

Effect of Water Content Chemical Composition and Antioxidant Activity from Volatile Generated from Beef Fat in Maillard Reaction

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THE PRESENT work was aimed to examine the chemical composition and antioxidant activity of the reaction between beef fat and cysteine with and without water model systems. Fifty two and thirty three volatile compounds were isolated and identified with the predominance of esters and sulfur-containing compounds. Sensory evaluation was also performed for the model systems according to (ISO) and the results revealed that the presence of volatiles having roasted meat-like aroma might be due to certain components such as pyrazine and thiazoles derivatives as the main compounds. The radical scavenging activity of the model systems was quantified spectrophotometrically, using DPPH radical and β -carotene bleaching assays.

Keywords: Maillard reaction, Water content, Beef fat, Meat, Like aroma –antioxidant activity.

Many types of chemical reactions are responsible for meat flavor formation, and over 1000 volatile compounds have been reported in beef, pork, lamb, and chicken. Sulfur-containing heterocyclic aroma compounds are known to play an important role in contributing meaty flavor to roasted and cooked meats. Cysteine is an important precursor for the formation of these sulfur compounds and has been extensively used in the manufacturing of reaction flavors. During the cooking of meat, hydrogen sulfide is liberated from the cysteine residues of the meat proteins or peptides and participates in subsequent thermal reactions to generate a number of sulfur-containing volatile compounds. The Maillard model system involving ribose and cysteine has been used widely to study generation of meaty flavors⁽¹⁻⁴⁾.

Over 180 compounds have been identified from these reaction systems, and the key odorants elicit an overall roasty, meat-like odour⁽¹⁾.

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During the cooking of foods, flavor is generated from the interaction of a number of intermediates produced by the thermal degradation of natural flavor precursors. The Maillard reaction, between amino acids and reducing sugars, and the thermal degradation of lipid are the major reactions involved. Model systems have been used to elucidate the intermediates and the pathways involved in the formation of some of the flavor volatiles found in cooked foods ⁽⁵⁾.

Schieberle and Hofmann⁽⁶⁾ compared the character impact compounds of cysteine-based process flavours formed in aqueous solution and under dry condition. In cysteine /ribose model system, dry heating yielded higher amounts of key odorants with roasty notes such as 2-furfurylthiol, 2-acetyl-2-thiazoline and 2-propionyl-2-thiazoline as well as 2-ethyl and ethienyl-3,5-dimethylpyrazine, whereas the meat-like sulphur compounds such as 2-methyl-3-furanthiol and 3-mercapto-2-pentanone were found in comparable or lower concentration, respectively and also revealed that the amounts of 3-deoxysone derived compounds, 2-furfural and 5-methylfuran-2-aldehyde were significantly higher in the dry-heated model system, whereas the formation of the 1-deoxysone-derived 4-hydroxy-2, 5-dimethyl-3-(2*H*)-furanone was enhanced in aqueous solution. An explanation of this finding could be that, under dry heating conditions, caramelisation process is favoured relative to MR. In caramelization process 1, 2-enolisation of the sugar molecule is preferred over 2, 3-enolisation, leading to the formation of high amount of 3-deoxysones.

It has been reported that the overall odor profiles of such reaction flavor and the volatiles formed are affected by a number of factors such as sugar moiety, temperature, reaction time, and the presence or absence of water (a_w) as well as the pH of the reaction solution ^(7, 3). Water activity (a_w) and temperature are the two major identified conditions, which could effect in generation of meaty aroma in a meat model system ⁽⁸⁾. Water activity has been found to have a pronounced influence on the rate of flavour formation as well as flavour character. Vauthery *et al* ⁽⁹⁾, reported the generation of roast-chicken aroma using a cubic phase system. This system was prepared by introducing a melted monoglyceride (saturated C₁₆ and C₁₈) into an aqueous phosphate buffer solution. Compared to the same reaction in phosphate buffer, the flavour formed in the cubic system was more intense, corresponding to higher amounts of sulphur compounds like 2-methyl-3-furanthiol.

Shu and HO⁽¹⁰⁾ investigated the reaction of cysteine and 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone in varying proportions of water and glycerol. They found that a superior roasted/ meaty character was obtained in the aqueous system.

Animal processing by-products retain a substantial amount of fat and protein that could be exploited in a thermal generation of meat aroma ⁽¹¹⁾.

In recent years, an increasing attention to the search for antioxidant protecting factors and more particularly natural inhibitors in the prevention of lipids and polyphenols oxidation, either by autoxidation or after enzymatic action (lipoxygenase, lipase..., etc) has been paid, owing to their involvement in the processed foods stability.

In this area, Maillard Reaction Products (MRPs) which are naturally produced in food during thermal processing and home cooking operations by reducing sugars interacting with available amino groups, modify important food properties such as colour, flavour and stability during processing and storage.

Antioxidant activity of MRPs derived from a protein-sugar system has been studied⁽¹²⁾. There are reports about the use of preformed MRPs as antioxidants in cooked meats from different species⁽¹³⁾. The application of food-processing practices to derive (MRPs) could improve the oxidative stability of food products⁽¹⁴⁾. MRPs used as food ingredients with their functional properties might preserve food from oxidation and microorganism contamination⁽¹⁵⁾. Therefore, protein sugar conjugates could be expected to have significant potential for use in food processing and storage. There are several factors influencing flavour formation and thus the sensory properties of Maillard reaction, are the types of sugar and amino acid, pH, solvent, water activity as well as temperature and time^(16,17).

The present study aimed to investigate the effect of water content in meat-like flavour generated from different model system using beef fat and cysteine with or without water and their antioxidant activity.

Materials and Methods

Chemicals

L-Cysteine (L-Cys), β -carotene, linoleic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH⁰), tert-butyl hydroquinone (TBHQ), polyoxyethylene sorbitan monopalmitate (Tween-80), chloroform (99%) and sodium sulphate anhydrous (Na₂SO₄) were purchased from Merck (Darmstadt, Germany). Dichloromethane (DCM) (99.8%), pressurized sealed bottles: with thermal taps were purchased from Aldrich and Sigma company (Germany). Authentic samples of volatile compounds purchased from laboratory chemical suppliers or were obtained as gifts from flavour companies.

Beef-like aroma model system

Two reaction mixtures were made up in presence or absence of distilled water; each mixture containing L-Cys (1 mmol) and beef fat (10 g). The two mixtures were as follows:

A: Beef fat + L-Cys + water, B: Beef fat+ L-Cys + without water

Each reaction mixture was heated at fixed degree for 30 min in a 1000 ml sealed bottle (Sigma-Aldrich.), fitted with an airtight and was then allowed to cool and subjected to the further study.

Extraction of volatile compounds of Maillard reaction model systems

The reaction mixtures obtained from pressurized bottle after reaction complete were subjected to a simultaneous steam distillation (1 L of water) and solvent (DCM, 200 ml) extraction. The dichloromethane extract was dried over anhydrous sodium sulfate. After the solvent was removed by rotary evaporator, the obtained concentrates were analyzed using gas chromatography and gas chromatography-mass spectroscopy⁽¹¹⁾.

Gas chromatography and gas chromatography-mass spectrometry

The obtained volatile samples were thermally desorbed, using a modified injector port, directly on the front of a (DB5) (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 40 °C -240 °C by the rate 2°C / min. Kovat's indices were determined by co-injection of the sample with a solution containing homologous series of n-hydrocarbons (C₆-C₂₆) 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 namely beef fat / cysteine/ water or without water 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) DB5 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 40 to 240 °C at 2 °C / min. the quantitative determination was carried based on peak area integration.

Antioxidant activity of Maillard reaction products (MRPs)

β- Carotene bleaching assay

Antioxidant activity of the aqueous solution was determined by a β-carotene/ linoleic acid system,⁽¹⁸⁾.

DPPH free radical scavenging assay

DPPH free radical scavenging assay carried out according to Yara *et al.*⁽¹⁹⁾.

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, pungent, burning; 7 = earthy, musty, moldy, sweat, 8 = malty, sweet; 9 = solvents, synthetic, chemicals; 10 = others-specify which); in the profile evaluation: 0mm = absent, 100mm = very strong. Odour profiles were tested by sniffing from ground wide-neck glass bottles⁽²⁰⁾.

Statistical analysis

All analyses were performed in triplicate. The data were recorded as means \pm standard deviations and analyzed by SPSS (version 10.1 for windows 98, SPSS Inc.). One-way analysis of variance (ANOVA) and Turkey multiple comparisons were carried out to test for any significant differences between the means; the mean value of antioxidant activities and sensory analyses of model systems.

Results and Discussion

Table 1 shows the identified volatile compounds with relative area percentages and Kovat indices of the beef fat/ cysteine/ water and beef triglycerides/ cysteine/ without water model systems. Sixty five and seventy five volatile compounds were isolated in the two model systems, respectively including carbonyls, esters, mercaptoalcohols, mercaptoketones, sulphur-containing thiols, thiophenes, disulphides and others.

TABLE 1. Volatile compounds of beef model systems .

No.	Identified compounds ^b	KI ^a	A	B	Method of identification
1	2- Butanone	604	1.2	2.75	K.I
2	ethylacetate	610	1.57	2.01	K.I &MS
3	methylbutenol	624	0.19	0.12	K.I &MS
4	2-methylbutanal	644	33.7	37.0	K.I &MS
5	1-penten-3-ol	672	0.61	0.43	K.I &MS
6	2-ethylfuran	682	0.21	0.33	K.I
7	Pentanal	696	0.05	0.71	K.I &MS
8	2,3-pentanedione	726	0.15	0.15	K.I
9	3-penten-2-one	731	nd	nd	K.I &MS
10	Pyrazine	747	0.16	nd	K.I
11	Pyrrole	760	nd	nd	K.I
12	dimethyldisulphide	783	0.87	0.04	K.I
13	2-pentanal	790	0.29	nd	K.I &MS
14	3-mercapto-2-butanone	798	0.2	0.1	K.I

TABLE 1. Cont.

No.	Identified compounds ^b	KI ^a	A	B	Method of identification
15	1-pentanol	805	nd	nd	K.I &MS
16	2-methylthiophene	814	0.1	0.15	K.I &MS
17	2-furfural	826	0.2	0.53	K.I &MS
18	2-methylthiazole	833	3.5	0.2	K.I &MS
19	2-methylcyclopentanone	838	nd	0.14	K.I
20	methylpyrazine	841	0.1	nd	K.I
21	2,4-dimethylfuran	853			K.I &MS
22	2-furylmethanol	858	nd	0.15	K.I &MS
23	2-methyl-3-furanthiol	870	0.1	0.18	K.I &MS
24	3-ethylthiophene	872	0.25	nd	K.I &MS
25	2,5-dimethylthiophene	876	1.1	nd	K.I
26	4-Hydroxy-5-methyl-3-(2H)-furanone	879	0.1	nd	K.I &MS
27	1-Heptanol	883	0.3		K.I
28	2-Heptanone	886	0.1	nd	K.I &MS
29	2-butylfuran	894	0.1		K.I
30	3-mercapto-2-pentanol	897	0.1		K.I
31	3-(methylthio)propanol	906	0.5	0.03	K.I
32	2-furylmethanethiol	911	0.3	0.44	K.I &MS
34	2-Acetylpyrrole	926	0.2	nd	K.I
36	methyl-dihydrofuranthiol	935	0.1	0.12	K.I &MS
37	mercaptomethylpentanone	940	0.27	nd	K.I &MS
38	2-pentanol	949	0.08	nd	K.I
39	4,5-dihydro-3-(2H)-thiophenone	955	0.2	nd	K.I
40	7-methyl-3-methylene-1,6-octadiene	959	0.2	nd	K.I
41	3-mercaptothiophene	961	0.1	0.04	K.I
42	2-methyl-4,5-dihydro-3-furanthiol	966	0.07	nd	K.I
43	2-thiophenethiol	981	0.2	0.66	K.I &MS
44	2-octanol	983	0.04	nd	K.I
45	3-hydroxy-(2H)-pyran-2-one	987	0.06	nd	K.I
46	2-octanone	991	0.02	nd	K.I
47	2,4,5-trimethylthiazole	1000	0.08	nd	K.I &MS
48	2-furylmethylsulphide	1007	nd	0.54	K.I
49	2-acetylthiazole	1020	0.69	1.14	K.I &MS
51	1,3-dithiane	1030	0.1	nd	K.I &MS
52	4-hydroxy-5-methyl-3-(2H)-thiophene	1044	0.04	nd	K.I
53	methylethylpyrazine	1057	nd	0.34	K.I

TABLE 1. Cont.

No.	Identified compounds ^b	KI ^a	A	B	Method of identification
54	2-ethyl-3,6-dimethylpyrazine	1063	0.08	nd	K.I
55	3-methyl-1,2-dithiolan-4-one	1071	0.02	0.08	K.I & MS
56	3,6-nonadienal	1075	0.7	0.09	K.I & MS
57	Methylcyclohexanone	1131	0.14	nd	K.I
58	2-acetyl-5-methylthiophene	1142	1.06	nd	K.I
60	2-acetyl-3-methylthiophene	1163	0.11	nd	K.I
61	2-methyl-3-(methylthio)furan	1166	0.2	nd	K.I & MS
62	2,3-diethyl-5-methylpyrazine	1172	nd	1.18	K.I & MS
63	2,5-dimethyl-3-isobutylpyrazine	1189	nd	0.08	K.I
64	3,5-dimethyl-1,2-dithiolan-4-one	1199	0.79	0.3	K.I
65	3-methyl-2-formylthiophene	1204	0.26	1.42	K.I
66	2-decanal	1258	0.2	nd	K.I & MS
67	dimethylpyridine	1264	0.16	1.37	K.I & MS
68	dihydrothienothiophene	1290	0.27	0.06	K.I
69	tridecane	1304	0.08	0.26	K.I
70	methylthienothiophene	1317	0.34	0.34	K.I

A: Beef fat/ cysteine/ with water, B: beef fat/ cysteine/ without water
a: Kovat indices, b: identified compounds

Aldehydes

Table 1 shows the aldehydes identified in the two systems. All aldehydes were detected in (A and B) model systems. The major aldehydes are 3, 6-nonadienal, (A=0.7%,B=0.09%), 2-methylbutanal (A=33.7%, B=37.0%), pentanal (A=0.05%, B=0.71%), 2-decanal and 2-pentanal were identified in A model system only with 0.2 and 0.29% respectively. Most of the straight chain aldehydes are derived from the oxidation of unsaturated fatty acids or may be due to thermal degradation of lipid in (A and B) model system ⁽²¹⁾. Clearly, when water is present in a very excess, the volatile products of MR differ greatly from those of low-water system. The conditions also, affect the formation of lipid oxidation products, the dry and low-moisture systems producing low concentration of lipid-derived volatiles. The role of moisture in the oxidation lipids indicates that the oxidation decreases with decreasing water activity ⁽²¹⁾, and the ability of water to mobilize reactants and catalysts may be important. Other affects of water such as hydrogen bonding to lipid hydro-peroxides and the reaction with free radical oxidation intermediates. The aqueous system plays important role in determining the amount and type of lipid oxidation products ⁽¹⁶⁾.

Ketones

Many ketones were generated in (A and B) model system except 2-heptanone was released in A model system with low concentration (0.1%) may be due to the thermal degradation of fatty acids in the buffalo fat or may be attributed to the presence of (a_w). The presence of other short chain ketones, *e.g.* 2-butanone in (A and B) model systems with (1.2, 2.75 %), respectively can be explained by Maillard-type reaction or (SD) of cysteine. However, the formation of other ketones such as 2-octanone (A = 0.02%, B = 0.03%), and 2-methylcyclopentanone (A=0.1%, B=0.14%) can not be explained by these mechanism; they may be have been formed by the condensation reaction between other carbonyl products that derived from lipid oxidation. Dicarboxyl ketone as 2, 3-pentanedione was detected in (A and B) model system with (2.1%, 0.03%) is important intermediate in the formation of other volatile products, as thiophenes, thiazoles and poly-sulphur volatiles⁽²²⁾. Dicarboxyls can react with hydrogen sulphides heading to the formation of mercaptoketones in (A and B) model systems, *e.g.* 3-mercapto-2-butanone with (0.2 and 0.1%) in (A and B) model systems, which can produce important meat-like volatiles. The meaty flavour of (A) model system was characterized in comparison with (B) model system may be due to the presence of 2-methylcyclopentanone. It was reported to contribute to increase the meatiness of the meat flavour⁽²³⁾.

Alcohols

Alcohols, derived mainly from decomposition of fats⁽²⁴⁾. In this study methylbutenol (A =0.19%, B =0.12%), 1-penten-3-ol (A =0.61%, B =0.43%), 1-heptanol (A =0.3%, B =0.05%), 2-pentanol (A =0.27%, B =0.12%), 2-octanol (A=0.04%, B=0.03%) and nonanol (A=0.2%, B=0.28%). The flavour note of straight-chain primary alcohols has been reported as greenish, woody and fatty-floral⁽²²⁾.

Mercaptoalcohols were formed in the two systems (A and B) including 3-mercapto-2-pentanol was identified in A and B model systems with (0.2% and 0.57%) respectively, where the mercaptoalcohols were formed via the reaction of the corresponding alkanols with hydrogen sulphides⁽²⁵⁾.

Esters

Esters are also thought to have a little impact on uncured meat aroma, that are generally associated with fruity aroma, but known to be associated with cured pork flavour as well, chicken and beef⁽¹¹⁾. Esters were found in (A and B) model systems such as ethylacetate (A=1.57, B= 2.01) and methylcyclohexanoate was generated in the two model system with the same concentration (0.14%).

Hydrocarbons

Both saturated and unsaturated aliphatic hydrocarbons were identified in the two systems such as tridecane (A=0.08 and B=0.26%) and heptadecane (A=0.2%, B=1.2%). While 7-Methyl-3-methylene-1, 6-octadiene (A=0.2%) was identified in (A) model system may be formed due to the thermally degraded fat

in low (a_w). All of these compounds have also been reported to be found in cooked beef⁽²³⁾.

Furans and sulphur-containing compounds

2-Ethylfuran (A = 0.2%, B = 0.33%), 2-furylmethanol (A = 0.06, B = 0.15%), 2,4-dimethylfuran (A = 0.1%, B = 0.08%), 2-furfural (A = 0.2%, B = 0.53%), 2-butylfuran (A = 0.1, B = 0.05%) and 4-hydroxy-5-methyl-3-(2*H*)-furanone (A = 0.1%, B = 0.2%) were identified in meat flavour and produced in (A and B) model systems and they were also reported to be found in cooked meat flavor .i.e., 2-ethylfuran and 2,4-dimethylfuran produced in canned meat of beef flavour⁽²⁷⁾.

Even though none of various furan compounds has been attributed as being crucial to meaty flavour, they have been regarded to contribute to the overall odour of boiled or roasted meaty aroma⁽²⁷⁾. In addition, there are eight thiol compounds such as 2-methyl-3-furanthiol (A = 0.1%, B = 0.18%), 2-furylmethanethiol (A = 0.3%, B = 0.44%), 3-(methylthio)propanol (A = 0.5%, B = 0.03%), methyl-dihydrofuranthiol (A = 0.3%, B = 0.08%) methyl-dihydrofuranthiol (A = 0.3%, B = 0.4%), 2-methyl-4, 5-dihydro-3-furanthiol (A = 0.1%, B = 0.08%), 2-thiophenethiol (A = 0.2%, B = 0.66%) and 2-methyl-3-(methyl-dithio)furan (A = 0.07%, B = 0.1%). Sulphur-containing furans, thiophenes and disulphides known to possess strong meat-like aromas and exceptionally low odour threshold values⁽²⁾. While methanethiol occurred in cooked boiled model system (A), in large concentration than roasted model system (B) which smells like cooked cabbage was also reported to be found in cooked beef meat⁽²³⁾.

Guth and Grosch⁽²⁸⁾ concluded that methanethiol derivatives is one of the character impact odour compounds have been reported to be important in cooked meaty flavour since some heterocyclic Sulphur compounds were described as possessing meat-like aromas^(2,3). It has been postulated that these sulphur-containing heterocyclic compounds could be generated either from thermal degradation of cysteine or cystine through the interaction between carbonyl compound from lipid and fatty acid degradation products⁽²⁹⁾ and sulphur-containing amino acids⁽³⁰⁾, the former pathway might be more important in flavour development in meaty flavour. 2-Methyl-3-furanthiol, methyl-dihydrofuranthiol, 2-methyl-4,5-dihydro-3-furanthiol, bis-(2-methyl-3-furyl) disulphide and 2-methyl-3-(methyl-dithio)furan are found in the two model systems (A and B).

MacLeod⁽²⁹⁾ reported that most of the sulphur-containing compounds including 2-methyl-3-furanthiol have meaty aroma. Gasser and Grosch⁽³¹⁾ also demonstrated that 2-methyl-3-furanthiol along with bis-(2-methyl-3-furyl) disulphide, was one of the odour compounds with high aroma values in cooked meat and which possessed meat-like odour. MacLeod *et al.*⁽²³⁾ suggested that 2-methyl-3-furanthiol, bis-(2-methyl-3-furyl) disulphide, and 2-methyl-3-(methylthio) furan were found in relatively large amounts in beef extract while these compounds existed in pork and chicken in only trace amounts. Most of the

thiophenes found in meats were identified to be substituted at the 2 or 3 position such as 2-methylthiophene (A = 0.1%, B = 0.15%), 3-ethylthiophene (A = 0.25%, B = 0.1%), 2,5-dimethylthiophene (A = 0.08%, B = 1.16%), 4,5-dihydro-3-(2*H*)-thiophenone (A = 0.08%, B = 0.1%), 3-mercaptothiophene (A = 0.1, B = 0.04%), 2-formylthiophene (A = 0.26%, B = 1.42%), 4-hydroxy-5-methyl-3-(2*H*)-thiophene (A = 0.04%, B = 0.06%), 2-acetyl-5-methylthiophene (A = 1.06%) and 2-acetyl-3-methylthiophene (A = 0.11%). 3-Mercaptothiophene was identified in (A & B) model system may be regarded to the decomposition of ribonucleotides of lipids in absence of water and it have roasted note. However, it has been stated that, 3-thiol-substituted thiophene such as 3-mercaptothiophene are the only thiophenes with meaty aroma⁽⁴⁾ but other thiophenes have sulphur⁽⁸⁾ or green/ sweet odour⁽³²⁾. Meanwhile, Van den Ouweland *et al.*⁽³³⁾ reported that a complex mixture of compounds including mercapto-substituted furan and thiophene derivatives possessing roasted meat odour were generated from the reaction of 4-hydroxy-5-methyl-3-(2*H*)-furanone or its thio analog with hydrogen sulphide such as 4-hydroxy-5-methyl-3-(2*H*)-thiophene.

4-hydroxy-5-methyl-3(2*H*)-furanone could be a degradation product of ribonucleotides such as robose-5-phosphate indicating that beefy aromas could possibly be generated from sugar degradation in addition to (MR).

Sulphides or disulphide compounds have been regarded to be associated with meat-like aroma. The presence of thiol substituted furans and thiophenes gave rise to a number of important disulphides in the reaction mixture of (A and B) model systems. sulphides derivatives were detected in (A and B) model systems such as dimethyldisulphide (A = 0.87%, B = 0.04%) and 2-furylmethylsulphide (B = 0.54%). These results confirm previous observations that the formation of furyl and thienyl sulphide and disulphides⁽³⁴⁾.

Several di-tri- and tetra-sulphur containing five or six membered rings were identified in the (A and B) systems, such as 1, 3-dithiane (A = 0.1, B = 0.19%), 3-methyl-1, 2-dithiolan-4-one (A = 0.02%, B = 0.08%).

Shu *et al.*⁽³⁵⁾ described the formation of dithiaolane and aliphatic sulphur compounds via the reaction between breakdown of fats and cysteine and they are likely to be formed from thermal degradation of cysteine or can be generated from the reaction of aldehydes/hydrogen sulphide⁽⁸⁾.

Bicyclic sulphur-compounds were generated in the two system (A and B), *i.e.* dihydrothienothiophene (A = 0.27%, B = 0.06%) and methylthienothiophene (A = 0.34%, B = 0.38%).

Moon *et al.*⁽³⁾ was reported a number of alkylthienothiophenes were generated in the volatile compounds of cysteine/ribose model system. Thiazoles such as 2-methylthiazole (A = 3.5%, B = 0.2%), 2,4,5-trimethylthiazole (A =

0.08%, B = 0.03%), 2-acetylthiazole (A = 0.69%, B = 1.14%) were reported in beef cooked at 140°C^(26, 37).

Nitrogen-containing compounds

Among the eleven nitrogen-containing compounds which identified in (A and B) systems, where nine of them belonged to the pyrazine class. Pyrazine derivatives as pyrazine (A = 0.16), methylpyrazine (A = 0.1%), 2-ethyl-3,6-dimethylpyrazine (A = 0.08%), methylethylpyrazine (B = 0.34%), 2,3-diethyl-5-methylpyrazine (B = 1.18%), and 2,5-dimethyl-3-isobutylpyrazine (B = 0.08%) and 2-acetylpyrazine (B = 0.6%), which also reported to be found in cooked beef⁽³⁸⁾.

2-Acetyl pyrazine was reported to be found in fresh, frozen beef and canned beef, shallow fried beef and this study it was found in two systems (A and B)⁽³⁹⁾.

The odour of pyrazines has been traditionally regarded as nutty, roasted or burnt⁽⁴¹⁾. While the other nitrogen compounds are dimethylpyridine (A = 0.16%, B = 1.37%) and 3-hydroxy-(2*H*)-pyran-2-one was identified in A model system with (0.06%) which can be from the (SD), that involved due to the interaction of nitrogen containing molecules (α -amino acid) with lipid degradation products⁽¹⁷⁾.

Odour sensory characteristics

Pronounced differences were observed in the odour profiles. As expected, intensities of roasted, burnt, caramel and sweet notes were higher in (A) model system than (B) which may be attributed to presence of thiazole, pyrazines and thiol compounds in (A) 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⁽²⁰⁾.

Other descriptors gave insignificant results, as the ratings were too low; therefore, they are not included in Fig.1 and 2.

Antioxidant activity of (A and B) model systems

It is well known that natural antioxidative food compounds are important for food technology, because they prolong the shelf life of processed food stuffs. More recently they also gained more interest because it was suggested that, their intact is beneficial for health and they are protective, e.g. against coronary heart diseases. The radical scavenging activity of the two model systems were measured by DPPH and β -carotene methods. As shown in (Fig. 3 & 4) the model system of beef_{fat} / cysteine in presence of water has higher antioxidant activity (71.06% at 400 μ g/ml) than beef_{fat} / cysteine/ without water model system (62.51% at 400 μ g/ml) in comparison with TBHQ (98.73% at 400 μ g/ml).

The MRPs of beef fat model system was found to be higher efficient than beef triglyceride model system may be due to the presence of phospholipid in (A) model system that, participate in MR to generate several heterocyclic volatiles with antioxidant activity as sulphides, thiazoles, pyrazines and thiols derivatives. As expected, (A) model system has higher antioxidative efficiency than (B) model in β -carotene-linoleate method, (A) model system inhibited the bleaching by (71.06% at 400 $\mu\text{g}/\text{ml}$) compared TBHQ with (98.86% at 400 $\mu\text{g}/\text{ml}$).

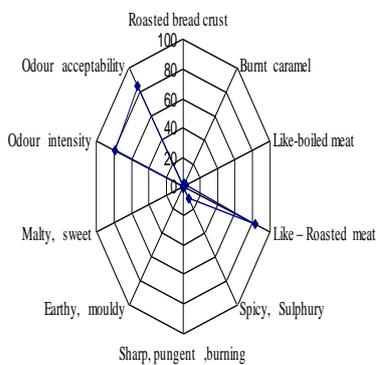


Fig.1. Sensory profile of A model system .

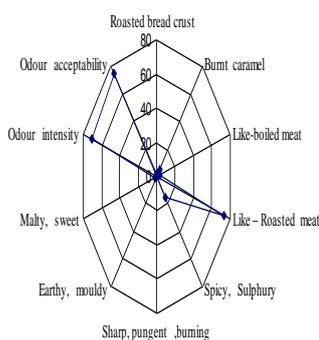


Fig.2. Sensory profile of B model system .

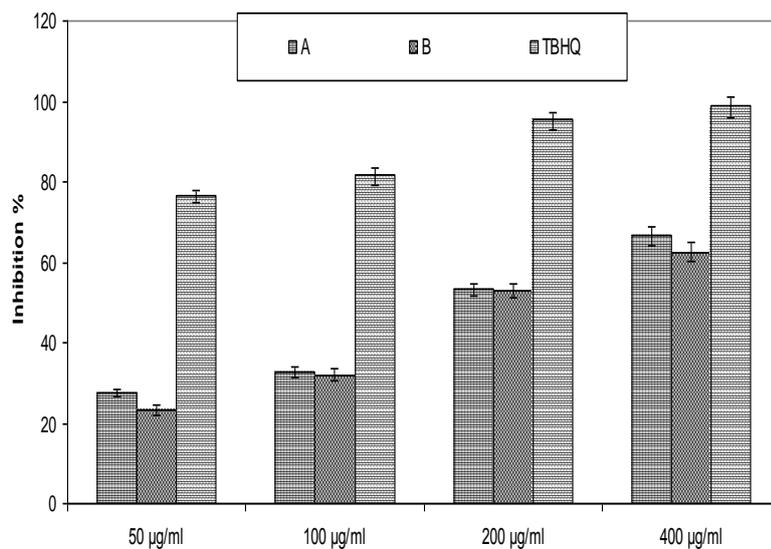


Fig.3. DPPH scavenging activity of A and B model systems .

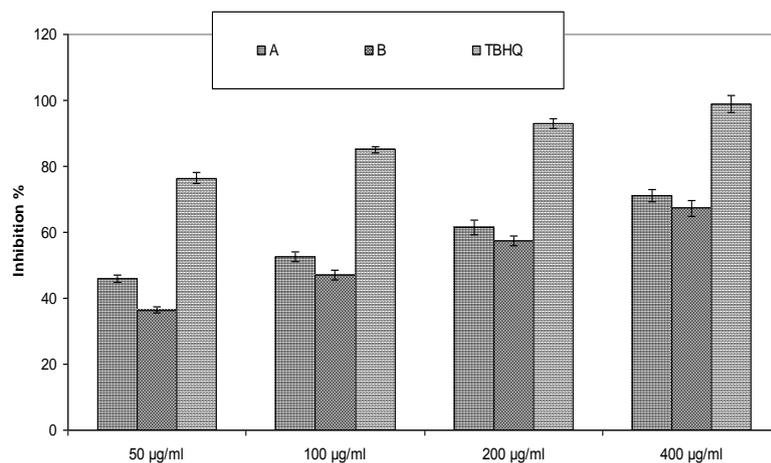


Fig.4. Beta – carotene bleaching scavenging activity of A and B model systems .

References

1. **Hofmann, T. and Schieberle, P.**, Evaluation of the key odorants in a thermally treated solution of ribose and cysteine by aroma extract dilution techniques. *J. Agric. Food Chem.* **43**, 2187-2194 (1990).
2. **Mottram, D. S. and Whitfield, F. B.**, Maillard-lipid interaction in nonaqueous systems: volatiles from the reaction of cysteine and ribose with phosphatidylcholine. *J. Agric. Food Chem.* **43**, 1302-1306 (1995a).
3. **Mottram, D. S. and Whitfield, F. B.**, Volatile compounds from the reaction of cysteine, ribose, and phospholipids in low-moisture system. *J. Agric. Food Chem.* **43**, 984-988 (1995b).
4. **Werkhoff, P., Bruning, J., Emberger, R., Guntert, M., Kopsel, M., Kuhn, W. and Surburg, H.**, Isolation and characterization of volatile sulphur-containing meat flavour components in model systems. *J. Agric. Food Chem.* **38**, 777-791 (1990).
5. **Vernin, G. and Parkanyi, C.**, Mechanisms of formation of heterocyclic compounds in Maillard reaction and pyrolysis reactions. In: *Chemistry of Heterocyclic Compounds in Flavour and Aromas*; G. Vernin, ED.; Ellis, Horwood: Chichester, U.K, pp.151-207 (1982).
6. **Schieberle, P. and Hofmann, T.**, Characterisation of key odourants in dry-heated cysteine-carbohydrate mixtures: Comparison with aqueous reaction systems, In: *Flavour Analysis*, C.J. Mussinan and M.J. Morello, (Ed). ACS, Washington. DC, pp. 320-330 (1998).
7. **Lane, M.J. and Nursten. G.A.**, The variety of odours produced in Maillard model systems and how they are influenced by reaction conditions, In: *Maillard Reaction in Food and Nutrition*. (G.R.Waller and M.S.Feather) Ed., American Chemical Society, Washington, DC, pp. 141-158. (1983).
8. **Kavitha, S. and Modi, V. K.**, Effect of water activity and temperature on degradation of 5'-inosine monophosphate in a meat model system. *J. Food Sci. and Technol.* **40**, 250-259 (2007).
9. **Vauthey, S., Leser, M. and Mllo, C.**, An aroma product comprising saturated C₁₆ – C₁₈ monoglycerides. *European patents*, 1008305 (1998).
10. **Shu, C. K. and Ho, C. T.**, Parameter effect on the thermal reaction of cystine and 2, 5-dimethyl-4-hydroxy-3(2H)-furanone, In: *Thermal Generation of Aromas*. T.H. Parliament, R. J, McGorin and C.T. Ho, Ed. ACS, Washington, DC, pp. 229-241 (1989).
11. **Wettasinghe, M., Shahidi, F., Amarowicz, R. and Abou-Zaid, M.**, Phenolic acids in defatted seeds of borage (*Borago officinalis* L.) *.Food Chem.* **75**, 49-59 (2001).
12. **Jing, H. and Kitts, D.D.**, Chemical and biochemical properties of casein sugar Maillard reaction product. *Food and Chemical Toxicology*, **40**, 1007-1015 (2002).
13. **Jayathilakan, K. and Sharma, G.K.**, Role of sugar-amino acids interaction products as antioxidants in a methyl linoleate model system. *Food Chem.* **95**, 620-626 (2006).

14. **Wijewickreme, A.N. and Kitts, D.D.**, Metal chelating and antioxidant activity of model Maillard reaction product. *Advances in Experimental Medicine and Biology*, **434**, 245-254 (1998b).
15. **Chevalier, F., Chobert, J. M., Genot, G. and Haertle, T.**, Scavenging of free radicals, antimicrobial, and cytotoxic activities of Maillard reaction products of lactoglobulin glycosylated with several sugars. *J. Agric. Food Chem.* **49**, 5031-5038 (2001).
16. **Reineccius, G.A.**, The influence of Maillard reactions on the sensory properties of food. In: *The Maillard Reaction in Food Processing, Human Nutrition and Physiology*. P.A.Finot.H.U.Aeschbacher, R.F.Hurrell and R. Liardon), Ed. Birkhauser Verlag, Basel, pp. 157-170 (1990)
17. **Shibamoto, T.**, Heterocyclic compounds in browning and browning/ nitrit model systems, In: *Instrumental Analysis of Foods*, **1**. Ed .G. Charalambous and G. Inglett) Academic press, New York, pp 229-277 (1983).
18. **Matthus, B.**, Antioxidant activity of extracts obtained from residues of different oilseeds. *J. Agric. Food Chem.* **50**, 3444-3452 (2002).
19. **Yara, S. Q., Emilio, Y. I., Deborah, H. M., Geni, R. S. and Elizabeth, A. F.**, Garlic (*Allium Sativum L.*) and ready to eat garlic products: *in vitro* antioxidant activity. *Food Chem.***115**, 371-374 (2009).
20. **El-Massry, K., Farouk, A. and El-Gorab, A.**, Volatile constituents of glutathione/ ribose model system and its antioxidant activity. *Amino Aci.* **24**, 171-177 (2003).
21. **Elmore, J.S., Mottram, D.S., Enser, M.B. and Wood, J.D.**, Novel thiazoles and 3-thiazolines in cooked beef aroma. *J.Agric. Food Chem.* **45**, 3603-3607 (1997).
22. **Peterson, R. J. and Izzo, H. J., Jungermann, E. and Chang, S. S.**, Changes in volatile flavour compounds during the retorting of caned beef stew. *J. Food Sci.* **40**, 948-954 (1975).
23. **Macleod, G. and Ames, J.M.**, 2-methyl-3-(methylthio) furan: a meaty character impact aroma compound identified from cooked beef. *Chem. Ind., (London)*, 175-176 (1986).
24. **Watanabe, K. and Sato, Y.**, Some alkylsubstituted pyrazine and pyridine in the flavour components of shallow fried beef. *J.Agric.Food. Food Chem.* **19**, 1017-1019 (1971).
25. **Farmer, L.J., Mottram, D.S. and Whitfield, F.B.**, Volatile compounds produced in Maillard reactions involving cysteine, ribose and phospholipid. *J. Sci. Food Agric.*, **49**, 347-368 (1989).
26. **Mottram, D.S.**, Flavour compounds formed during the Maillard reaction. In: *Thermally Generated Flavours: Maillard, Microwave, and Extrusion processes*; Parliament, T. H., Morello, M.J., McGorin, R.J., ACS Symposium Series, 543, ACS: Washington DC, pp. 104-126 (1994).
27. **Shahidi, F.**, Flavour of muscle foods- an overview. In *flavour of meat, meat products and seafoods*; Shahidi, F., Ed, Blackie Academic and Professional: New York, pp 1-4 (1998).

28. **Guth, H. and Grosch, W.**, Identification of the character impact odourants of stewed beef juice by instrumental analysis and sensory studies. *J. Agric. Food Chem.* **42**, 2862-2866 (1994).
29. **MacLeod, G.**, The flavour of beef. In: *Flavour of Meat And Meat Products*, F. Shahidi (Ed.), pp. 4-37. Glasgow: Chapman and Hall. (1994).
30. **Zhang, Y. and Ho, C. T.**, Volatile compounds formed from thermal interaction of 2, 4-decadienal with cystine and glutathione. *J. Agric. Food. Chem.* **37**, 1016-1020 (1989).
31. **Gasser, U. and Grosch, W.**, Identification of volatile flavour compounds with high aroma values from broiled beef. *Z. Lebensm. Unter. Forsch.* **186**, 489-494 (1988).
32. **Shahidi, F. and Rubin, L.J.**, Meat flavour volatiles: A review of the composition, techniques of analysis, and sensory evaluation. *CRC Crit. Revs. Food Sci. and Nutri.* **24**, 141-243 (1986).
33. **Van den Ouweland, G.A.M. and Peer, H.G.**, Compounds contributing to beef flavour. Volatile compounds produced by the reaction of 4-hydroxy- methyl-3-(3H)-furanone and its thioanalogue with hydrogen sulphide. *J.Agric.Food. Chem.* **23**, 501-505 (1975).
34. **Madruga, M.S. and Mottram, D.S.**, The effect of pH on the formation of volatile compounds produced by heating a model system containing 5- IMP and cysteine. *J. Barz.Chem.Soc.* **9** (3), 261-271 (1998).
35. **Shu, C. K., Hagedorn, M. L., Mookherjee, B. D. and Ho, C. T.**, Volatile components of thermal degradation of cysteine in water. *J. Agric. Food. Chem.* **33**, 438-442 (1985).
36. **Moon, S. Y., Margaret, A. C. and Eunice, C. Y.** Odour active components of simulated beef flavour analyzed by solid phase microextraction and gas chromatography-mass spectroscopy and olfactometry. *Food Res. Inter.* **39**, 294-308 (2006).
37. **Madruga, M.S. and Mottram, D.S.**, The effect of pH on the formation of Maillard-derived aroma volatiles using a cooked meat system. *J. Sci. Food Agric.* **68**, pp. 305-310 (1995).
38. **Cerny, C. and Grosch, W.**, Evaluation of potent odourants in roasted beef by aroma extract dilution analysis. *Zeitschrift FurLebensmitteluntersuchung und Forschung A*, **194**, 322-325 (1992).
39. **Peterson, R.J. and Chang, S.S.**, Identification of volatile flavour compounds of fresh, frozen beef stew and a comparison of these with those of canned beef stew. *J. Food Sci.* **47**, 1444-1448 (1982).
40. **Mussinán, C. J., Wilson, R. A. and Katz, I.**, Isolation and identification of pyrazines present in pressure-cooked beef. *J. Agric. Food. Chem.* **21**, 871-872 (1973).

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تأثير المحتوى المائي على التركيب الكيماوى ونشاط تضاد الأكسدة للمركبات الطيارة الناتجة من تفاعل ميلارد مع دهن الأبقار

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تهدف الدراسة الي توضيح تأثير المحتوى المائي على التركيب الكيماوى ونشاطية التضاد للأكسدة للمركبات الطيارة المسئولة عن رائحة اللحم أثناء تفاعل ميلارد بين الحمض الأميني السيستئين في وجود الماء او عدم وجود الماء مع دهن الأبقار كمصدر اقتصادي لمكسبات الطعم و الرائحة و ايضا كمضاد أكسدة طبيعية فعالة.

تم استخدام التحليل الغازي الكروماتوجرافي- طيف الكتلة لكل من تفاعل السيستئين مع دهن الأبقار في وجود الماء و مرة اخري في غياب تأثير الماء وتم التعلاف على ٥٢ و ٣٣ مركب علي التوالي .

أظهرت الاختبارات الحسية فاعلية النواتج ذات رائحة اللحم المحضرة لوجود المركبات الطيارة المسئولة عن نكهة اللحم مثل السلفيد و الثيولات و الثيوفينات. وتم عمل دراسة نشاط تضاد الأكسدة للنواتج و اظهرت النتائج نشاطية النواتج كمضادات أكسدة طبيعية مقارنة بمضاد أكسدة صناعي مثل TBHQ.

يمكن استخدام مركبات النكهة الناتجة من هذه تفاعلات ميلارد من الناحية التطبيقية كمكسبات للطعم و الرائحة طبيعية لها نشاط مضاد للاكسدة و خواص حسية مرغوبة .