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Volatile constituents of glutathione – ribose model system and its antioxidant activity

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Summary. Reaction between glutathione and ribose was carried out to study the volatiles formed via Maillard reaction and their antioxidant activity as well as their role in inhibition of LDL oxidation. The simultaneous distillation – extraction technique was used for trapping the volatile components followed by GC – MS analysis. Thirty six compounds were identified with the predominance of carbonyls and sulfur – containing compounds in the volatiles of this model system. Sensory evaluation was performed for the model system product according to the International Standard Methods (ISO). The results showed a high decrease in roasted and burnt attributes and remarkable increase in the like – boiled and roasted meat attributes. The sensory results of the model system product were confirmed by the presence of high concentrations of some volatile compounds having meat – like aroma such as 2-methyl-3-furanthiol and 2-furylmethanethiol. The radical scavenging activity of glutathione – ribose model system was quantified spectrophotometrically, using DPPH radical. The activity of the model system product was found to be slightly lower than that of gallic acid and BHA, but it was much higher than that of cinnamic acid (200 ppm. for each). A highly antioxidative activity was recorded by the model system product during the inhibition of LDL – oxidation in comparison with L-ascorbic acid as well as reduced glutathione (as a concentration of 0.5 μmol/L, for each) which may be due to the presence of some compounds such as 2-furylmethanethiol, 2-acetyl thiazole, 4-hydroxy-5-methyl-3(2H)-furanone.

Keywords: Glutathione – Meat-like aroma – Maillard reaction – Sensory evaluation – LDL – DPPH

Introduction

The Maillard reaction is of major technological importance in the development and imitation of desirable flavours and aromas of the processed foods (Ledl and Schleicher 1990). It has been industrially used for the production of imitated flavours and aromas especially meat (Taiji et al., 1996). Most subsequent patent proposals for 'reaction product' meat flavourings have involved sulfur – containing amino acids, usually cysteine or hydrogen sulfide (MacLeod and Seyyedain – Ardebili, 1981; MacLeod and Ames, 1986). Heterocyclic compounds, especially those containing sulfur, are very important flavour compounds produced in the Maillard reaction, providing savoury, meaty, roast and boiled flavours. These later compounds, together with carbonyl compounds produced in the Maillard reaction lead to many important classes of flavour compounds including: furans, pyrazines, pyrroles, oxazoles, thiophenes, thiazoles and many other heterocyclic compounds (Mottram, 1998). Ohloff et al. (1985) were reported the responsibility of cysteine which found in muscle protein as the main precursor for this aroma chemical in meat on prolonged heating. Effect of hydrogen sulfide concentration evolved from glutathione or cysteine on the volatile formation was studied (Ho et al., 1997). However, the formation of meat – like flavour via interaction of glutathione and pentoses was not reported before. The main source of pentoses in meat are ribonucleotides, where 5′-IMP is the principal ribonucleotide in post – mortem meat (Madruga and Mottram, 1998).

Many attentions have been paid recently to the antioxidative food components, which may have a protective effect against coronary heart diseases (CHD) by inhibiting LDL oxidation *in vivo*. LDL plays a major role during the development of atherosclerotic plaques and as a result of cardiovascular disease (Steinberg et al., 1989). The protective role of natural antioxidants from plant sources, particularly polyphenols on LDL has been thoroughly investigated. Although, it has been often shown that Maillard products which are formed from different sugars and amino

acids or proteins have antioxidative activity (Monti et al., 1999), hardly anything is known about their role in LDL-oxidation. Also, the complexity of the mechanisms involved in the Maillard reaction and the uncertainty of melanoidin formation, the exact structure of those compounds responsible for the antioxidative effect have not yet been fully determined (Eiserich et al., 1992).

In this study, we report the formation of meat like – flavour via glutathione – ribose interaction, the sensory evaluation of the product as well as the identification of the volatile constituents formed via Maillard reaction and its activity to scavenge the radicals by DPPH. In addition, the ability of Maillard products to inhibit LDL oxidation *in vitro* was investigated.

Materials and methods

All chemicals used in this study were purchased from E. Merck (Darmstadt, Germany). An equal molar ratios of D-ribose and glutathione amino acids content (glutamic acid, cysteine and glycine, as an equal molar ratios of each) were dissolved in 1 L. of deionized water and adjusted to pH 7.1 with 0.1 M sodium phosphate buffer solution. The reaction mixture was refluxed for 3 hours, then the reaction flask was cooled to room temperature using running cool tap water. The reaction mass was stored in a refrigerator until the isolation.

Volatiles were extracted from the aqueous solution using simultaneous steam distillation solvent extraction in a modified Likens – Nickerson apparatus. Extraction was carried out for 2 h using 100 mL. of methylene chloride. The distillates were dried over anhydrous sodium sulfate and concentrated to about 0.5 mL using a Vigreux column (50 cm × 1 cm).

Gas Chromatography – Mass Spectrometry (GC / MS) analysis

GC-MS analysis of the glutathione-ribose model system was performed on a Varian gas chromatograph interfaced to Finnigan SSQ 7000 mass selective detector (MSD) with ICIS V2.0 data system for MS identification of the GC components. The column used was DB-5 (J & W Scientific, Folsom, CA) cross – linked fused silica capillary column (30 m, 0.25 mm i.d.) coated with polydimethylsiloxane (0.5 μm film thickness). The oven temperature was programmed from 50°C for 3 min, isothermal, then heating by 7°C/min to 250°C, and isothermally for 5 min at 250°C. Injector temperature was 200°C and the volume injected was 0.5 μL. Transition – line and ion source temperatures were 250°C and 150°C, respectively. The mass spectrometer had a delay of 3 min to avoid the solvent peak and then scanned from *m/z* 40 to *m/z* 350. Ionization energy was set at 70 eV. Identifications were based on the comparison with the MS computer library (NIST – Software package, Finnigan), and on the respective retention indicies with those of authentic components and with puplished data (Adams 1995). The quantitative determination was carried out based on peak area integration.

Sensory analysis

The sensory analysis was carried out under the conditions specified by the International Standards (International Standardization Organization, ISO); general guidelines after ISO 6658-1985; unstructured graphical scales (ISO 4121-1988) were presented as straight lines 100 mm long, provided with descriptors on either end (odour acceptability: 0 mm = very little agreeable, 100 mm = very agreeable; odour intensity: 0 mm = very weak, 100 mm = very strong). The sensory profile was based on free choice profiling, and the following descriptors were retained (out of 32 collected descriptors): 1 = roasted, bread crust, roasted peanuts; 2 = burnt, caramel, bitter; 3 = like – boiled meat; 4 = like – roasted meat; 5 = spicy, sulphuric, onion, garlic; 6 = sharp, pungent, burning; 7 = earthy, musty, moldy, sweat, wet dog; 8 = malty, sweet; 9 = solvents, synthetic, chemicals; 10 = others – specify which); in the profile evaluation: 0 mm = absent, 100 mm = very strong. Odour profiles were tested by sniffing from ground wide-neck glass bottles.

Scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical

The effect of glutathione / ribose model system on DPPH radical was estimated according to Hatano et al. (1988). Glutathione / ribose model system (200 ppm) was added to a methanolic solution (1 mL) of DPPH radical (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and left to stand at room temperature for 30 min; the absorbance of the resulting solution was measured spectrophotometrically in triplicates at 517 nm. In this test, the percentage of DPPH reduction by the model system was compared to that of gallic acid, BHA and cinnamic acid (negative control) (200 ppm of each).

LDL assay

Blood samples were collected after an overnight fasting from healthy male volunteers. Plasma was separated by low speed centrifugation at 600 × g for 10 min at 4°C. After separation of fresh plasma, LDL samples were isolated immediately using single – step ultracentrifugation. Plasma (2 ml) was adjusted to a density of 1.21 g/ml with KBr and layered under 10 ml saline (density 1.006 g/ml) containing 0.01% EDTA in 12 ml quick – seal tubes (Beckman Instruments, Munich, Germany). The tubes were centrifuged at 65,000 rpm for 6 h at 4°C using a Ti75 fixed angle rotor in a Beckman L5-75 ultracentrifuge (Beckman Instruments, Munich, Germany) (Abbey et al., 1993). The yellow LDL-band was collected and immediately prior to the oxidation incubations, separated from EDTA by gel filtration using Econo-Pac 10 DG columns (BioRad GmbH, Munich, Germany) (Cominacini et al., 1991; McDowell et al., 1995). LDL concentration was determined by measuring total cholesterol using CHOD-PAP-method since more than 98% purity of LDL-solution after LDL preparation could be obtained (Siedel et al., 1983). The LDL-oxidation was initiated by addition of a freshly prepared aqueous $CuSO_4$ solution, which diluted with oxygen-saturated phosphate buffer solution (PBS) pH 7.4.

In all experiments, the final conditions were: Temperature 30°C, 0.08 g/L LDL-cholesterol and 5 μmol/L $CuSO_4$, final test volume 1 ml. The LDL-solution was supplemented with 0.5 μmol/L of the test substances which added to the LDL-solution prior to the initiation of the oxidation of LDL by copper ions. LDL-oxidation was spectro-photometrically monitored in triplicates, by measurement of the change in absorbance at 234 nm in 1 ml quartz cuvettes in a Perkin Elmer Lambada 2 ultraviolet / vis spectrophotometer (Perkin-Elmer, Ueberlingen, Germany) with absorbance readings made every 5 min at 37°C until there was no further increase in the formation of conjugated dienes. The duration of the lag-phase was determined as described by Esterbauer in (1989). LDL susceptibility to oxidation was measured by continuous monitoring of conjugated diene formation and is characterized by lag-time, which seems to be

an oxidation resistance marker (Ramos et al., 1995; Schreier et al., 1996).

Results

Figure 1 shows the Gas Chromatography – Mass Spectrometry (GC – MS) profile of the volatiles isolated from the refluxed glutathione – ribose model system. Thirty six volatile compounds were identified including carbonyls, mercaptoketones, thiophenes, furanthiols, and others. The identified components along with their relative area percentages and retention indicies were reported in Table 1.

Figure 2 demonstrates the sensory profile of the glutathione – ribose model system extract as well as the intensity of the developed odour and the odour acceptability.

The radical scavenging activity of glutathione – ribose model system was quantified spectrophotometrically using DPPH radical (61%) in comparison with gallic acid (80%), BHA (85%) and cinnamic acid (3%) (negative controle), as a concentration of 200 ppm of each.

However, in the course of studying the effect of glutathion model system to prevent the LDL – oxidation, in terms of the prolongation of lag – time (min), was measured. It was found that the activity of model system (940 min) higher than that of the reduced glutathione (259 min) and L-ascorbic acid (311 min) (0.5 μmol/L of each).

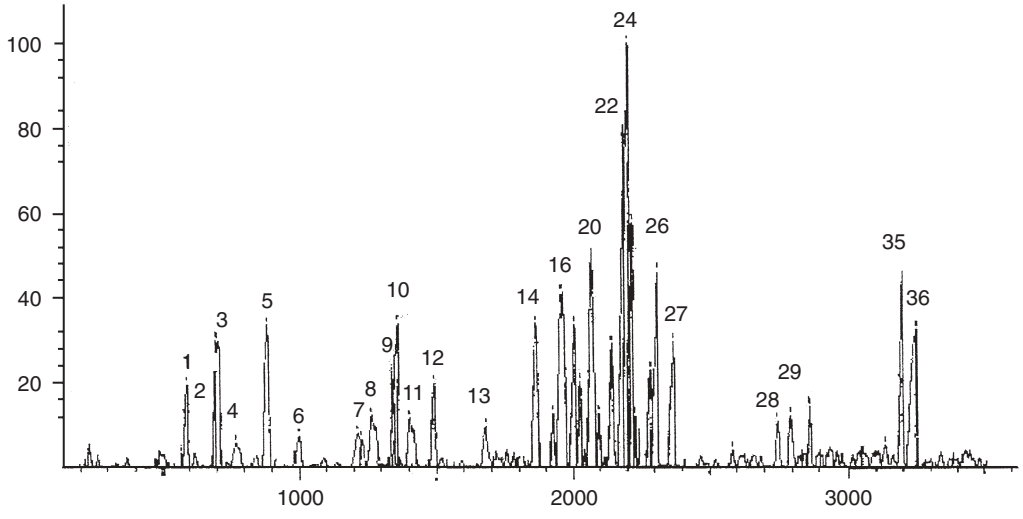

Fig. 1. GC/MS Chromatogram of glutathione/ribose model system

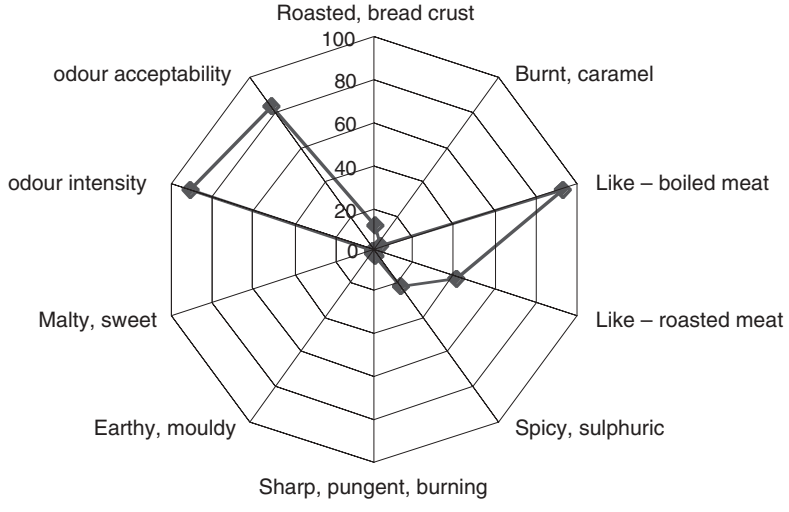

Fig. 2. Aroma profile diagram of the flavour concentrate obtained through glutathione – ribose model system

Table 1. Volatile compounds obtained from reaction between glutathione and ribose

No.	Structure	Retention time	Retention index	Area%
1	2,3-butanedione	8.00	592	1.814
2	2-butanone	8.15	603	0.271
3	3-pentanone	8.52	694	4.466
4	2,3-pentanedione	9.31	725	1.001
5	3-penten-2-one	10.26	728	3.789
6	unknown	10.48	752	0.295
7	2-methylthiophene	11.26	777	0.866
8	2-hexanone	13.23	798	2.544
9	hexanol	13.26	799	1.083
10	3-mercapto-2-butanone	14.26	817	4.006
11	Butanoic acid	14.49	821	1.245
12	2-furfural	15.33	829	1.868
13	2-methylthiazole	16.04	832	0.623
14	2-methylcyclopentanone	17.07	836	1.272
15	unknown	17.48	849	0.112
16	2-methylfuranthiol	18.44	870	9.773
17	2-ethylthiophene	18.57	871	1.029
18	4-hydroxy-5-methyl-3(2H)-furanone	19.14	880	0.785
19	2,5-dimethylthiophene	19.28	882	0.786
20	2-heptanone	19.51	890	3.059
21	2-butylfuran	20.03	893	1.435
22	3-mercapto-2-pentanone	20.26	898	7.336
23	2-acetylfuran	20.40	904	0.785
24	2-furylmethanethiol	21.29	913	20.583
25	2-acetyl-1-pyrroline	21.47	926	0.379
26	2(3)-thiophenethiol	22.00	977	3.438
27	3-hydroxy-2H-pyran-2-one	22.14	989	1.543
28	2-formylthiophene	22.25	1,000	4.196
29	2-acetylthiazole	22.57	1,020	4.358
30	unknown	25.06	1,049	0.183
31	unknown	26.08	1,077	0.779
32	unknown	26.30	1,096	1.276
33	2-formyl-5-methylthiophene	27.43	1,118	2.734
34	unknown	29.23	1,126	0.245
35	(E)-3,5-dimethyl-1,2,4-trithiolane	29.58	1,140	5.685
36	(Z)-3,5-dimethyl-1,2,4-trithiolane	30.17	1,151	4.358

Discussion

Thirty six components of the model system involving ribose and glutathione at pH 7.1 are represented in Table 1, while their GC – MS chromatogram is presented in Fig. 1. Carbonyls and sulphur – containing compounds are the predominates in the volatiles of this model system.

3-Pentanone (4.47%), 3-penten-2-one (3.79%), 2-hexanone (2.54%) and 2-heptanone (3.06%) were the main constituents of the identified carbonyl class. The presence of ketones in this model system can be explained by the interaction between glutamate residue; the free amino group in glutathione mixture and ribose (Mottram and Madruga, 1998). However, the formation of relatively longer carbonyl compounds e.g. 2-hexanone and 2-heptanone may be explained by the condensation reaction between shorter carbonyl compounds or fragments.

Hydrogen sulfide which evolved upon heating of glutathione can react with the reactive carbonyl intermediates to form mercaptoketones e.g. 3-mercapto-2-butanone (4.01%) and 3-mercapto-2-pentanone (7.34%). Mercaptoketones have been identified in meat aroma model system (Farmer et al., 1989; Zhang and Ho, 1989 and Zhang and Ho, 1991) and in the volatiles of boiled meat and chicken broth (Gasser and Grosch, 1990).

Three alkylthiophenes and two formylthiophenes were identified in the system volatiles. The main route for their formation in glutathione / ribose model system is through the exchange of oxygen – atom found in

furans and furanones with sulphur – atom via hydrogen sulfide (Zheng et al., 1997).

One trithiolanes with 2 isomers (E) and (Z) were detected as 5.68% and 4.36%, respectively. Generally, five- and six-membered rings which possess 1, 2 or 3 sulphur atoms are more predominant in boiled meat (Fors, 1983).

The thiols identified included two furanthiols: 2-methyl-3-furanthiol (9.77%) and 2-furylmethanethiol (20.58%) and one thiophenethiol: 2(3)-thiophenethiol (3.44%). It was reported that, 2-methyl-3-furanthiol possess meaty, roasty and boiled notes (Madruga, 1994), while 2-furylmethanethiol is considered to play an important role in chicken and bovine broths (Gasser and Grosch, 1990). The routes involved in the formation of furanthiols are likely to be the interaction of hydrogen sulfide with dicarbonyls or furaneol (a dehydration product of ribose) to form 2-methyl-3-furanthiol (Madruga, 1994; van den Ouweland and Peer, 1975) or with furfural to form 2-furylmethanethiol (Shibamoto, 1977). The 2(3)-thiophenethiol which has been reported in pork flavour (Werkhoff et al., 1993), was also probably formed via the same routes of furanthiols (Shu et al., 1985).

Roasted notes in foods are usually associated with the presence of certain classes of heterocycles e.g. pyrazines and thiazoles. However, mode of glutathione molecule reactivity during Maillard reaction may inhibited the pyrazine formation. 2-Methyl thiazole (0.62%) and 2-acetylthiazole (4.36%) were detected among the volatiles produced by this model system.

Odour sensory characteristics

Pronounced differences were observed in the odour profiles. As expected, intensities of the roasted, burnt, caramel and sweet notes were weak in glutathione / ribose model system where pyrazines were replaced by sulphur derivatives, which were produced from the same bicarbonylic presursors as pyrazines (Table 1). The lower content of furans generated via this model system is also in agreeable with the lower intensities of the above – mentioned notes due to the replacing of Oxygen – atom in furans by sulphur which leads to more sulphur – derivatives.

The aroma of like – boiled meat was intensive in the volatiles produced through glutathione – ribose model system, due to the preponderance of sulphur – containing compounds (Table 1). Mottram (1991) reported that, boiled meat contains more aliphatic thiols, sulfides as well as heterocyclic compounds with 1, 2 or 3 sulfur atoms in 5- and 6-membered rings, in comparison to roasted meat. Lower intensity of roasted meat note is reasonable, due to the absence of pyrazines and oxazoles and the lower content of thiazoles, which were reported as the responsibles for the roasted note in meat (Farmer and Patterson, 1991). Other descriptors gave insignificant results, as the ratings were too low; therefore, they are not included in Fig. 2.

Antioxidant activity of glutathione – ribose model system

It is well known that free radicals are of the causes of several diseases, such as Parkinson disease (Adams and Odunze, 1991), Alzheimer type dementia (Chia et al., 1984). Natural antioxidative food components are important for food technology, because they prolong the shelf life of processed food stuffs. More recently they also gained interest because it was suggested that, their intact is beneficial for health and they are protective, e.g. against coronary heart diseases (CHD).

The radical scavenging activity of glutathione – ribose model system was quantified in spectrophotometric assay using the DPPH radical. In this test, the percentage of DPPH reduction by this model system was compared to that of gallic acid, BHA and cinnamic acid (negative control) (200 ppm of each). The radical scavenging activity of glutathione – ribose model system (61%), was found to be slightly lower than that of gallic acid (80%), a well known antioxidant agent and BHA (85%), a synthetic antioxidant widely used in food technology (Potterat, 1997).

LDL – oxidation *in vivo* plays a major role during the development of atherosclerosis and CHD and the inhibition of such diseases by isolated antioxidant(s) remains to be established by ongoing and future clinical trials. Although the antioxidative activity of Maillard reaction products (MRPs) is well established, hardly anything is known about their role in the LDL oxidation.

In the course of studying the effect of MRPs on LDL-oxidation, the activity of glutathione-model system to prevent oxidation of human LDL was measured. LDL – oxidation was initiated by the addition of copper ions and recorded by measuring UV – absorbance at 230 nm. The antioxidative activity was recorded by the length of lag – time and compared to the antioxidative activity of commercial reduced glutathi-

one and L-ascorbic acid. It was found that this model system exhibited a highly antioxidative activity by inhibiting LDL – oxidation by prolonging the lag – time (940 min) as compared to L-ascorbic acid (311 min) as well as the commercial glutathione (259 min) (0.5 μmol/L. of each). The antioxidative activity of the glutathione – ribose model system may be attributed to the presence of some active components such as 2-furylmethanethiol (20.58%), 2-acetylthiazole (4.4%), 2,3-thiophenethiol (3.4%), 4-hydroxy-5-methyl-3(2H)-furanone (0.8%) and methylthiazole (0.6%). Eiserich et al. (1992) reported that the five-membered hetero-cyclyic compounds such as furanones, oxazoles and thiazoles have a remarkable antioxidant activity through radical scavenging.

In summary, the generation of the characteristic meat like-aroma of glutathione – ribose model system was observed due to the formation of 2-Methyl-3-furanthiol and 2-furylmethanethiol. The radical scavenging activity of this model system was found to be slightly lower than that of gallic acid and BHA, but it was much higher than that of cinnamic acid (200 ppm for each). This study showed for the first time that glutathione – ribose model system products can have beneficial influence on LDL – oxidation *in vitro*. Also, the present results strongly propose that the glutathione – ribose model system have antioxidant and anti-lipoperoxidant activity, which indicates its effectiveness in diseases caused by overproduction of radicals. Further studies are needed to evaluate the *in vivo* potential of this extract in various animal models.

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