of cysteine and ribose with phosphatidylcholine. Journal of Agricultural and Food Chemistry, **43**: 1302–1306.

Mottram D.S., Madruga M.S., Whitfield F.B. (1995): Some novel meatlike aroma compounds from the reactions of alkanediones with hydrogen sulfide and furanthiols. Journal of Agricultural and Food Chemistry, **43**: 189–193.

Oakes R.S., Clifford A.A., Rayner C.M. (2001): The use of supercritical fluids in synthetic organic chemistry. Journal of the Chemical Society, Perkin Transactions, **1**: 917–941.

Parker J.K., Hassell G.M.E., Mottram D.S., Guy R.C.E. (2000): Sensory and instrumental analyses of volatiles generated during the extrusion cooking of oat flours. Journal of Agricultural and Food Chemistry, **48**: 3497–3506.

Sihvonen M., Järvenpää E., Hietaniemi V., Huopalahti R. (1999): Advances in supercritical carbon dioxide technologies. Trends of Food Science and Technology, **10**: 217–222.

Van Den Ouweland G.A.M., Peer H.G. (1975): Components contributing to beef flavor. Volatile compounds produced by the reaction of 4-hydroxy-5-methyl-3(2H)-furanone and its thio analog with hydrogen sulfide. Journal of Agricultural and Food Chemistry, **23**: 501–505.

Vemavarapu C., Mollan M.J., Lodaya M., Needham T.E. (2005): Design and process aspects of laboratory scale SCF particle formation systems. International Journal of Pharmaceutics, **292**: 1–16.

Whitfield F.B., Mottram D.S. (1999): Investigation of the reaction between 4-hydroxy-5-methyl-3(2H)-furanone and cysteine or hydrogen sulfide at pH 4.5. Journal of Agricultural and Food Chemistry, **47**: 1626–1634.

Xu H., Gao Y., Liu X., Zhao J. (2008a): Effects of supercritical carbon dioxide on volatile formation from Maillard reaction between ribose and cysteine. Journal of the Science of Food and Agriculture, **88**: 328–335.

Xu H., Liu X., Zhao J., Gao Y. (2008b): Effects of ribose to cysteine ratios on the formation of volatile compounds from the Maillard reaction in supercritical carbon dioxide. Food Research International, **41**: 730–737.

Yalpani M. (1993): Supercritical fluids: puissant media for the modification of polymers and biopolymers. Polymer, **34**: 1102–1105.

Zhang Y., Chien M., Ho C.T. (1988): Comparison of the volatile compounds obtained from thermal degradation of cysteine and glutathione in water. Journal of Agricultural and Food Chemistry, **36**: 992–996.

Received for publication October 20, 2008
Accepted after corrections March 17, 2010

Corresponding author:

Prof. Yanxiang Gao, China Agricultural University, No. 17, Tsinghua East Road, Haidian District, P.O. Box 112, Beijing, 100083, P. R.China
tel.: + 86 106 273 70 34, e-mail: gyxcau@126.com