GENERATION OF MEAT-LIKE VOLATILES FROM CYSTEINE, GLUCOSE AND SERINE MODEL SYSTEM AND ITS ANTIOXIDANT ACTIVITY

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Abstract

Reaction between Cysteine, Glucose and Serine was carried out to study the volatiles formed via Maillard reaction and their antioxidant activity. 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 increase in roasted and burnt 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 attributes and remarkable increase in the like– roasted meaty aroma. The radical scavenging activity of cysteine, glucose and serine 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 which may be due to the presence of some compounds such as 2-furylmethanethiol, 2-acetylthiazole, 4-hydroxy-5-methyl-3(2H)-furanone.

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

Introduction

The Maillard reaction is of major technological importance in the development and imitation of desirable flavours and aromas of the processed foods [(1)]**.** It has been industrially used for the production of imitated flavours and aromas especially meat [(2).] **The mechanistic pathway of maillard reaction shows in figures (1-4)**

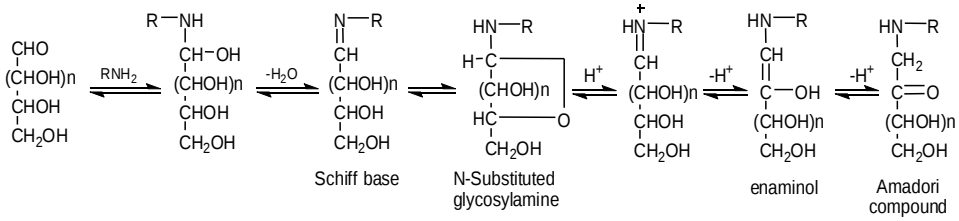

Fig.1. Initial steps in the Maillard reaction showing the formation of an Amadori compound

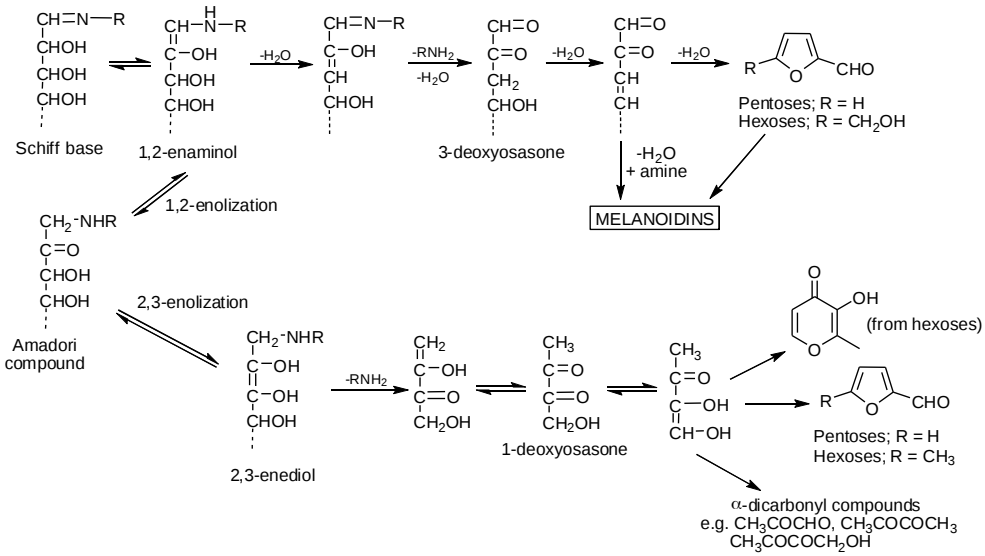

Fig. 2. Intermediate stages of the Maillard reaction showing the formation of carbonyl compounds.

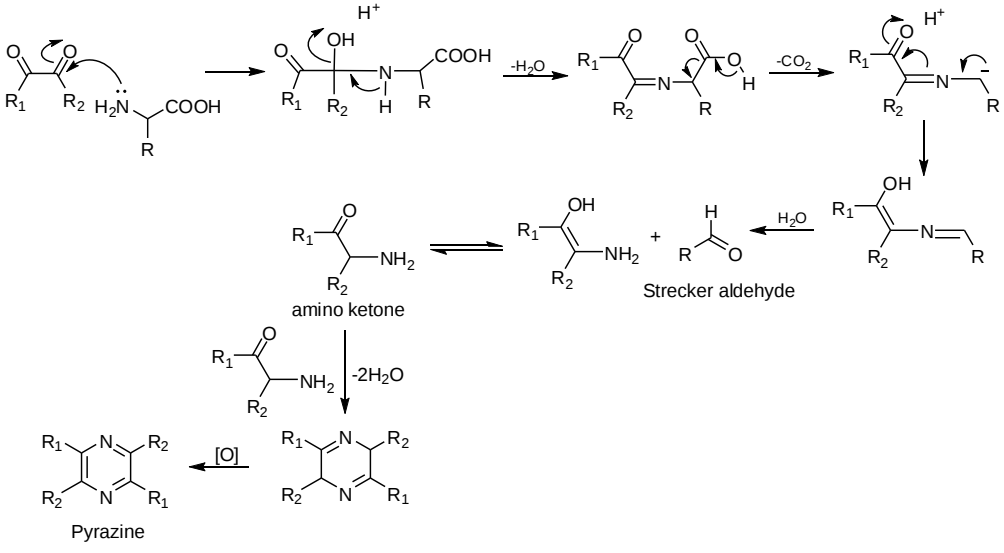

Fig. 3. Strecker degradation

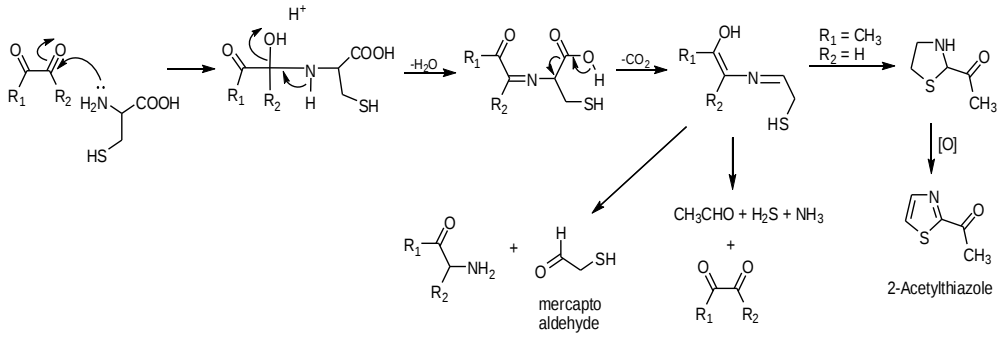

Fig. 5. Routes to the formation of compounds with bread-like aromas from the reaction of proline with 2-oxopropanal

Most subsequent patent proposals for 'reaction product' meat flavourings have involved sulfur – containing amino acids, usually Cysteine or hydrogen sulfide [3, 4]. 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 [5]. **Ohloff et al.**[6] were reported the responsibility of Cysteine which found in muscle protein as the main precursor for this aroma chemicals in meat on prolonged heating. Effect of hydrogen sulphide concentration evolved from cysteine on the volatile formation was studied [7]. However, the formation of meat – like flavour via interaction of cysteine 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 [8].

Although, it has been often shown that Maillard products which are formed from different sugars and amino acids or proteins have antioxidative activity [9], 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 [10]. In this study, we report the formation of meat like–flavour *via* cysteine, glucose and serine 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.

Materials and methods

All chemicals used in this study were purchased from Merck (Darmstadt, Germany). An equal molar ratios of cysteine, glucose and serine 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 used. 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 using100 mL. of methylene chloride. The distillates were dried over anhydrous sodium sulfate and concentrated to about 0.5 mL using rotary evaporator

Gas Chromatography–Mass Spectrometry (GC/MS) analysis

GC-MS analysis of the Cysteine, glucose and serine model system was performed on a Varian gas chromatograph interfaced to Finnigan SSQ7000 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 andthen 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 published data [11].

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 cysteine, glucose and serine model system on DPPH radical was estimated according to (Hatano et al., 1988). cysteine, glucose and serine 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).

Results

Figure 51 shows the Gas Chromatography– Mass Spectrometry (GC–MS) profile of the volatiles isolated from the refluxed cysteine, glucose and serine 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 and 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 cysteine, glucose and serine 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.

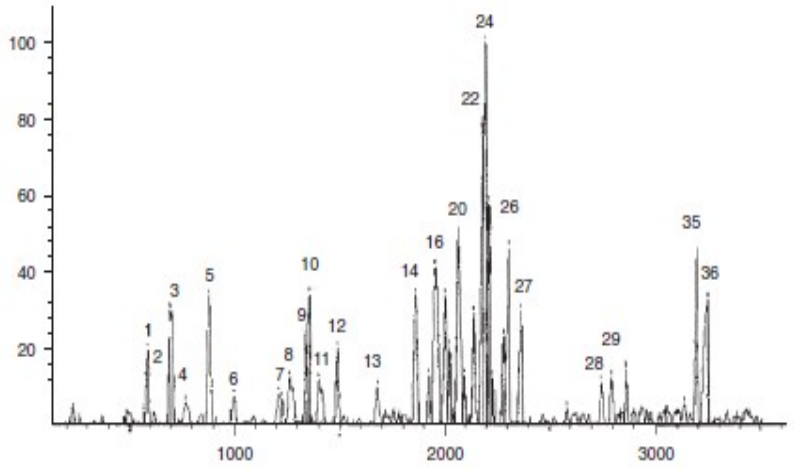

Fig.5 . GC/ MS Chromatogram of cysteine/ glucose/ serine model system

Table 1. Volatile compounds Cysteine, Glucose and Serine model System

Peak No.	Compounds name	K.I [a]	Area %	Identification method
1	2,3-butanedione	592	1.8	KI
2	2-butanone	603	0.277	KI
3	3-pentanone	603	4.466	KI&MS
4	2,3-pentanedione	725	1.011	KI&MS
5	3-pentane-2-one	728	3.789	MS
6	Pyrazine	752	0.298	KI
7	2-methylthiophene	777	0.889	KI, MS& St
8	2-hexanone	798	2.455	KI&MS
9	Hexanol	799	1.083	KI&MS
10	3-mercapto-2-butanone	817	4.006	MS
11	Butanoic acid	821	1.245	MS
12	2-furfural	829	1.868	KI
13	2-methylthiazole	832	0.625	KI
14	2-methylcyclopentanone	836	1.272	KI
15	2,3-dimethylpyrazine	849	0.112	KI&MS
16	2-methylfuranthiol	870	9.773	KI&MS
17	2-ethylthiophene	871	1.029	MS
18	4-hydroxy-5-methyl-3(2H)-furanone	880	0.789	KI
19	2,5-dimethylthiophene	882	0.785	KI, MS& St
20	2-heptanone	890	3.025	KI&MS
21	2-butylfuran	893	1.426	MS
22	3-mercapto-2-pentanone	898	7.336	KI
23	2-acetylfuran	904	0.786	KI, MS& St
24	2-furylmethanethiol	913	20.584	KI&MS
25	2-acetyl-1-pyrroline	926	0.371	KI&MS
26	2(3)-thiophenethio	977	3.428	MS

Con. Table 1.

27	3-hydroxy-2H-pyran-2-one	989	1.534	MS
28	2-formylthiophene	1000	4.196	KI
29	2-acetylthiazole	1020	44.358	KI
30	2-Methylthiazole	1049	0.183	KI
31	Furfurylthiol	1077	0.779	KI&MS
32	2,5-Dimethylthiophene	1096	1.276	MS
33	2-formyl-5-methylthiophene	1118	2.768	KI
34	unknown	1126	0.4457	KI, MS& St
35	(E)-3,5-dimethyl-1,2,4-trithiolane	1140	5.359	KI&MS
36	(Z)-3,5-dimethyl-1,2,4-trithiolane	1151	4.358	KI&MS

Discussion

Thirty six components of the model system involving cysteine, glucose and serine at pH 7 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 Cysteine, Glucose and Serine; the free amino group in Cysteine, Glucose and Serine [8]. 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 cysteine 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 [11-14] and in the volatiles of boiled meat and chicken broth [15]**.** Three alkylthiophenes and two formylthiophenes were identified in the system volatiles. The main route for their formation in cysteine/ glucose and serine model system is through the exchange of oxygen – atom found in furans and furanones with sulphur–atom via hydrogen sulfide [16]**.** One trithiolanes with 2 isomers (E) and (Z) were the presence of certain classes of heterocycles e.g. 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 [17]**.** 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 [18]**,** while 2-furylmethanethiol is

considered to play an important role in chicken and bovine broths [15]. 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 [18, 19] or with furfural to form 2-furylmethanethiol [20]. The 2(3)-thiophenethiol which has been reported in pork flavour [21], was also probably formed via the same routes of furanthiols (Shu et al., 1985). Roasted notes in foods are usually associated with pyrazines and thiazoles. However, mode of cysteine molecule reactivity during Maillard reaction may inhibited the pyrazine formation. 2-Methylthiazole (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 strong in cysteine, glucose and serine 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** [22] 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 cysteine, glucose and serine model system

It is well known that free radicals are of the causes of several diseases, such as Parkinson disease [23], Alzheimer type dementia [24]. 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 cysteine/ glucose and serine 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 [25]**.** The antioxidative activity of the cysteine/ glucose and serine 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%). [26] reported that the five-membered heterocyclyic compounds such as furanones, oxazoles and thiazoles have a remarkable antioxidant activity through radical scavenging.

Conclusion

In summary, the generation of the characteristic meat like-aroma of Cysteine, Glucose and Serine 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).

References

1. LEDL F, SCHLEICHER E (1990). New aspects of the Maillard reaction in food and the human body. Angew Chem Int Ed Engl 29: 565–706.
2. TAIJI T, KOJI T, YUZO I (1996). US Patent 5 565 234.
3. MACLEOD GM, SEYYEDAIN-ARDEBILI M (1981). Natural and simulated meat flavors (with particular reference to beef). CRC Crit Rev Food Sci Nutr 14: 309–437.
4. MACLEOD GM, AMES JM (1986). 2-Methyl-3-(methylthio)furan: a meaty character impact aroma compound identified from cooked beef. Chem Ind (London): 175–176.
5. MOTTRAM DS (1998). Flavour formation in meat and meat products:a review. Food Chem 62: 415–424.
6. OHLOFF G, FLAMENT I, PICKENHAGEN W (1985). Flavor chemistry (Review). Food Rev Int 1: 99–148.
7. HO, C.T.; CARLIN, J. T. Formation and aroma characteristic of heterocyclic compounds in foods. In flavour chemistry trends and developments; Treanishi, Buttery, R., Buttery,

R.G., Shahedi, F., (Eds.; ACS symposium) series 388; American Chemical Society: Washington DC, 1989; pp 92-104.

8. MADRUGA MS, MOTTRAM DS (1998). The effect of pH on the formation of volatile compounds produced by heating a model system containing 5-IMP and cysteine. J Braz Chem Soc 9: 261–271.

9. HSIU, L. K., MING, T. C., DENG, C. L., AND LIEH, C. L. (2005). Relationship between thermal prosperities of muscel proteins and pork quality. Asian Australian Journal of animal science, 18(3), 427-432.

10. EISERICH JP, MACKU C, SHIBAMOTO T (1992) Volatile antioxidants formed from an L-cysteine / D-glucose Maillard model system. J Agric Food Chem 40: 1982–1988.

11. DAMS RP (1995) Identification of essential oil components by Gas chromatography/Mass spectroscopy. Allured Publishing Corporation, USA

12. HATANO T, KAGAWA H, YASUHARA T, OKUDA T (1988). New aspects of the Maillard reaction in food and the human body. Angew Chem Int Ed Engl 29: 565–706.

13. FARMER LJ, MOTTRAM DS, WHITFIELD FB (1989). Volatile compounds produced in Maillard reaction involving cysteine, ribose and phospholipid. J Sci Food Agric 49: 347–368

14. ZHANG Y, HO C-T (1989). Volatile compounds formed from thermal interaction of 2,4-decadienal with cysteine and glutathione. J Agric Food Chem 37: 1016–1020.

15. ZHANG Y, HO C-T (1991). Formation of meat – like aroma compounds from thermal reaction of inosine 5-monophosphate with cysteine and glutathione. J Agric Food Chem 39: 1145–1148.

16. GASSER U, GROSCH W (1990). Primary odorants of chicken broth. A comparative study with meat broth from cow and ox. Z Lebensm Unters Forsch 190: 3.

17. ZHENG, Y., BROWN, S., LEDIG, W. O., MUSSINAN, C., HO, C-T (1997). Formation of sulfur – containing flavor compounds from reactions of furaneol and cysteine, glutathione, hydrogen sulfide, and alanine / hydrogen sulfide. J Agric Food Chem 45: 894–897.

18. FORS S (1983) Sensory properties of volatile Maillard reaction products and related compounds. In: Waller G, Feather MS (eds) The Maillard reaction in foods and nutrition. ACS, Washington DC, pp 185–286.

19. MADRUGA MS (1994). Studies on some factors affecting meat flavour formation. PhD Thesis, The University of Reading, UK

20. VAN DEN OUWELAND GAM, PEER HG (1972). Mercaptofurane and mercaptothiophene derivatives. GB Patent 1,283,912

21, SHIBAMOTO T (1977). Formation of sulfur- and nitrogen-containing compounds from the reaction of furfural with hydrogen sulfide and ammonia. J Agric Food Chem 25:206–208

22. WERKHOFF P, BRUNING J, EMBERGER R, GUNTERT M, HOPP R (1993). Flavor chemistry of meat volatiles: new results on flavor components from beef, pork and chicken. In: Hopp R, Mori K (eds) Recent developments in flavor and fragrance chemistry. VCH, Weinheim, pp 183–213.

23. SHU C-K, HAGEDORN ML, MOOKHERJEE BD, HO C-T (1985). pH effect on the volatile components in the thermal degradation of cysteine. J Agric Food Chem 33: 442–446.

24. MOTTRAM DS (1991). Meat. In: Maarse H (ed) Volatile compounds in foods and beverages. Marcel Dekker, New York, pp 107–177.

25. FARMER LJ, PATTERSON RLS (1991) Compounds contributing to meat flavour. Food Chem., 40: 201–205.

26. ADAMS JD, ODUNZE IN (1991) Oxygen free radicals and Parkinson disease. Free Radicals Biol Med 10: 161–169.

27. CHIA LS, THOMSON JE, MOSECARELLO MA (1984). X-ray diffraction evidence for myelin disorder in brain humans with Alzheimer ‘s disease. Biochem Biophys Acta 775: 308–312..