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Chemical Composition and Antioxidant Activity of Maillard Reaction Products Generated from Glutathione or Cysteine/Glucose

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Abstract: The present investigation aimed to study the chemical composition of volatile compounds generated from either glutathione or cysteine with glucose model systems are evaluated the antioxidant activity of these volatiles compounds. The simultaneous distillation–extraction technique was used for trapping the volatile components followed by GC/ MS analysis. Twenty seven volatile compounds were isolated and identified in glutathione or cysteine/ gluose model systems, with the predominance of esters and sulfur-containing compounds. The radical scavenging activity of the model systems was quantified spectrophotometrically, using DPPH radical and β-carotene bleaching assays.

Key words: Glutathione · Cysteine · Glucose · Maillard reaction · Antioxidant activity.

INTRODUCTION

The Maillard reaction is a well-known reaction that occurs in food during cooking, because of the complexities of the Maillard reaction, many investigations have been aimed to understanding the mechanisms of the reaction. The pathways for the Maillard reaction originally proposed by Hodge [1] have gained wide acceptance. The studies on the generation of aroma compounds from the Maillard reaction were mostly concerned with simple model systems using amino acids. Some of the amino acids used in model systems were proline [2], hydroxyproline [3], serine and threonine [4], cysteine [5, 6], leucine [7] and glycine [8]. For flavour studies, D-glucose and L-cysteine have been most commonly used as reactants in Maillard model systems mainly because D-glucose is one of the most abundant reducing sugars found in food materials and L-cysteine contains nitrogen and sulfur, necessary atoms in heterocyclic flavour chemicals [9].

Antioxidants can play a major role in the prevention of autoxidation. However, there may be two major problems for the use of conventional antioxidants such as butyl hydroxy tolouene (BHT) and butyl hydroxy anisole (BHA). They are approved for use in a limited number of food products and each new synthetic antioxidant must be subjected to a lengthy process of evaluations for proof of safety. Consumers are increasingly concerned about chemical additives used in foods [10]. The antioxidant capacity of MRPs was observed for the first time by Franzke and Iwainsky [11] and some fractions were reported to have strong antioxidant properties comparable to those of commonly used food antioxidants [12]. Eiserich *et al.* [13] identified volatile compounds from a Maillard model system contain cysteine/glucose that possessed antioxidative properties. Their results showed that volatile compounds displayed the strongest antioxidative activity. According to Lee and Nagy [14], 2-acetylfuran and furfuryl alcohol were also the degradation products of sugars. In the Maillard model system consisting of cysteine and glucose, 2-acetylfuran is one of the major thermal degradation products of glucose [9].

Maillard reaction products (MRPs), especially melanoidins, have been reported to have antioxidant activity through scavenging oxygen radicals or chelating metals. MRPs from histidine (basic amino acid) had the highest antioxidant activity determined by conjugated diene formation from peroxidation of linoleic acid among MRPs from either dipeptides of histidine–phenylalanine or lysine–alanine, amino acids histidine, lysine, or ascorbic acid when glucose was used as a reducing sugar. Compounds in the MRPs with amino reductone structures may have both antioxidant and pro-oxidant activities depending on the reaction conditions. MRPs obtained from heated histidine and glucose exhibit copper ion binding ability in oil/water mixtures [15].

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The 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 [16]. The present study aimed to identify the volatiles generated from tripeptide (glutathione) or cysteine with glucose as well as to evaluate the antioxidant activity of.

MATERIALS AND METHODS

Chemicals: Glutathione, L-Cysteine, (D)-Glucose, β-carotene, methanol, 2, 2-diphenyl-2-picrylhydrazyl, tert-butyl hydroquinone (TBHQ), were purchased from Sigma-Aldrich company.

Methods

Preparation of Glutathione or Cysteine/ Glucose Model Systems: Model mixtures containing glutathione or cysteine and glucose at similar molar ratios were used for the preparation of model aqueous MRPs. Each mixture was dissolved in phosphate buffer (pH 7) and allowed to react at 90°C under efficient reflux. The flasks containing the aqueous solutions of MRPs were immediately cooled in ice bath.

Extraction of Volatile Compounds from Model Systems: The reaction mixtures obtained from pressurized bottle after the reaction was completed were subjected to a simultaneous steam distillation (1 L of water) and solvent (dichloromethane, 200 mL) extraction. The dichloromethane extract was dried over anhydrous sodium sulfate.

Gas Chromatography and Gas Chromatography- Mass Spectrometry (GC/ MS): The obtained volatile samples were thermally desorbed, using a modified injector port, directly on the front of a (DB-5) (60 m x 0.32 mm i.d) fused silica capillary column, in the oven of a Perkin-Elmer XL gas chromatography and temperature increase from 50°C to 220°C by the rate 4°C / min. Kovat's indices were determined by co-injection of the sample with a solution containing homologous series of n-hydrocarbons (C_6-C_{26}) under the same conditions as described above. The separated components were identified by matching with NIST mass-spectral library data and by comparison of Kovat's indices with those of authentic components and with published data. GC/MS analysis of the two model systems were performed on An HP model 6890 GC interfaced to an HP 5791A mass selective detector (GC/ MS) was used for mass spectral identification of the GC components at (MS) ionization voltage of 70 eV. A 30 m x 0.25 mm i.d. (DF = 0.25 lm) DB-5 bonded-phase fused-silica capillary column was used for (GC). The linear velocity of the helium carrier gas was 30 cm/s. The injector and the detector temperatures were 250 °C. The oven temperature was programmed from 50 to 220 °C at 4 °C / min [17].

Determination of the Antioxidant Activity of MRPs: Scavenging Effect on 2, 2-diphenyl-2-picrylhydrazyl (DPPH) Radical:

The effect of glutathione or cysteine/gluose model systems on DPPH radical was estimated according to Hatano *et al.* [18]. Aqueous portion of model systems (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 BHA and TBHQ (200 ppm of each).

β-Carotene Measurement: Antioxidant activity of the aqueous solution was determined by a β-carotene/Linoleic acid system, as described by Matthaus [19]. Briefly, 2 ml of β–carotene solution (5 mg /1 ml chloroform), 40 mg of linoleic acid were transferred to a round – bottom flask. Chloroform from the sample was evaporated using a stream of nitrogen. Then 100 ml of oxygenated distilled water were added slowly to the residue and vigorously agitated to give a stable emulsion. To an aliquot of 2 ml of this emulsion, different volumes of the samples were added with the same volumes of standard mixture. Absorbance was measured at 470 nm after incubate the reaction mixtures in water bath (50°C) and the readings takes from zero time till 120 min. Antioxidant activity index(AAI) was calculated as: Equation: (2).

$$AoA = [1-(A_{S\,(0)} . A_{S\,(120)}/A_{b\,(0)} - A_{b\,(120)}] \times 100$$

Where $A_{S\,(0)}$ is absorbance of sample at 0 min, $A_{S\,(120)}$ is absorbance of sample at time (t).

Sensory Evaluation: The sensory analysis was carried out under the conditions specified by the international standards (international standardization organization, ISO); guidelines after ISO 6658-1985;unstructured graphical scales(ISO 4121-1988) were presented as straight lines 100 mm long, provided with descriptions on either end (odour acceptability; 0mm = very little agreeable, 100 = very strong agreeable); odour intensity: 0 = very weak, 100mm = 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, sulphury, onion, garlic; 6 = sharp, punget, burning; 7 = earthy, musty, moldy, sweat, 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 [23].

Statistical Analysis: All measurements were carried out in triplicate and presented as mean values ± S.D (standard deviation of the mean). Statistical significance level was fixed at ($p < 0.05$).

RESULTS AND DISCUSSION

Twenty seven compounds generated from of the model systems of glutathione or cysteine with glucose are represented in Table 1, while their antioxidant activities are presented in Fig. 1. Hydrogen sulfide which evolved upon heating of glutathione or cysteine can react with the reactive carbonyl intermediates to form mercaptoketones *e.g.* 3-mercapto-2-butanone and 3- mercapto-2-pentone in glutathione/ glucose and cysteine/ glucose model systems with (4.2%, 2.06% and 0.84%, 0.39%), respectively, have been identified in meat aroma model system [20] and in the volatiles of boiled meat and chicken broth [21].

Many of the heterocyclic compounds identified in the present investigation possess aromatic characteristics. In both five- and six-membered heterocyclic compounds, such as 3-methyl-1, 2-dithiolan-4-one with (0.258 % and 0.324%) in glutathione and cysteine model system respectively, was identified in the model system and six electrons are delocalized over the ring through resonance. In case of pyrazine, the two nitrogen atoms withdraw π-electrons from the remaining four carbon atoms, rendering them u-electron deficient.

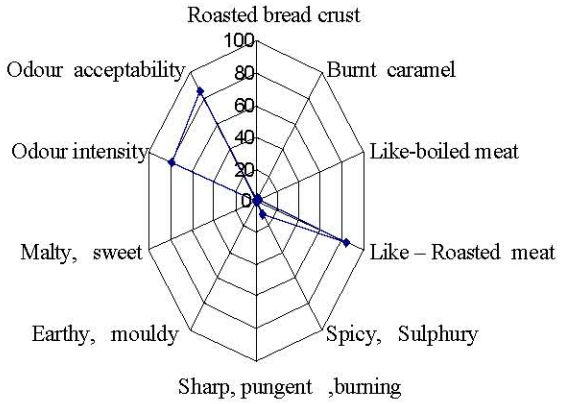

Fig. 1: Sensory profile of glutathione/ glucose model system

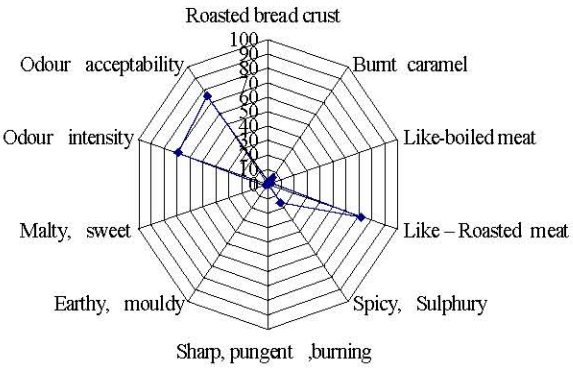

Fig. 2: Sensory profile of cysteine/ glucose model system

However, in the case of furan, oxazole and thiazole, the electrons are distributed among only five atoms, which subsequently results in carbon atoms that are_π-electron excessive. The higher π-electron densities of the five-membered heterocyclic compounds render them open to electrophilic addition by radical species. By the same token, the u-electron-deficient pyrazine does not readily undergo electrophilic addition, which explains its inability to exhibit antioxidative activity [13].

Roasted note in foods are usually associated with the presence of certain classes of heterocyclic compounds e.g. pyrazine derivatives such as pyrazine, methylpyrazine, 2, 5-dimethylpyrazine, 2-ethyl-3,6-dimethylpyrazine and 2, 3-diethyl-5-methyl pyrazine and thiazoles such as 2,5-dimethyl-4-ethylthiazole and 2-acetylthiazole were identified in the glutathione/ glucose model system where pyrazine and thiazoles derivatives represented with high concentrations in glutathione/ glucose model system rather than cysteine/ glucose model system..

Table 1: Aroma compounds identified in volatiles generated from model systems containing glutathione or cysteine/glucose

Peak no.	KI [a]	Identified compounds [b]	Glutathione	Cysteine	RT[b]	MS[c]
1	747	Pyrazine	1.2	0.041	+	+
2	783	dimethyl disulphide	0.9	0.052	+	+
3	798	3-mercapto-2-butanone	4.2	2.06	+	+
4	826	2-furfural	2.3	1.03	+	+
5	838	dihydro-2-methyl-3- (2H)Furanone	1.16	0.841	+	+
6	841	Methylpyrazine	3.2	2.113	+	+
7	853	2.4-dimethylfuran	0.72	0.029	+	+
8	868	methyl furanthiol	1.23	0.082	+	+
9	870	2-methyl-3-furanthiol	0.76	0.187	+	+
10	872	3- ethylthiophene	0.249	0.152	+	+
11	876	2,5-dimethylthiophene	0.019	0.042	+	+
12	879	4-hydroxy-5-methyl-3(2H)furanone	4.22	1.932	+	+
13	897	3- mercapto-2-pentone	0.84	0.39	+	+
14	911	2-furfuryl thiol	0.53	0.164	+	+
15	928	2,5-dimethylpyrazine	0.775	0.352	+	+
16	955	4,5-dihydro-3-(2H)thiophenone	0.243	0.164	+	+
17	959	7-methyl-3-methylene-1,6-octadiene	0.192	0.019	+	+
18	961	3-mercaptothiophene	0.245	0.132	+	+
19	966	2-methyl-4,5-dihydro-3-furanthiol	0.182	0.017	+	+
20	1005	2-formylthiophene	1.09	0.831	+	+
21	1016	2-propionylfuran	1.21	0.752	+	+
22	1020	2-acetylthiazole	0.332	1.21	+	+
23	1063	2-ethyl-3,6-dimethylpyrazine	0.812	0.275	+	+
24	1071	3-methyl-1,2-dithiolan-4-one	0.258	0.324		+
25	1076	2,5-dimethyl-4-ethylthiazole	0.621	0.187		+
26	1166	2-methyl-3-(methyldithio)furan	0.534	0.314		+
27	1172	2,3-diethyl-5-methyl pyrazine	0.712	0.234		+

[a]: LRI: Linear retention index; [b]: Retention time; [c]: Mass spectra.

Unsaturated hydrocarbon was identified in the glutathione/ glucose and cysteine/ glucose model systems such as 7-methyl-3-methylene-1,6-octadiene with (0.192% and 0.019%), respectively, where it was identified in the broth roasted meaty aroma [22].

The identified thiols in the two model systems included 2-furfurylthiol (0.53% and 0.164%), 2-methyl-4,5-dihydro-3-furanthiol (0.182% and 0.017%), methylfuranthiol (1.23% and 0.082%), 2-methyl-3-furanthiol (0.76% and 0.187%) and 2-methyl-3-(methyldithio)furan (0.534% and 0.314%) were identified in glutathione/ glucose and cysteine/ glucose model system, respectively. It was reported that 2-methyl-3-furanthiol, 2-furfurylthiol, 2-methyl-4, 5-dihydro-3-furanthiol possess meaty and boiled notes, while 2-methyl-3-(methyldithio) furan is considered to play an important role in chicken broth [16]. The routes involved in the formation of thiol derivatives are likely to be the interaction of hydrogen sulphide with dicarbonyls or with furfural to form 2-furfurylthiol, 2-methyl-4, 5-dihydro-3-furanthiol [16]. Thiophene derivatives were detected in the two model systems as shown in the Table 1. Most of thiophene derivatives derived from the pyrolysis of carbonyl compounds and hydrogen sulphide [23] and all thiophene derivatives have sulphurous odour except 3-mercaptothiophene have meaty aroma [16].

Alkylated furans were released in the model system such as 2.4-dimethylfuran, 2-propionylfuran, 4-hydroxy-5-methyl-3-(2H)-furanone, dihydro-2-methyl-3-(2H)-furanone and 2-furfural. Mottram [16] was reported alkylfuran and 4-hydroxy-5-methyl-3(2H)-furanone may be formed via glucose decomposition and associated with roasted note.

Elizalde *et al.* [24] found that hydroxyl radicals (OH°) react with oxazoles by exclusive addition to the carbon at the 5-position of the ring, producing an adduct containing an allylic radical at the 4-position. This can be explained by the higher u-electron density at the 5-position of the oxazole ring. This type of addition leaves the unsubstantiated oxazole with a secondary allylic radical, whereas OH addition to an oxazole substituted at the 4-poeition (2, 4, 5-trimethyloxazole and 4, 5-dimethyloxazole) would result in a more stable tertiary allylic radical. The highest antioxidant activity in case of glutathione may explained by the fact that 2, 5-dimethyl-4-hydroxy-3-furanone, is higher in model system contain

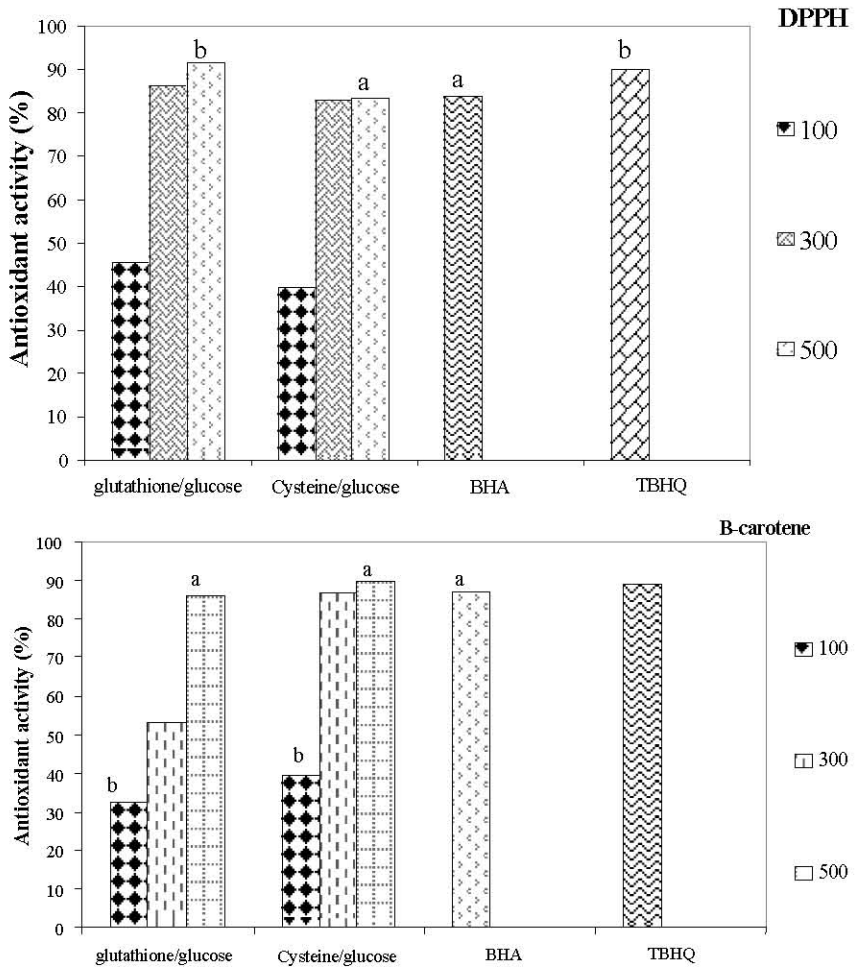

Fig. 3: Antioxidant activity of volatiles generated from glutathione or cysteine/glucose model systems using different assays (DPPH and β-carotene). Columns with the same letter are not significant ($P < 0.05$).

the former the electron-withdrawing effects of the oxygen heteroatom and the hydroxyl substituent oriented in the tram configuration allow for rapid radical addition. The oxygen heteroatom in oxazole is more electronegative than the sulfur in thiazole, which suggests that radical addition to the former will proceed at a faster rate.

It is well known that Maillard reaction products exhibited high efficiency as natural antioxidant and that was clear in all the model systems under investigation using different assays. The radical scavenging activity of glutathione or cysteine/glucose model system was quantified spectrophotometrically using DPPH radical (86.6%) and (82.8%), respectiely in comparison with BHA (85%) and TBHQ (87.3%). as a concentration of 200 ppm of each. 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 [23].

The radical scavenging activity of glutathione–ribose model system was quantified in spectrophotometric assay using the DPPH radical(Fig.3) [24]. 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. Volatile compounds, in particular heterocyclic flavor chemicals, obtained from a sugar/ amino acid model system have been reported to inhibit the oxidation of lipids [24, 25]. These studies clearly indicate that some flavor chemicals possess antioxidative activities [26-28].

The volatile extracts that exhibited high antioxidative activities (glucose/ asparagine, glucose/ histidine and glucose/tryptophan). The effect of irradiation on the formation of MRPs during irradiation in whey protein

suspension as well as antioxidant activity was studied using monitoring spectrophotometric and chemical changes. A dose-dependent increase in UV absorbance and development of fluorescence was observed. Formation of brown pigments was established by increased A_{420} nm. These MRPs exhibited antioxidant activity as measured by 1,1-diphenyl-2-picrylhydrazyl and β-carotene bleaching assays. These MRPs were able to scavenge hydroxyl and superoxide anion radicals under *in vitro* conditions. Dose-dependent decrease in free amino groups and lactose suggested the formation of glycated proteins upon irradiation treatment [15].

The mixtures of casein and glucose with and without antioxidants were prepared by Faist and Erbersdobler [28] to give a moisture content of 11% and stored at 37°C. Under 60% relative humidity for 0-60 days. The effects of antioxidants on the Maillard reaction were monitored by browning, available lysine and furosine level. Browning decreased with the addition of α-tocopherol, BHT, BHA or propyl gallate but not ascorbic acid in mixtures stored 60 days. All antioxidants improved the retention of available lysine and lowered the concentration of furosine compared to the control.

Odour Sensory Characteristics: Pronounced differences were observed in the odour profiles. As expected, intensities of roasted, burnt, caramel and sweet notes were higher in glutathione/ glucose model system than cysteine/ glucose which may be attributed to presence of thiazole, pyrazines and thiol compounds with high concentration in glutathione/ glucose model system, respectively (Table 1).

Roasted meaty aroma contains more thiazoles, pyrazines in comparison to boiled meat. Higher intensity of roasted meat note is responsible, due to the presence of pyrazines and thiazole derivatives, which were reported as the responsible for the roasted aroma in meat accordance to El-Massry *et al.* [23]. Other descriptors gave insignificant results, as the ratings were too low; therefore, they are not included in Figs.1 and 2.

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