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Chemical Composition and Antiradicals Activity of the Volatile Compounds from Reaction of Cysteine / Ribose and Beef Fat

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Abstract: Reaction between beef fat and triglycerides with cysteine and ribose model systems were carried out to study the volatiles formed *via* Maillard reaction and their antiradical activity as well as their safety as a meaty flavour additive. Sixty nine and sixty three volatile compounds were isolated and identified in beef fat/ cysteine/ ribose and beef triglyceride/ cysteine/ ribose model systems, respectively 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 such as pyrazine and thiazole derivatives as the main compounds. The radical scavenging activity of the model systems was quantified spectrophotometrically, using DPPH radical and β-carotene bleaching assays. Biological evaluation was carried out to determine the safety of meaty flavour by studying the Maillard reaction products(MRPs) also, supplementation on body weight, relative organs weight, liver and kidney functions, as well as level of total antioxidant capacity as antioxidant biomarker were investigated.

Key words: Maillard reaction · Meat-like aroma · Sensory evaluation · DPPH · Biological evaluation

INTRODUCTION

The Maillard reaction (MR), also known as non-enzymatic browning, is one of the most common reactions that occur in food products during their processing and storage. The reaction occurs between reducing sugars, amino acids and lipids producing a wide range of colouring (typically brown) and aromatic compounds. Therefore, it can have a strong impact on the appearance, flavour and nutritional value of many food products [1]. The Maillard reaction has also been employed by the food industry to produce processed flavours, especially savoury notes, for application in numerous food staffs [2, 3]. Heterocyclic compounds, especially that containg sulfur, are very important flavour compounds in MR, providing savoury, meaty, roast and boiled flavours. These later compounds, together with carbonyls produced in the MR lead to many important classes of flavour volatiles including: furans, pyrazines, pyrroles, oxazoles, thiophenes, thiazoles and many other heterocyclic compounds [4]. Ohloff *et al.* [5] reported the responsibility of cysteine found in muscle protein as the main precursor of meat-like aroma on prolonged heating. Volatile compounds were formed from the thermal interaction between cysteine and carbohydrates have been studied extensively. However, few studies on the volatiles produced from cysteine/ fat interaction have been reported. A range of volatile aroma compounds have been detected depending on interaction between lipid degradation and MR for their formation.In addition, the presence of lipid has been shown to affect the formation of volatile products of the MR. Over 180 compounds have been identified from cysteine/ribose model system [6], including a number of furanthiols and disulphides which have strong meat-like aromas. In the presence of lipids or fatty acids, a number of thiophene derivatives were arising from the interaction of fatty acids degradation products with the MR [7]. Saturated and unsaturated aldehydes, from lipid oxidation, are major contributors to the volatile profile of the cooked meats [8]. Oxidative reactions occurring in a variety of foods can highly affect their sensorial and nutritional qualities. Synthetic antioxidants, such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and tertiary butyl

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hydroxyl quinoline (TBHQ) are commonly used as food preservatives. However, the use of such compounds has been related to health risks resulting in strict regulations of their use in foods. According to Baardseth [9], they have carcinogenic effects on living organisms. Because of the growing concern for the potential health hazard of synthetic antioxidants, there is renewed much interest in the use of naturally occurring substances.

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. MRPs formed from the condensation of sugars and amino acids have been reported to possess promising antioxidative activity [10]. The MR produced from an amino acid/ sugar model system has been associated with the formation of compounds with strong antioxidative activity. MRPs used as food ingredients with their functional properties might preserve food from oxidation and microorganism contamination [11]. Therefore, protein sugar conjugates could be expected to have significant potential for use in food processing and storage. The production of MRPs is widely affected by temperature, pH, reaction time and reactants, which in turn result in different chemical composition and variable antioxidant activity of MRPs.

The relationship between the chemical compositions of MRPs and antioxidant activity remains unclear [12]. The purpose of this study was to investigate the possible relationship between different compositions of MRPs and the antioxidant activity of MRPs derived from different model systems. Also, the complexity of the mechanisms involved in MR and the uncertainty of melanoidin formation, the exact structure of those compounds responsible for the antioxidative effect have not yet been fully determined [13].

Consumers allover the world is becoming more conscious of the nutritional value and safety of their food and its ingredients. At the same time, there is a preference for natural foods ingredients that are believed to be safer, healthier and less subject to hazards than their artificial counterparts. The safety of food flavours has been highly concerned in recent years. Compared with synthetic flavour compounds, natural flavours are more acceptable to consumers. So it has been one of hot topics in flavour chemistry to obtain natural flavours [14]. Meat-like aromas are known to make a significant improvement in many savoury foods such as soups, gravies, snakes and in a variety of other prepared foods. Almost of these food types can be purchased in a final cooked form so that it requires only heating. It should be noted that, although meat-like flavourings developed so far are reasonably satisfactory, they are still far from real meat flavours.These products do not yet have the unique characteristic flavour profile, the meat component of beef, chicken or veal are in general clearly distinguishable from each other organoleptically.

Egypt has to import large quantity of food flavours annually. If these food flavours can be produced in Egypt, it not only reduces the importation of food flavours but it can also be able to export to other countries. After the animal slaughters as beef, buffalo and chicken many by-products such as fats and blood that contaminate the environment so that, in this study, we report the formation of meat-like flavour via beef fat and triglycerides with cysteine and ribose model systems, the sensory evaluation of MRPs and identification of the volatiles formed *via* MR and its activity to scavenge the free radicals by DPPH and β-carotene methods. In addition, the safety assessment of MRPs *in vivo* was investigated.

MATERIALS AND METHODS

Chemicals: L-Cysteine (L-Cys), L- Serine (L-Ser), L-Leucine (L-Leuc), (D)- Ribose (D-Rib), β-carotene, linoleic acid, 1,1-diphenyl-2-picrylhydrazyl (DPPH°), tert-butyl hydroquinone (TBHQ), polyoxyethylene sorbitan monopalmitate (Tween-80), chloroform (99%) and anhydrous sodium sulphate (Na_2SO_4) 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.

Fats: Fresh beef fat was obtained after animal slaughtered and carefully packed in glass containers and stored in freezer at (-18°C) until used according to Farmer and Mottram, [15].

Preparation of Maillard Reaction Model Systems

Beef -Like Aroma Model System: The reaction mixtures were made up in presence of distillated water; each mixture containing Cysteine (I mmol), (D)-ribose (0.005mmol) and beef fat or triglycerides (10 g). The two mixtures were as follows, (A): Beef fat/ cysteine/ ribose and (B): Beef triglycerides/ cysteine/ ribose.

Extraction of Volatile Compounds of Maillard Reaction Model System: The reaction mixtures obtained from pressurized bottle after reaction complete 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 sulphate.

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 (DB5) (*60 m x 0.32 mm i.d*) fused silica capillary column, in the oven of a Perkin-Elmer outosystem 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 (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 [16]. GC/MS analysis of the two model systems beef $_{fat}$/ cysteine/ ribose and beef $_{tg}$/ cysteine/ ribose 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 out based on peak area integration [17].

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,sulphuric,onion,garlic; 6 = sharp, punget,burninig;7 earthy,musty,moldy,sweat, wet dog; 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 [17].

Antioxidant Activity of Maillard Reaction Products (MRPs)

β- Carotene bleaching assay: Antioxidant activity of the aqueous solution was determined by β-carotene/linoleic acid system, as described by Matthaus [18]. Antioxidant activity (AA) was calculated by equation [1].

$$AA = [1-(A_{S(0)}-A_{S(120)})/ A_{b(0)}-A_{b(120)}] \times 100 \quad [1]$$

DPPH Free Radical Scavenging Assay: DPPH free radical scavenging assay was carried out according to Yara, *et al* [19].

Safety Evaluation

Experimental Animal Design: A total of 30 male Albino rats, *Sprague dewley* with an average weight of 60-70 g were housed individually in well aerated cages with screen bottoms and fed with basal diet as recommended by Tebib *et al.* [20]. Rats were fed with basal diet for one week as adaptation period. Temperature and humidity were maintained as 25°C and 60%, respectively and water was provided. Rats were randomly divided into three main groups, negative control group (G1) (10 rats) which were fed only on basal diet while the other two groups fed on beef$_{fat}$ / cysteine/ ribose concentrate (G2) and beef $_{tg}$ / cysteine/ ribose concentrate (G3).

Blood Sampling: Blood samples were collected from each rat by orbital puncture and withdrawn on heparinized tubes, plasma were collected after centrifugation at 3000 r.p.m for ten min at 4°C and divided into aliquots to avoid freezing and thawing. Aliquots were then stored at -20°C pending assay.

Biochemical Assays: Tranaminases (ALT and AST), alkaline phosphatase (ALP), γ-glutamyl-transferase (γ-GT), activities were determined by the methods described by Bergmeyer *et al.* [21], Rosalki *et al.* [22], Szasz [23] and Anon [24], respectively. Triglycerides (TG) and total cholesterol levels in plasma were carried out according to the methods of Wahefeld [25] and Allain *et al.* [26], respectively. Plasma samples were analyzed for urea [27] and creatinine [28]. The activity of total antioxidant capacity (TAC) was measured using method described by Koracevic *et al.* [29].

Statistical Analysis: All analyses were performed in triplicate. The data were recorded as means ± standard deviations and analyzed by SPSS (version 10.1 for windows 98, SPPS Inc.). One-way analysis of variance (ANOVA) and Tukey 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. Correlations were obtained by Pearson correlation coefficient in bivariate correlation. Differences between means at 5% ($p < 0.05$) level were considered significant. Each experiment was replicated three times.

RESULTS

Table 1 shows the identified volatile compounds with relative area percentages and Kovat indices in the beef fat and beef triglyceride model systems. Sixty nine and sixty four volatile compounds were isolated in $beef_{fat}$ / cysteine/ ribose and $beef_{tg}$ / cysteine/ ribose model systems, respectively including carbonyls, esters, mercaptoalcohols, mercaptoketones, thiols, thiophenes, disulphides and others. Figure 1 and 2 demonstrate the sensory profiles of the two model systems (A and B) extract roasted meat-like aroma as well as the intensity of the developed odour and the odour acceptability with (79±1.8, 70.0±2.4 and 85.0 ± 2.4, 75.0 ± 3.2), respectively. $Beef_{fat}$ / cysteine/ ribose model system was recorded low increasing in its antioxidant activity with (66.6 ± 0.9, 62.51 ± 1.08 and 71.06 ± 1.06, 67.23 ± 0.87 % at 400 µg/ml) in DPPH and β-carotene assays in comparison with standard TBHQ with (98.73 ± 0.64 % at 400 µg/ml). However, in the course of studying the biochemical effect of MRPs of (A and B) model systems on body weight, relative organs weight, liver functions, kidney functions, glucose level Hb% as well as level of triglycerides, total cholesterol and total antioxidant activity were investigated. No significant change occurred

Table 1: Volatile compounds of beef fat and triglyceride model systems

		Relative area percentages (%)			
No.	Identified compound [b]	K.I[a]	A	B	Method of identification
1	2- Butanone	604	nd	0.08	KI
2	Ethyl acetate	610	0.19	nd	MS
3	Methylbutenol	624	Nd	0.1	KI and MS
4	2-Methylbutanal	644	0.25	0.1	KI
5	2,3-Pentanedione	726	0.1	0.1	KI and MS
6	3-Penten-2-one	731	0.2	nd	MS
7	Pyrazine	747	0.56	0.08	KI
8	Pyrrole	760	0.1	nd	KI and MS
9	Dimethyldisulphide	783	0.08	0.02	KI
10	2-Pentanal	790	0.06	nd	KI and MS
11	3-Mercapto-2-butanone	798	0.16	0.03	KI
12	2-Methylthiophene	814	0.09	0.01	KI
13	2-Furfural	826	0.1	0.02	KI and MS
14	2-Methylthiazole	833	0.11	0.02	MS
15	Methylpyrazine	841	Nd	0.03	KI
16	2.4-Dimethylfuran	853	Nd	0.1	KI
17	2-Furylmethanol	858	0.24	nd	KI and MS
18	2-Methyl-3-furanthiol	870	0.06	0.05	KI and MS
19	3- Ethylthiophene	872	0.04	0.02	KI and MS
20	2,5-Dimethylthiophene	876	0.1	0.1	KI
21	1-Heptanol	883	0.09	nd	KI
22	2-Heptanone	886	0.1	nd	KI
23	2-Butylfuran	894	Nd	0.33	KI
24	3- Mercapto-2-pentanol	897	0.39	0.05	MS
25	3-(methylthio) Propanol	906	0.05	0.01	KI and MS
26	2-Furylmethanethiol	911	0.07	0.09	MS
27	2-Acetylpyrrole	926	0.1	0.21	KI
28	2,5-Dimethylpyrazine	928	Nd	0.02	KI
29	Methyldihydrofuranthiol	935	0.08	0.03	KI and MS

Table 1: Continued

30	Mercaptomethylpentanone	940	0.02	0.01	KI
31	7-Methyl-3-methylene-1,6-octadiene	959	0.45	nd	KI
32	3-Mercaptothiophene	961	0.02	nd	KI
33	2-Methyl-4,5-dihydro-3-furanthiol	966	0.32	0.37	KI
34	2-Thiophenethiol	981	0.09	0.1	MS
35	2-Octanol	983	0.45	0.4	KI
36	3-Hydroxy-(*2H*) pyran-2-one	987	0.27	2.23	KI
37	2-Octanone	991	0.97	3.16	KI
38	2,4,5-Trimethylthiazole	1000	0.33	3.14	KI
39	2-Furylmethylsulphide	1007	1.17	2.02	KI and MS
40	2-Acetylthiazole	1020	6.73	1.86	KI and MS
41	Acetylpyrazine	1023	1.22	0.43	KI and MS
42	1,3-Dithiane	1030	0.53	2.07	KI
43	4-Hydroxy-5-methyl-3-(*2H*)-thiophene	1044	0.67	0.79	KI
45	3-Methyl-1,2-dithiolan-4-one	1071	0.09	0.27	KI
46	3,6-Nonadienal	1075	0.59	nd	KI
47	2-Acetyl-5-methylthiophene	1142	0.03	nd	KI and MS
48	2- Acetyl-3-methylthiophene	1163	0.01	nd	KI
49	2-Methyl-3-(methyldithio) furan	1166	0.1	0.03	KI
50	2,3-Diethyl-5-methyl pyrazine	1172	Nd	0.02	KI and MS
51	2,5-Dimethyl-3-isobutylpyrazine	1189	0.59	0.48	MS
52	(E)or(Z)-3,5-Dimethyl-1,2-dithiolan-4-one	1199	0.38	0.33	KI
53	3-Methyl-2-formylthiophene	1204	2.41	1.07	KI
54	Dihydrothienothiophene	1290	0.3	0.02	KI
55	Tridecane	1304	2	0.9	KI
56	Methylthienothiophene	1317	0.31	0.29	KI
57	1,2,4,5-Tetrathiane	1325	1.1	1.12	KI
58	Methylene *bis*-(methyl sulphide)	1399	0.27	nd	KI and MS
59	Methyldihydrothienothiophene	1422	0.99	0.91	KI
60	2-Ethylthienothiophene	1430	2.25	1.6	KI
61	2-Dodecanol	1467	nd	9.41	KI
62	Ethyl-(E)or(Z)-2,4-decadienoate	1473	nd	1.57	KI
63	3,4,6-Trimethyl-1,2,3-trithiane	1499	4.37	0.78	KI
64	2-Methyl-3-furyl-1-methyl-2-oxopropyldisulphide	1508	0.51	2.38	KI
65	Tridecanal	1526	1.99	2.77	KI
66	3-Mercaptohexanol	1531	10.59	12.3	KI and MS
67	Bis-(2-methyl-3-furyl) disulphide	1541	0.72	1.01	KI and MS
68	2-Methyl-3-furyl-1-ethyl-2-oxopropyldisulphide	1569	0.2	1.48	KI and MS
69	2-Methyl-3-furyl-1-methyl-3-oxobutyldisulphide	1580	0.07	nd	KI
70	2-Methyl-3-furyl-1-methyl-3-oxopropyldisulphide	1589	nd	0.46	KI and MS
71	2-Methyl-3-furyl-1-methyl-2-oxopropyldisulphide	1596	0.53	0.35	KI
72	2-Methyl-3-furyl-2-furylmethyldisulphide	1636	1.69	5.22	KI
73	2-Methyl-3-furyl-3-thienyldisulphide	1698	1.04	0.2	KI and MS
74	2-Methyl-3-furyl-2-methyl-3-thienyldisulphide	1740	0.5	1.27	KI
75	Methylfuraneol	1760	0.61	0.36	KI and MS
76	2-Furyl-methyl-thienyldisulphide	1788	0.88	nd	KI and MS
77	Hexadecanol	1858	0.18	0.17	KI
78	Ethylhexadecanoate	1905	33.62	34.64	KI
79	2-Methyl-3-thienyl-3-thienyldisulphide	1933	14	nd	KI and MS
80	Trimethylphenylbutenone	1942	0.52	0.41	KI

A: Beef fat/ cysteine/ ribose, B: beef triglyceride / cysteine/ ribose

a: Kovat indices, b: Identified compounds

Table 2: Different classes of identified volatile compounds

Total Volatiles	A	B
Aldehydes	1.89	0.10
Ketones	3.79	3.79
Hydrocarbons	1.46	0.90
Esters	35.80	38.98
Alcohols	11.70	22.43
Furans	0.95	0.81
Thiols	0.77	0.68
Thiophenes	3.37	1.99
Sulphides	21.66	14.41
Aliphatic-sulphur-compounds.	6.47	4.57
Bicyclic-sulphur-compounds.	3.85	2.82
Thiazoles	7.17	5.02
Pyrazines	2.37	1.06
Other-nitrogen-compounds	0.47	2.44

A: Beef fat/-cysteine/ribose, B: beef triglyceride /cysteine/ribose

Table 3: Effect of A and B MRPs on some biochemical markers

	G1		G2		G3	
Groups Parameters	Means ± SE	p=5	Means ± SE	p=5	Means ± SE	p=5
ALT (u/ml)	38.2 ±0.41	N.S	40.62±0.33	N.S	39.86±0.65	N.S
AST(u/ml)	43.82±0.75	N.S	44.24±0.90	N.S	40.11±0.88	N.S
ALP(u/ml)	296.28±2.34	N.S	288.78±4.98	N.S	283.60±6.86	N.S
(γ-GT) (u/ml)	2.34±0.24	N.S	2.28±0.96	N.S	2.30±0.12	N.S
Urea (mg/dl)	40.92±0.52	N.S	42.0±0.61	N.S	41.6±0.79	N.S
Creat. (mg/dl)	0.92±0.13	N.S	0.88±0.09	N.S	0.90±0.089	N.S
Hb (g/dl)	13.41±0.23	N.S	14.92±0.39	N.S	13.95±0.17	N.S
Gluc. (mg/dl)	76.22±1.32	N.S	81.61±1.21	N.S	78.92±0.95	N.S
T.Chol. (mg/dl)	124.31±2.4	N.S	126.23±2.81	N.S	130.61±3.61	N.S
Tri (mg/dl)	98.63±1.1	N.S	104.21±2.31	N.S	108.44±4.87	N.S
T.A.C. (mM /L)	0.76±0.03	N.S	0.75±0.031	N.S	0.71±0.028	N.S

N.S; No significant change compared to control (p≤ 0.05),ALT; alanine aminotransferase, AST; aspartate aminotransferase, ALP; alkaline phosphatase, (γ-GT);gamma-glutamyl transferase, Creat; creatinine, Hb%; hemoglobin, Gluc; glucose, T.Chol; total cholesterol, Tri-G; triglyceride and T.A.C; total antioxidant activity

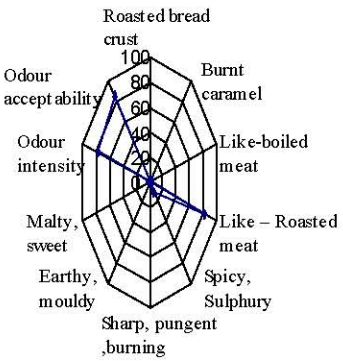

Fig. 1: Sensory profile of A model system

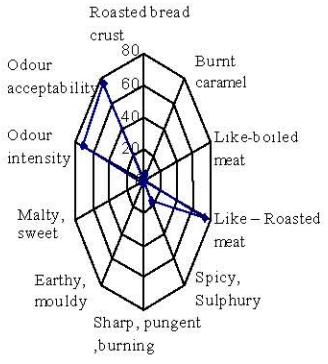

Fig. 2: Sensory profile of B model system

in activity of ALT, AST, ALP, γ-GT, urea and creatinine levels were normal in comparison with control rats group and the extract of the two model systems (A and B). Two MRPs model systems data are postulated in Table (3) Also, results of testing MRPs of (A and B) systems on blood hemoglobin (Hb%) and blood glucose as well as triglycerides, cholesterol and (TAC) showed no significant changes between mean values in control rats group and supplemented MRPs rats groups (A and B).

DISCUSSION

Sixty nine and sixty four components involving beef $_{fat}$ / cysteine / ribose and beef $_{tg}$/ cysteine/ ribose model systems at neutral medium are represented in (Table 1). Esters and sulphur-containing compounds predominate in the volatiles of (A and B) model systems.

Carbonyl Compounds: The contribution of aldehydes to the total volatile contents was (2.64 and 2.85%) for (A and B) models, respectively. Tridecanal was the dominant aldehyde in (A and B) model systems with (1.99 and 2.77%). Most of the straight chain aldehydes are derived from the oxidation of unsaturated fatty acids [30]. Formation of carbonyl compounds may result from thermal oxidative decomposition of lipids in food materials. Aldehydes are believed not only to contribute to the odour of foods including beef, but also to react with other compounds to produce flavour through amino-carbonyl reactions. Various research groups have reported a wide range of saturated and unsaturated aldehydes and ketones in cooked beef. 3, 6-Nonadienal and tridecanal and 2-pentanal have been reported to be identified in cooked beef by Moon *et al.* [31].

Ketones were identified in both (A and B) model systems with (2.07 and 5.86), respectively. 2-Octanone (0.97 and 3.16%), trimethylphenylbutenone (0.52 and 0.41%) and 2, 3-pentanedione (0.1 % in both A and B) were the main constituents of the identified ketones. The presence of ketones can be explained by the interaction of cysteine pyrrolysis and ribose [32]. Trimethylbutenone may be formed via fatty acids or lipid oxidation and have buttery aroma note in cooked meat. While 2, 3-pentanedione may be formed *via* thermal degradation of ribose and ribonucleotides and participate in the formation of pyrazine and thiophenes or it can react with hydrogen sulphide from cysteine to form mercaptoketones which can produce important meat-like volatiles, as 3-mercapto-2-butanone and mercaptomethyl pentanone with (0.16, 0.03 and 0.02, 0.01%) in (A and B) model systems[4]. Mercaptoketones were probably formed via the reaction of the corresponding alkanedione with hydrogen sulphide. Farmer *et al.* [33] mentioned that 3-mercapto-2-butanone and mercaptomethyl- pentanone have been identified in meat aroma model system and in the volatiles of boiled meat of chicken broth.

Alcohols: Different alcohols are detected in (A and B) model systems and the dominant alcohol was 3-mercaptohexanol with (10.59 and 12.3%) in (A and B), respectively. However, there was an obvious difference between alcohol concentrations in the two model systems. Among 55 alcohol compounds listed as components of cooked beef flavour in a review by Shahidi *et al.* [34], relatively large amount of saturated and unsaturated alcohols as 2-octanol and hexadecanol were reported as components of beef flavour. 3-Mercapto-2-pentanol and 3-mercapto- hexanol were formed in (A and B) models with (0.39, 0.05 and 10.59, 12.3%), respectively. Farmer *et al.* [35] reported that mercaptoalcohols have meaty flavour and alcohols are derived from oxidative decomposition of lipids.

Generally, esters have been associated with fruity notes but a considerable number of esters were found in beef flavour [36]. Ethylhexadecanoate was the predominant ester in (A and B) model systems with (35.61 and 37.41%), respectively.

Both saturated and unsaturated hydrocarbons were identified in (A and B) model systems as tridecane (2.0 and 0.9%), respectively and 7-methyl-3-methylene-1,6-octadiene (0.45%) in (A) model system only (table1). All of these compounds have also been reported to be found in cooked beef [37]. Meanwhile, tridecane was identified in both roasted model systems (A and B). Alkylated furan and furaneol were released in (A and B) systems with (0.95 and 0.81%) while the predominant furan derivatives was methylfuraneol (0.61, 0.36% in both A and B models). Mottram [4] reported methylfuraneol to be formed *via* ribose decomposition and associated with roasted note. The thiols identified included2-methyl-3-furanthiol (0.06, 0.05%), 2-(methylthio)propanol (0.05, 0.01%), 2-furylmethanethiol (0.07, 0.09%), methyldihydrofuranthiol (0.08, 0.03%), 2-methyl-4,5-dihydro-3-furanthiol (0.32, 0.37%), 2-thiophenethiol (0.09, 0.1%) and 2-methyl-3-methyldithio)furan (0.1, 0.03%) were determined in both (A and B) model system, respectively. It was reported that, 2-methyl-3-furanthiol possesses meaty, roasty and boiled notes [38], while 2-furylmethanethiol is considered to play an important role in chicken broth [39]. 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 furyl methanethiol [38]. Thiophene derivatives were detected in both (A and B) models (table 1) and the abundant of them was 2-methyl-2-formylthiophene with (2.41, 1.07%) in A and B models. Thiophenes were originated from thermal degradation of cysteine and carbonyl compounds from fatty acids degradation [40]. Most of thiophene

derivatives derived sulphurous odour except 2-mercaptothiophene with meaty aroma [41].The predominant bicyclic-sulphur compound was 2-ethylthienothiophene as shown in Table 1. Farmer *et al.* [35] reported that a number of alkylthienothiophene was released in meat model system of cysteine-ribose model system. Table (1) shows some of aliphatic poly-sulphur containing compounds that were detected in beef$_{fat}$ / cysteine/ ribose and beef $_{tg}$ / cysteine/ ribose model systems and their concentration ranged from (3.85 in A and 2.82% in B).Vernin [42] reported that 2, 4, 6-trimethyl-1, 2-dithiolan-4-one was identified in cooked beef while (E) or (Z)-3, 5-dimethy-1, 2-dithiolan-4-one was identified in chicken flavour.

Generally, sulphides and disulphides were generated in (A and B) model systems and have been regarded to be associated with meat-like aroma [4]. 2-Methyl-3-thienyl-3-thienyldisulphide was considered the predominant sulphide derivative in (A) model system with (14.0%) as well as 2-methyl-3-furyl-1-methyl-2-oxopropyldisulphide in (B) model system with (2.38%).Roasted notes in foods are usually associated with the presence of certain classes of heterocyclic compounds e.g. pyrazines and thiazoles (Table. 1). Beef $_{fat}$ / cysteine/ ribose model system may catalyze the formation of thiazole and pyrazine derivatives more than beef $_{tg}$ / cysteine/ ribose model system due to thermal decomposition of phospholipids in (A) model system.

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 the presence of thiazoles (7.17, 5.02%), pyrazines (2.37, 1.06%) and furans (0.95, 0.81%) in (A and B) model systems, respectively (Table 1). Mottram [43] reported that 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 responsibles for the roasted aroma in meat [33]. Other descriptors gave insignificant results, as the ratings were too low; therefore, they are not included in Figs. 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 interest because it was suggested that, their intact is beneficial for health and they are protective, e.g. against coronary heart diseases [44]. The radical scavenging activity of the two model systems were measured by DPPH and β-carotene methods. As shown in Fig. 3 and 4 the model system of beef $_{fat}$ / cysteine/ ribose has higher antioxidant activity (66.6% at 400 μg/ml) than beef tg/ cysteine/ ribose model system (62.51% at 400 μg/ml) in comparison with TBHQ (98.73% at 400 μg/ml). The MRPs of beef fat model system was found to be higher efficient than beef triglyceride model system which may be related to the presence of phospholipids in (A) model system that, participate in MR to generate several heterocyclic volatiles having antioxidant activity as sulphides, thiazoles, pyrazines and thiols derivatives [45]. 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 μg/ml) in comparison with TBHQ with (98.86% at 400 μg/ml).

Biological Evaluation: The safety assessment of MRPs on body weight, relative organs weight, liver and kidney functions as well as levels of total antioxidant capacity, hemoglobin %, glucose, total cholesterol and triglycerides were investigated.

Body and Relative Organs Weights: Cumulative body weight (initial, final and gain weight), organs weight (liver, spleen, heart, lung and pancreas) and relative organs weight (organ weight / body weight ratio) showed no significant difference in the mean value of body weigh gain between control rats and MRPs supplemented groups after eight weeks (at the end of experiment). No significant differences were noted in organs weight and relative organs weight in the three MRPs supplemented groups (G2 and G 3) compared to control rats group (G1). Bissery *et al.* [46] reported that any compound dosage that produces 20% loss in weight is considered as excessively toxic. The obtained results revealed high significant increase ($p \leq 0.01$) in the mean value of final body weight in both control and supplemented rats groups with respect to their initial body weight.

In early study, Feron [47] reported that in sub-chronic toxicity experiments, the weight of major organs of the body may serve as a useful index of toxicity. Our results pointed out to the safety effect of the two different concentrations of MRPs on body weight as well as organs weight.

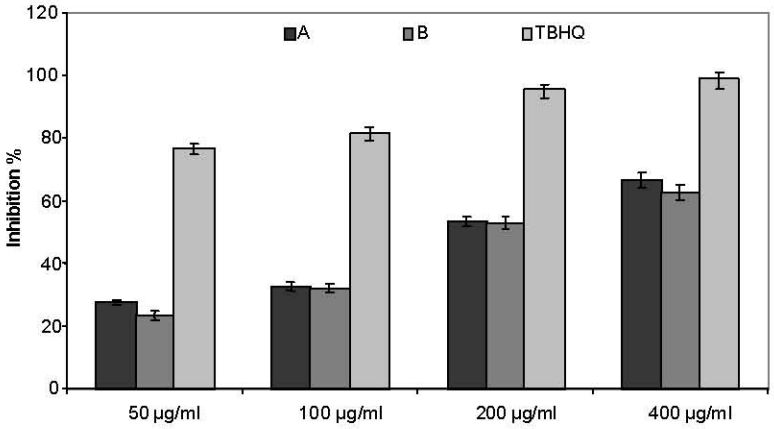

Fig. 3: DPPH scavenging activity of A and B model systems

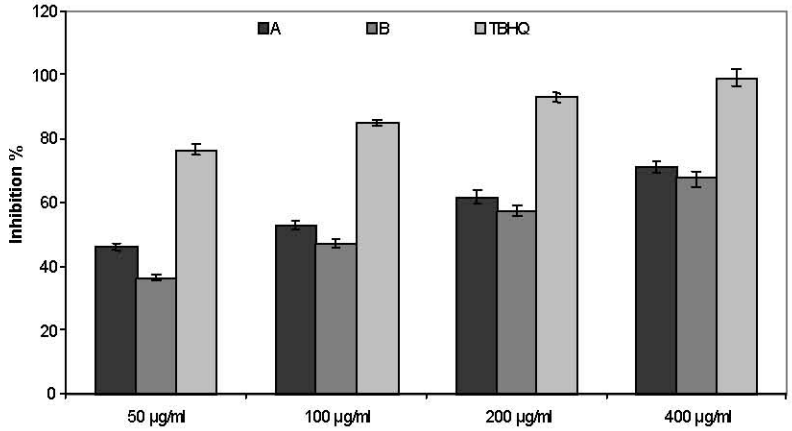

Fig. 4: Beta-carotene bleaching scavenging activity of A and B model systems

Liver, Kidney Functions and Some Other Biochemical Parameters: Activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (γ-GT) enzyme, sensitive indicator of liver function, were studied. Since they could be used as an indirect biochemical index of hepatocellular damage. The increase in the activities of these enzymes was directly proportional to the degree of cellular damage [48]. The mean (value ± SE) for each ALT, AST, ALP and (γ-GT) activities in the normal control rats group and the two concentrates of MRPs supplemented rats groups are presented in Table 3.ANOVA analysis indicated non significant changes in all tested liver function parameters, the present results demonstrated that supplemented normal rats with two model systems mention the activities of ALT, AST, ALP and γ-GT, which are used as the biochemical markers for early acute hepatic damage, in normal levels. Urea and creatinine concentrations were determined as an evidence for marked impairment of kidney function. These two parameters, which were evaluated, indicate renal disorders. The obtained results in (Table 3) revealed that neither urea nor creatinine levels were affected by the concentrates of model systems. The previous results pointed to the safety effect of MRPs on liver and kidneys functions. Also results of testing the effect of MRPs on blood hemoglobin (Hb %) and plasma glucose levels as well as lipids (total cholesterol and triglycerides) and also the total antioxidant capacity in plasma as antioxidant biomarker are illustrated in Table 3. No significant changes between means values in control and supplemented MRPs rats groups for hemoglobin, plasma glucose, total cholesterol, triglycerides and total antioxidant capacity were noted. These results revealed the non toxic effect and non significant alterations in all the studied biochemical markers. Finally we can conclude that the MRPs are safe dietary administration in high concentrations without harmful side effects.

In conclusion the results have shown that the reaction between beef fat or triglyceride with cysteine and ribose led mainly to the formation of heterocyclic compounds. Many of these were sulphur-containing volatiles, such as disulphides, thiophenes, thiazoles, other compounds identified were furans, mercaptoketones and thiols, bicyclic and cyclic sulphur-compounds. A clear result was observed for some classes of compounds to be formed in beef fat/ cysteine/ ribose model system at high concentration in comparison with beef triglyceride/ cysteine/ ribose, such pyrazine, thiazole, thiophene and disulphide derivatives which are responsible for roasted meat-like aroma and possess antioxidant activity.

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