52

Egypt. J. Chem. **Vol. 60**, No. 5, pp.753 - 767 (2017)

Natural Meat-Like Aroma with Antioxidant Potency Based on Bovine Fat by-product via Millard Reaction

Adel G. Abdel-Razek[1], El-Shahat H. A. Nashy[2*], Ahmed H. El-Ghorab[,3, 4] and Khaled F. El-Massry [3,4]
[1]Department of Fats and Oils, [2]Department of Chemistry of Tanning Materials and Leather Technology, [3]Department of Flavor and Aroma, National Research Centre, Dokki 12622 ,Cairo, Egypt and [4]Department of Chemistry, Faculty of Science, Aljouf University, Saudi Arabia.

FATS and fatty acids play a significant role as an influential source of the flavor components. The aim of this study was producing of natural meat like aroma components via the interaction of cysteine with bovine fat by product in two model systems. Gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) were used to identify the obtained volatile compounds. Thermal treatment of cysteine/ bovine fat by-product with or without water gave several thiol compounds such as 2-methyl-3-furanthiol, 2-furylmethanethiol and methyl-dihydrofuranthiol. A number of disulfides compounds were formed as 2-methyl-3-furyl-2-methyl-3-thienyldisulphide, bis-(2-methyl-3-furyl) disulphide, 2-methyl-3-furyl-methyl-2-xopropyldisulphide. Furans and sulfur-containing compounds are important precursors of meaty aromas.

Sensory evaluation of the aroma products was performed for cysteine and bovine fat by-product model systems according to International Standards Organization (ISO) method. The results revealed that the presence of volatiles having boiled note in presence of water may be due to the preponderance of thiol compounds (22.33 %) and roasted character attributes to the presence of higher content of pyrazine (1.28%), 2-methylthiazole (0.4%), furfural (3.0%) and methylfuraneol (31.47%). It was found that, the antioxidant activity of model system containing water has higher antioxidant activity (78.0±1.8%), in comparison with tert-butylhydroquinone (TBHQ) 98.73±2.3%. From the above results, the obtained natural identical meat-like aroma concentrate, could be applied as food additives.

Keywords: Meat like aroma, Bovine fat, Millard reaction, Antioxidant activity, Sensory evaluation.

Introduction

Many studies over several years ago have focused on the flavor and aroma of meat for human consumption in particular beef, which is the most preferable and highly valued meat in many countries [1-7]. Animal fats may affect food flavor in different ways: (I) Fatty-acids, on oxidation, can generate carbonyl components that are capable as a flavor contributor, as well (II) Lipids are original source of flavor ingredient, un-modified and indirectly (modified products). The released volatile components from fats or produced from triacylglycerol or phosphatide fractions may be effective for the certain species bovine or lamb flavors. Fat has the wonderful efficiency to absorb and preserve the flavors. Fats

as well include compounds that provide special flavors. Fat may work as store station for odorous compounds that are progressive during the heating process [8-10].

The non-volatile precursors having a low molecular weight, intrinsic for meat flavor, are water-soluble components, like free amino acids (FAAs), peptides, some organic acids, sugars, nucleotides and their metabolites [11-14].

The different flavors of meats depend on different culinary procedures are mainly due to the temperature of the cooking process and the water content of the meat. Subsequently, the flavor of boiled meat is generated in the presence of water at temperatures nearly 100°C. The

flavor and aroma of roasted meat, however, are produced at temperatures over 100°C in relatively dry conditions [15].

The Maillard reaction (MR) has a major technological importance in the development and imitation of desirable flavors and aromas for processed foods. It has been used in large scale for the manufacture of imitation flavors, particularly meat taste, and its aromas. Most patents proposed for meat flavors creation have involved sulfur-containing amino acids, usually cysteine [16]. Ohloff and coworkers [17] reported that the meaty aroma is attributed to the prolonged heating of cysteine which found in muscle protein [18].

It is well known that the cooking of meat results in oxidation of fat, which create a pleasant smell [19]. Raw meat emphasizes bland and metallic flavors and little aroma while cooked meat has a characteristic flavor. The precise sensory properties of meat were developed fully after heat treatment and its generation depends on the kind and intensity of cooking time and temperature. Flavor is one of the most critical odors because of its sensorial characteristics. The flavor precursors, when heated, of meat undergo a series of physical and chemical changes that are governed by temperature and water content.

Safety of food flavors has been highly concerned in recent years. So, the consumers around the world are becoming more aware of the nutritional value and safety of their foods and its constituent. In addition, there is a preference for ingredients natural food that is supposed to be safe, healthy and less subjected to hazards than those synthetic counterparts. However, more studies should be introduced in order to achieve an economical final product to meet the consumer's demands especially in the field of meat flavor [20].

The oxidative reactions which may occur in foods can be affected on their sensory and nutritional characteristics. Therefore, compounds having antioxidant potency possess a role for inhibition of the autoxidation. There is renewed more attention to use the natural products due to the expected potential health hazard effect of synthetic antioxidants [21].

The aim of the present work was devoted to maximizing the utilization of the bovine fat

by-product, to produce meat-like aroma, using Millard reaction. Physico-chemical characteristics of bovine fat were investigated. The chemical composition of the treated bovine fat and identification of the resulting volatile compounds model systems was studied using GC and GC-MS. Determination of antioxidant activity of the different model systems by using DPPH and β-carotene assays has been investigated as well.

Materials and Methods

Materials

- Chemicals used for different fat analysis from Merk, Germany and BDH, England.

-L-Cysteine (L-Cys), β-carotene, linoleic acid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), TBHQ, polyoxyethylene sorbitan monopalmitate (Tween-80), chloroform (99%) and anhydrous sodium sulfate, methylene chloride (MC), pressurized sealed bottles: with thermal taps and n-alkanes (C6-C24) were obtained from Aldrich and Sigma (Germany).

- Authentic samples of volatile compounds purchased from laboratory chemical suppliers and / or were obtained as gifts from flavor companies.

- Local bovine fat was used for the present study supplied by slaughterhouse, Cairo, Egypt.

Methods

Analysis of bovine fat

Chemical characteristics of bovine fat were analyzed according to the American Oil Chemists Society Methods [22]. Acid value, peroxide value, saponification value, iodine value and unsaponifiable matters were determined.

The ester value was estimated by subtracting the acid value from saponification value (SV-AV).

Fatty acid composition

Bovine fatty acid methyl esters were prepared according to AOCS Official Method Ce 1 k-07 **[23-25]. .** Determination of fatty acid composition was performed using Hewlett Packard HP 5890 series II gas chromatography, equipped with flame ionization detector (FID), operated under the following conditions: Detector, flame ionization (FID); column, capillary, 30.0 m x 530 μm, 1.0 μm thickness, polyethylene glycol phase (INNO Wax); N_2 with flow rate, 15 ml per min with average velocity 89 cm/s (8.2 psi); H_2 flow rate, 30 ml per min; air flow rate, 300 ml per min; split ratio, 8:1, split flow, 120 ml per min; gas saver, 20 ml per min. Detector temperature, 280ºC; column

temperature, 240ºC; injection temperature, 280ºC. Temperature programming starting from 100ºC to reach a maximum of 240ºC was used for eluting the fatty acid methyl esters. The identification of peaks was made as compared with chromatograms of standard fatty acids methyl ester (Sigma, USA).

Calculated Oxidizability (Cox) Value:

The Cox value of the oils was calculated by applying the formula proposed by Fatemi and Hammond [26]:

Cox value = (1 [18:1%] + 10.3[18:2%] + 21.6 [18:3]) / 100

Meat-like aroma model systems

Two reaction mixtures were made up in presence or absence of distilled water; **A:** fat/ L-Cys/ water, **B**: fat/ L-Cys/ without water, each mixture containing L-Cys (1 m mol) and bovine fat (10 g). The reaction mixtures were heated for 30 min in a 1000 mL sealed bottle (Merck Ltd.), 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 obtained reaction mixtures were subjected to a simultaneous steam distillation (1 L of water) and solvent (MC, 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 GC and GC/ MS according to the method reported by Wettasinghe [27].

Gas chromatography/ (Gas chromatography - Mass Spectrometric) analysis

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 ^{0}C to 240 ^{0}C by the rate 2 ^{0}C/ min. Kovat's indices were determined by co-injection of the sample with a solution containing homologous series of n-hydrocarbons (C_6-C_{26}) under the same conditions as described above. The separated components were identified by matching with NIST mass-spectral library data, and by comparison of Kovat's indices with those of authentic components and with published data. GC/MS analysis of the two model systems was performed on HP model 6890 GC interfaced to 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 ºC. The oven temperature was programmed from 40 to 240 ºC at 2 ºC/ 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 [28]. Two ml of β–carotene solution (5 mg / ml) and 40 mg of linoleic acid were transferred to a round–bottom flask. Chloroform was evaporated using a stream of nitrogen, after that, 100 ml of oxygenated distilled water were added slowly to the residue and vigorously agitated to give a stable emulsion. Aliquots of 2 ml of this emulsion with different volumes of the MRP concentrates were added. The same procedure was carried out with standard antioxidant (TBHQ). Absorbance was measured spectrophotometrically at 470 nm by using (UV-Visible spectrophotometer-Shimadzu, Japan) after incubating the reaction mixtures in water bath (50 ^{0}C) and the readings were taken from zero time till 120 min. Antioxidant activity (AA) was calculated as follow.

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

A. A: Antioxidant Activity

Where, $A_{S(0)}$ is absorbance of sample at 0 min.
$A_{S(120)}$ is absorbance of sample at 120 min.
$A_{b(0)}$: is absorbance of control at 0 min.
$A_{b(120)}$: is absorbance of control at 120 min.

DPPH free radical scavenging assay

DPPH free radical scavenging assay was carried out [29]. The various volumes of reaction mixture (MRPs, 50, 100, 200 and 400 µl) were added to 4 ml of DPPH solution (5 mg/500 ml MeOH) and the tubes vigorously shaken and incubated in the dark at room temperature for 30 min. After incubation, the absorbance of the reaction mixture was measured spectrophotometrically at 517 nm. All experiments were carried out in triplicate. The scavenging effect of DPPH free radical was calculated by using the following equation.

$$\text{Inhibition \%} = [(A_B-A_A)/A_B] \times 100$$

Where:

A_B: absorption of blank

A_A: absorption of sample

Sensory evaluation of meat-like model systems

The sensory analysis was carried out under the conditions specified by ISO method; guidelines after ISO 6658-1985; unstructured graphical scales (ISO 4121-2003) were presented as straight lines 100 mm long, provided with descriptions on either end. 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, pun get, burning; 7 = earthy, musty, moldy, sweat, 8 = malty, sweet; 9 = solvents, synthetic, chemicals; 10 = others-specify which); in the profile evaluation: 0 mm = absent, 100 mm = very strong. Odor profiles were tested [30].

Statistical analysis

The obtained results were recorded as means ± standard deviations and analyzed by SPSS (version 10.1 for windows 98, SPPS Inc**.**). All analyses were performed in triplicate. 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 evaluation of model systems.

Results and Discussion

Bovine fat is a by-product resulting from the meat processing industry (slaughter house) which would have caused environmental problems unless it's used. Such by-products are serious environmental load unless it is modified or utilized as intermediates components in other industries. Consequently, the overall economic return from such by-products would be attractive.

Analysis of fat

A.Proximate analysis

The proximate analysis (PV, AV, SV, unsap. and ester values) of the extracted bovine fat were investigated (Table1).

From Table 1 it was found that, SV 192.93 mg KOH/g oil. Also, it was noticed that PV, AV and unsap., values are 2. 1 m Eq/kg, 1.31 mg/g and 0.35%, respectively. Ester value (SV-AV) was 191.62 mg KOH / g oil.

TABLE 1. Chemical properties of bovine fat.

Properties	Bovine fat
Acid value, mg KOH / g oil	1.31
Peroxide value m Eq/Kg	2.10
Saponification value, mg KOH / g oil	192.93
Ester value (S.V.-A.V.) mg KOH / g oil	191.62
Unsaponifiable matter (%)	0.35

B. Composition of fatty acids

It's clear from Table 2 that, the saturated (SFA) and unsaturated fatty acids (USFA) contents are 56.2% and 43.8% respectively. In addition, it was observed that, SFA: USFA ratio is (1.28). Palmitic acid content more than 51.0% of the SF, while oleic acid represents more than 90.0% of the USFA. Furthermore, stearic acid makes more than 19.0% of the total bovine fatty acid composition.

With regard to the total fatty acids present in bovine fats are consists of SFA/ USFA. From the study conducted by Kosowska *et al.,* [8], which interpreted that the oxidation of USFA typically produces a variety of volatile compounds, some of which give the characteristic odors to specific types of meat.

TABLE 2. Fatty acids composition of bovine fat.

Fatty acids (%)	Bovine Fat
Saturated fatty acids:	
Lauric $(C_{12:0})$	02.1
Myristic $(C_{14:0})$	05.5
Palmitic $(C_{16:0})$	28.7
Stearic $(C_{18:0})$	19.3
Arachidic $(C_{20:0})$	01.6
Σ Saturated Fatty Acids	56.2
Unsaturated fatty acids:	
Palmetoleic $(C_{16:1})$	01.7
Oleic $(C_{18:1})$	39.6
Linoleic $(C_{18:2})$	02.5
Σ Un-Saturated Fatty Acids	43.8
SFA /PUFA	1.28
Cox Value	0.654

It is worthy to mention that the Cox value and SFA/PUFA ratio were considered as criteria for fatty materials susceptibility to oxidation [26, 31]. Increasing the content of SFA (palmitic & stearic) and monounsaturated fatty acids (MUFA) have a positive influence on the ratio of SFA/PUFA as well as Cox value oxidative stability [32]. Generally, a low content of PUFA (linoleic acid) and absence of linolenic acid in addition to the increasing of MUFA leads to prolonging the oxidative stability.

Meat- like aroma via Maillard reaction

The Maillard reaction has been widely used for aroma generation. Since it has a powerful effect on the outside appearance, flavor and nutritional characteristics for different foodstuffs. MR is a chemical reaction between reducing compounds (as carbonyl compounds) and amino acids and chiefly responsible for the development of aroma and taste during food processing. The primary

reactions in cooked meat are the oxidation of lipids lead to the formation of aroma compounds, moreover, high temperature can help the oxidation of USFA [33].

In this study, the cysteine was chosen as amino acid and bovine fat as carbonyl source in model systems to prepare meaty aroma as well as determine the antioxidant activity of two model systems. Thermal reaction of cysteine produced aldehyde, hydrogen sulfide and ammonia, Scheme (1).

The reaction between the produce hydrogen sulfide and ammonia with the generated volatile components which formed from the thermal oxidation of fat has been represented in schemes (2-5). The heterocyclic compounds, particularly those containing sulfur and/or nitrogen are very significant as a precursor for meat-like aroma production.
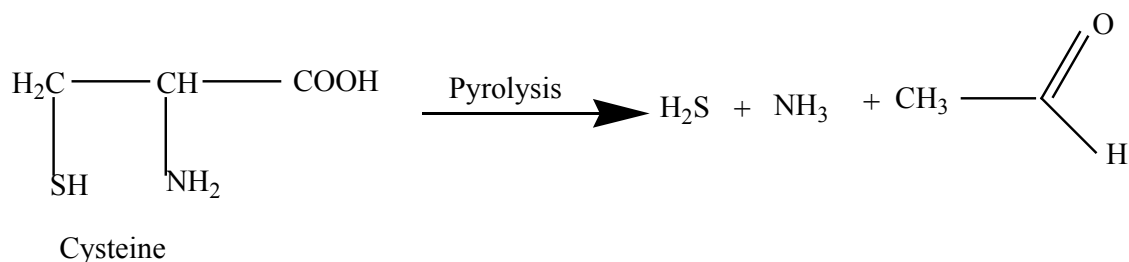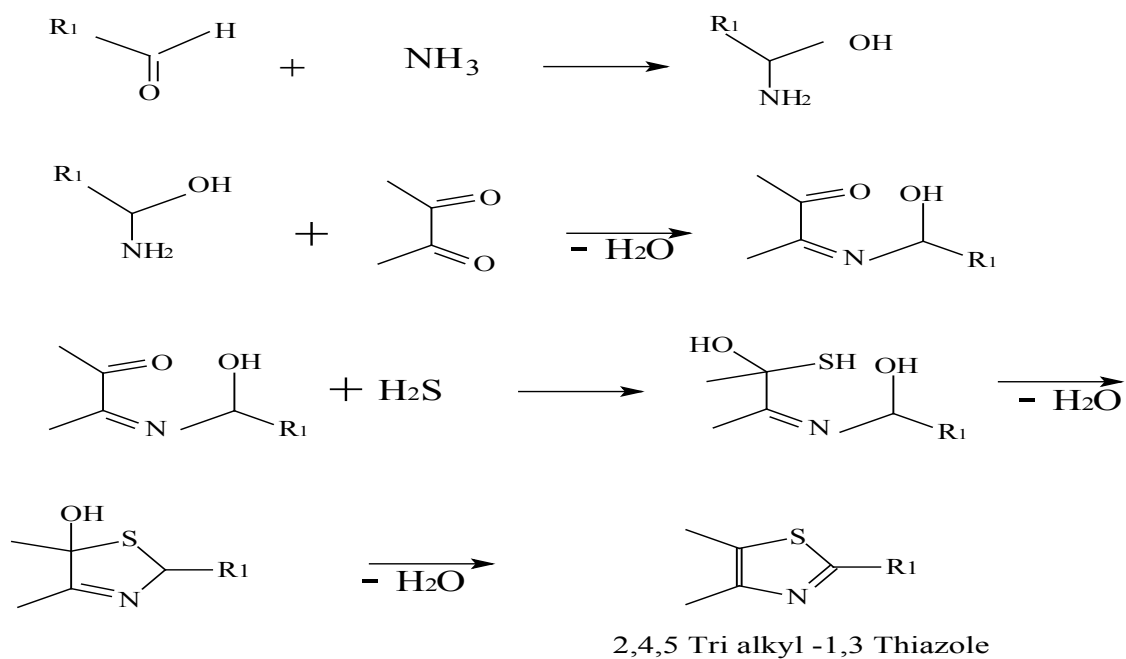

Scheme 1. Pyrolysis of cysteine.
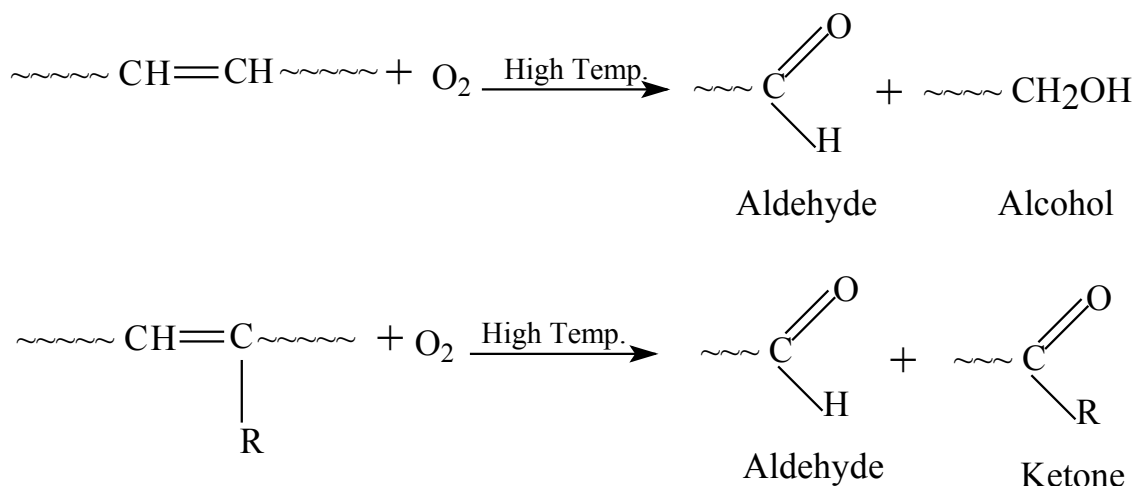
**Scheme 2. The representative oxidation reactions of fat**.
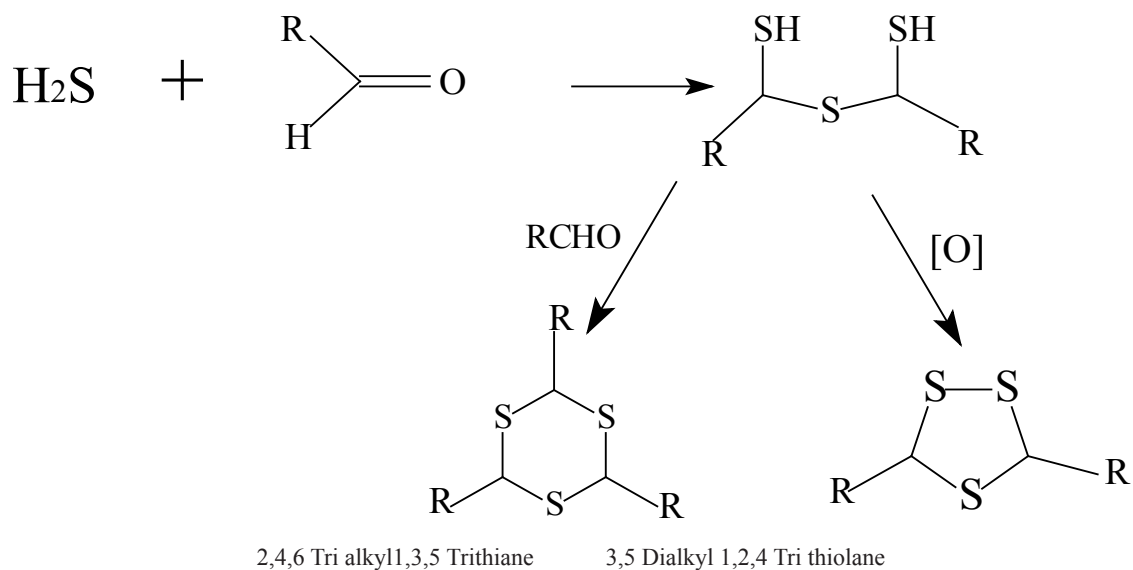
Scheme 3. Representative route for the formation of some sulfur heterocyclic compounds (thiane and thiolane) formed from milliared reaction (protein-fat).

Scheme 4. Representative route for the formation of sulfur and nitrogen heterocyclic compounds (thiazoles) formed from maillard reaction (protein-fat).
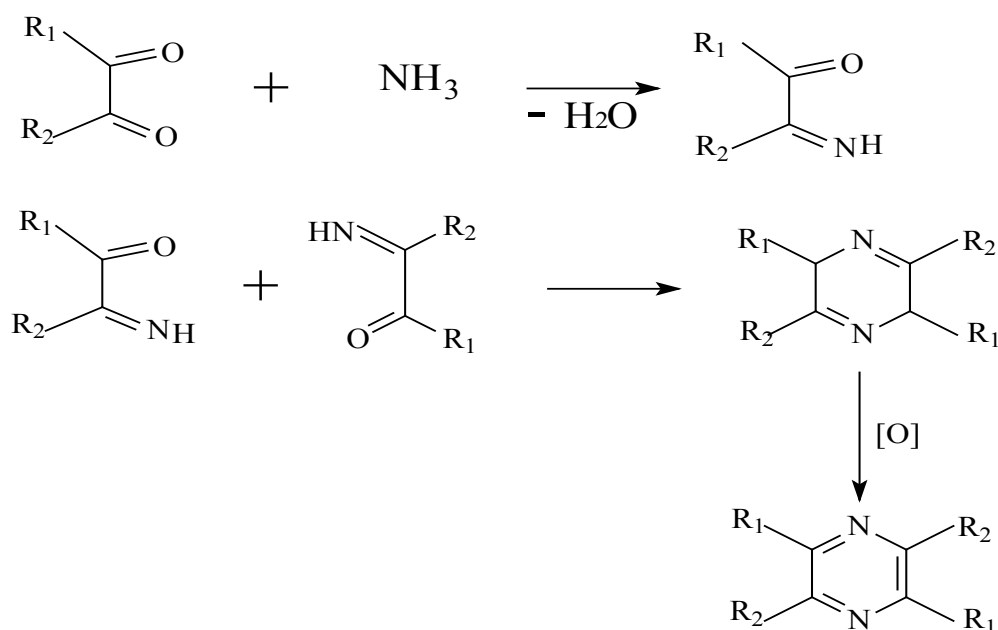
Scheme 5. Representative Route for the Formation of some Nitrogen Heterocyclic Compounds (pyrazoles) formed from Maillard Reaction (Protein-Fat).

Gas chromatographic analysis of meat-like aroma model systems

The volatile aldehydes and ketones compounds produced by the thermal oxidation are the main aroma substances derived from bovine fat oxidation. The identified volatile compounds were shown in Table (3). Thirty–five and twenty-four volatile compounds were recorded in fat/cysteine with or without water model systems A and B respectively, including aliphatic aldehydes, ketones, thiophenes, aliphatic alcohols, oxygen, nitrogen and sulfur containing compounds.

Carbonyl compounds

Short chain aldehydes, ketones and bi-carbonylic precursors were identified in the two model systems (A and B) with (16.40 and 11.53%), respectively, Table (3).

a. Aldehydes: The identified aldehydes and ketones in model systems (A & B) were shown in Table (3), 3, 6-nonadienal was the most abundant aldehydes in (A and B) model systems, while tridecanal was generated in (A) model system only with (0.64%). As illustrated in Table (3), the most obtained aldehydes are straight chain this due to the thermal oxidation of USFA, which is agreement with that previously reported [34]. It was observed that, the system conditions effect

on fat oxidation products. Where, the volatile products of MR in presence of water differ greatly from those in absence of water. The role of moisture in the oxidation of fat indicates that the oxidation decreases with decreasing water content due to the efficiency of water content to manage reactants and catalysts may be significant. The results obtained are in agreement with that obtained by Mottram and Whitfield [35]. The aqueous system plays a leading role in the amount and type of fat oxidation products. Whereas, the impact of water activity refers to its hydrogen bonding between fat hydroperoxides and reaction with free radical oxidation intermediates [35].

b. Ketones: 2-octanone was detected in the model system (A) only with (0.55%), which may be attributed due to the thermal degradation of fat in the presence of water. The presence of other short-chain ketones e.g. 2-butanone in (A and B) model systems with (0.37, 5.91 %), respectively can be explained by MR or Strecker degradation (SD) of cysteine. However, the formation of long-chain ketones such as trimethyl phenyl butenone (A = 0.07%, B = 0.1%) and 2-heptanone (A = 24.13%, B = 5.51%) could be formed due to the condensation between other carbonyl products that derived from fat oxidation.

TABLE 3. Volatile compounds of a and b model systems.

Peak No.	Compound name	K.I	A	B	Method of Identification
	Carbonyls				
1	2- Butanone	604	0.37	5.91	K.I
2	2,3-pentadion	726	1.8	3.0	K.I
3	3-mercapto-2-butanone	790	0.81	0.1	K.I
4	2-Heptanone	886	24.13	5.15	K.I &MS
5	mercaptomethylpentanone	940	11.66	1.82	K.I
6	2-octanone	991	0.55	nd	K.I &MS
7	trimethylphenylbutenone	1942	0.07	0.1	KI
8	3,6-nonadienal	1075	0.50	0.6	K.I
9	tridecanal	1526	0.64	nd	K.I
	Alcohols				
10	methylbutenol	624	4.43	nd	K.I &MS
11	1-Heptanol	883	0.68	nd	K.I
12	3-mercapto-2-pentanol	897	2.39	nd	K.I &MS
13	2-dodecanol	1467	1.34	nd	K.I
14	3-mercaptohexanol	1531	0.45	1.8	K.I &MS
15	hexadecsanol	1858	0.92	nd	K.I
	Esters				
16	Ethylhexadecanoate	1905	0.1	0.08	KI
	Furan and sulphur containing compounds				
17	2-furfural	814	2.58	3.0	K.I &MS
18	methylfuraneol	1760	5.33	31.47	K.I
19	2-methylthiazole	826	0.4	nd	K.I
20	3-ethylthiophene	872	0.59	0.03	K.I &MS
21	3-merecaptothiophene	961	1.8	0.3	K.I &MS
22	2-methyl-3-furanthiol	870	3.47	1.63	K.I
23	2-furylmethanethiol	906	1.0	0.04	K.I &MS
24	methyldihydrofuranthiol	935	0.75	0.28	K.I
25	1,3-dithiane	1030	0.31	nd	K.I &MS
26	3-methyl-1,2-dithiolan-4-one	1071	4.18	13.03	K.I &MS
27	1,2,4,5-tetrathiane	1325	2.26	1.2	K.I
28	3,4,6-trimethyl-1,2,3-trithiane	1499	0.24	2.0	K.I
29	2-ethylthienothiophene	1430	2.75	0.6	K.I &MS
30	dihydrothienothiophene	1290	2.64	nd	K.I &MS
31	Bis-(2-methyl-3-furyl)disulphide	1541	0.31	1.6	K.I
32	2-methyl-3-furyl-methyl-2-oxopropyldisulphide	1596	0.1	0.12	K.I &MS
33	2-methyl-3-furyl-2-methyl-3-thienyldisulphide	1740	0.62	7.72	K.I &MS
	Nitrogen –containing compounds				
34	Pyrazine	747	1.28	nd	K.I &MS
35	2-acetylpyrrole	926	0.72	0.2	K.I &MS

c. Dicarbonyls : 2, 3-pentanedione was detected in both model systems (A and B) with 1.8% and 3.0% which can be an important intermediate compound in the formation of other volatile products, as thiophenes, thiazoles and poly-sulfur volatiles [36], as shown in scheme (3-5). Where, dicarbonyl compound leads to the formation of mercapto ketones, when react with hydrogen sulphide, as 3-mercapto-2-butanone and mercaptomethyl pentanone with (0.81, 0.1 11.66, and 1.82%) in model systems (A and B), respectively. These mercapto ketones and sulfur compounds can produce important meat-like volatiles.

The characterized meaty flavor of model system (A) in comparison with model system (B) due to the presence of mercaptoketones, which contribute to increase the meatiness of meat flavor [36].

Alcohols
Alcohols derived mainly from thermal decomposition of fat. Different alcohols were detected in model system (A) only**.** In this study methyl butenol, 1-heptanol, 2-dodecanol and hexadecsanol were identified (4.43, 0.68, 1.34 and 0.92%) respectively. Mercapto alcohol as 3-mercapto-2-pentanol was generated in fat/ cysteine in presence of water (2.39%) and it was formed via the reaction of the corresponding alkanols with hydrogen sulphide **[30].** While 3-mercaptohexanol was detected in both systems (A&B) 0.45 and 1.8 respectively.

Esters
Ethylhexadecanoate (0.1 and 0.08%) was released in fat/ cysteine with and without water model systems, respectively. Also, it's thought that esters have a little impact on uncured meat aroma that was generally related with fruity aroma but known to be associated with meaty flavor [34].

Furan/ sulphur- containing compounds
2-Furfural (A = 2.58%, B = 3.0%) and methylfuraneol (A =5.33%, B = 31.47%) were identified in model systems (A and B). Although none of the various furan compounds has been referred to meat-like flavor, they have been considered to contribute to the overall odor of boiled or roasted meaty aroma **[17]**. In addition, there are three thiol compounds such as 2-methyl-3-furanthiol (A=3.47%, B=1.63%), 2-furylmethanethiol (A=1.0%, B=0.04%), methyldihydrofuranthiol (A = 0.75%, B = 0.28%).

It is well known that, sulphur-containing furans, thiophenes and disulphides have known to possess strong meaty-like aromas [37]. While 2-methyl-3-furanthiol occurred in cooked boiled model system (A), in large concentration than roasted model system (B) which have characteristics smells like cooked cabbage. 2-furylmethanethiol and methyl dihydro furanthiol were found in the two model systems (A and B). The character impact odor compounds as thiol derivatives have been reported to be important in cooked meaty flavor [38].

The essential components of thiophenes present in meat flavor were identified to be substituted at the 2- or 3-position such as 3-ethylthiophene (A = 0.59%, B = 0.03%) and 3-mercaptothiophene (A = 1.8%, B = 0.3%). 3-Mercaptothiophene was identified in (A and B) model systems which could be formed due to the decomposition of ribonucleotides of fats in absence of water and it has a roasted note. The results obtained agreed with that obtained by MacLeod et al., [16]. It has been assuming that these sulphur containing heterocyclic compounds could be generated either from thermal degradation of cysteine [25] through the interaction between carbonyl compound from fat and fatty acid degradation products and sulphur-containing amino acids [38]. However, it has been stated that, 3-mercaptothiophene is the only thiophenes with meaty aroma but other thiophenes have sulphur or green / sweet odor [39-40].

Disulphide compounds have been attributed with the meat-like aroma. The presence of thiol-substituted furans and thiophenes grant rising to a number of significant disulfides in the reaction mixture of model systems (A and B). Three disulphide derivatives were detected in model systems (A and B) such as 2-methyl-3-furyl-1-methyl-2-oxopropyldisulphide (A = 0.1%, B = 0.12%), *bis-* (2-methyl-3-furyl) disulphide (A = 0.31%, B = 1.6%) and 2-methyl-3-furyl-2-methyl-3-thienyldisulphide (A = 0.62%, B = 7.72%). The obtained results of the formation of furyl and thienyl sulphide and disulphides were agreement with that previously reported [41 and 42].

Di-and tetra-sulphur containing five or six membered rings were identified in the (A and B) systems, such as 3-methyl-1, 2-dithiolan-4-one (A = 4.18%, B = 13.03%) and 1, 2, 4, 5-tetrathiane (A = 2.26%, B = 1.2%), while 1, 3-dithiane was

identified in fat/cysteine/water model system with 0.31 [43] described the formation of dithiaolane and aliphatic sulphur compounds via the reaction between the breakdown of fats and cysteine and they are likely to be produced through thermal degradation of cysteine or can be created from the reaction of aldehydes with hydrogen sulphide.

2-Ethylthienothiophene was generated in (A and B) model systems with (2.75 and 0.6%), respectively, While dihydrothieno-thiophene was detected only in A model system with 2.64%. Madruga and Mottram [41] reported that a number of alkylthieno-thiophenes were generated in the volatile compounds of cysteine/ ribose model system. In addition, 2-Methyl-thiazole was evolved in model A system only with 0.4% and this compound was reported in beef cooked at 140°C.

Nitrogen-containing compounds

2-acetylpyrrole was identified in model systems (A and B) with (0.72% and 0.2%), respectively, while pyrazine was detected only in the model A (1.28%). Farmer *et al,* (44) were reported that 2- acetyl-pyrrol was found in fresh, frozen beef and canned beef, shallow fried beef and it was found in two systems (A and B). The odor of pyrazine has been traditionally regarded as nutty, roasted or burnt. 2–Acetylpyrrol can be formed from the SD that involved due to the interaction of molecules containing nitrogen (α-amino acid) with fat degradation products.

Sensory evaluation of (A and B) model systems

Figures 1 and 2 demonstrate the sensory profiles of (A and B) model systems as well as the intensities of the developed odors and the odor acceptability. Pronounced differences were observed in the odor profiles of (A and B) model systems. As expected, intensities of the roasted, burnt, caramel and sweet notes were weak in (A) models where pyrazine was occurred in low concentration and sulphur derivatives, which were produced from the same bi-carbonylic precursors, occurred in high concentration (Table 3). The lower content of furans generated *via* these model systems is also in agreeable with the lower intensities of the above-mentioned notes due to the presence of more sulphur-derivatives [38].

The boiled like meat aroma was intensive in the volatiles produced through model system (A) due to the preponderance of sulphur-containing compounds. Mottram [40] reported that, boiled

meat contains more aliphatic thiols, sulphides as well as heterocyclic compounds with 1, 2 or 3 sulphur atoms in 5- and 6- membered rings, in comparison with model system (B) where the intensities of the roasted, burnt, caramel and sweet notes were strong due to the presence of higher content of pyrazine, thiazole and furfural.

The high content of furans, thiazole and pyrazine is also agreeable with the higher intensities of the above- mentioned notes. Higher intensity of roasted meat note is reasonable, due to the presence of higher content of pyrazine, thiazole and furfural, which were reported previously [43]. While other descriptors gave insignificant results, as the ratings were too low, therefore, they are not included in Fig. 1 and 2.

Antioxidant activity of Maillard model systems (A and B).

It is worthy to mention that natural antioxidative components are very respectable for food technology because they prolong the shelf life of fatty foods. Nowadays they are also obtained more interest because it was supposed that, their impact is beneficial for health and they are protective too, e.g. against coronary heart diseases [44]. The radical scavenging activity of the two model systems were measured by DPPH and β-carotene methods [29, 45, 46], as clarify in (Fig. 3 & 4) the model system in entity of water has a high antioxidant potency (78.1% at 400μg/ml) than fat/ cysteine without water model system (65.2% at 400μg/ml) in comparison with the synthetic antioxidant TBHQ (98.75% at 400μg/ml). The products of MR concerning to the system in appearance of water was observed to be higher efficacy than that without water may be due to the sharing of phospholipids in model system (A) that, contribute in MR to produce several heterocyclic components having antioxidant potency as sulphides, thiazoles, pyrazines and thiol derivatives [42]. As predictable, model system (A) has higher antioxidative efficiency than the model (B) in the β-carotene-linoleate method, (A) model system inhibited the bleaching by (73.6% at 400 μg/ ml) compare with TBHQ (98.88% at 400 μg/ml) (Fig. 3 & 4).

Conclusion

● This study has proposed a way of converting, previously non-marketable, residual bovine fats to producing meat-like aroma as a value added marketable products. In addition to produce
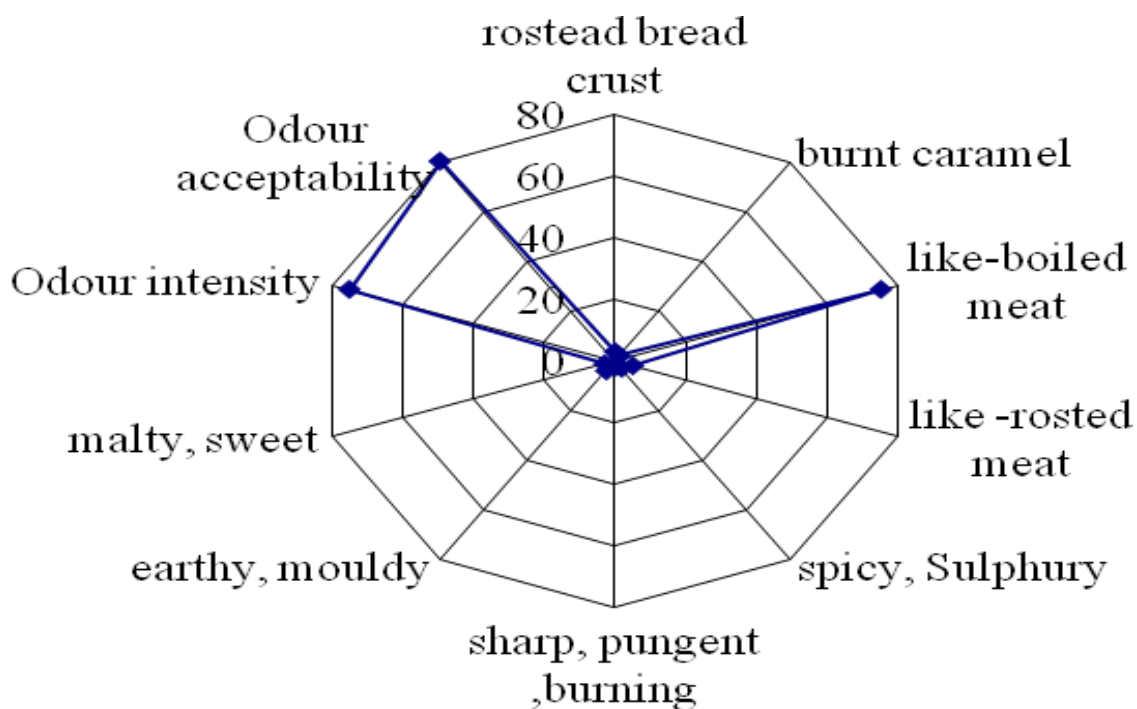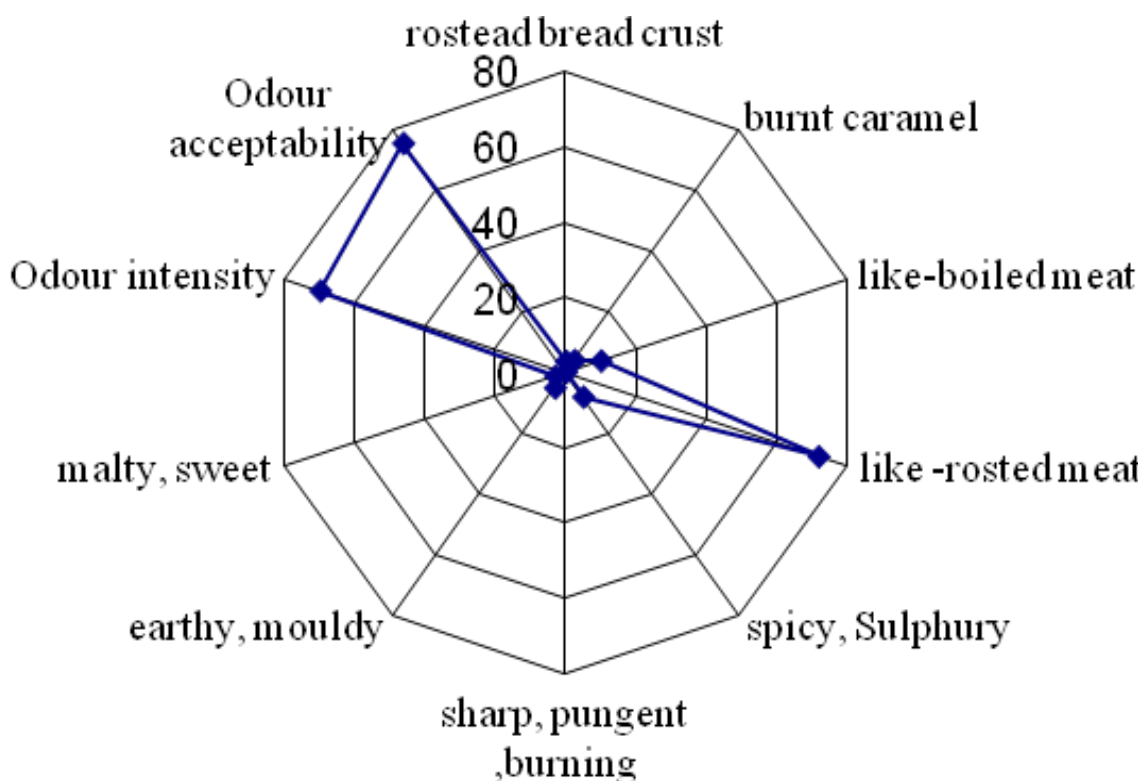
Fig. 1. Aroma profile of A model system.
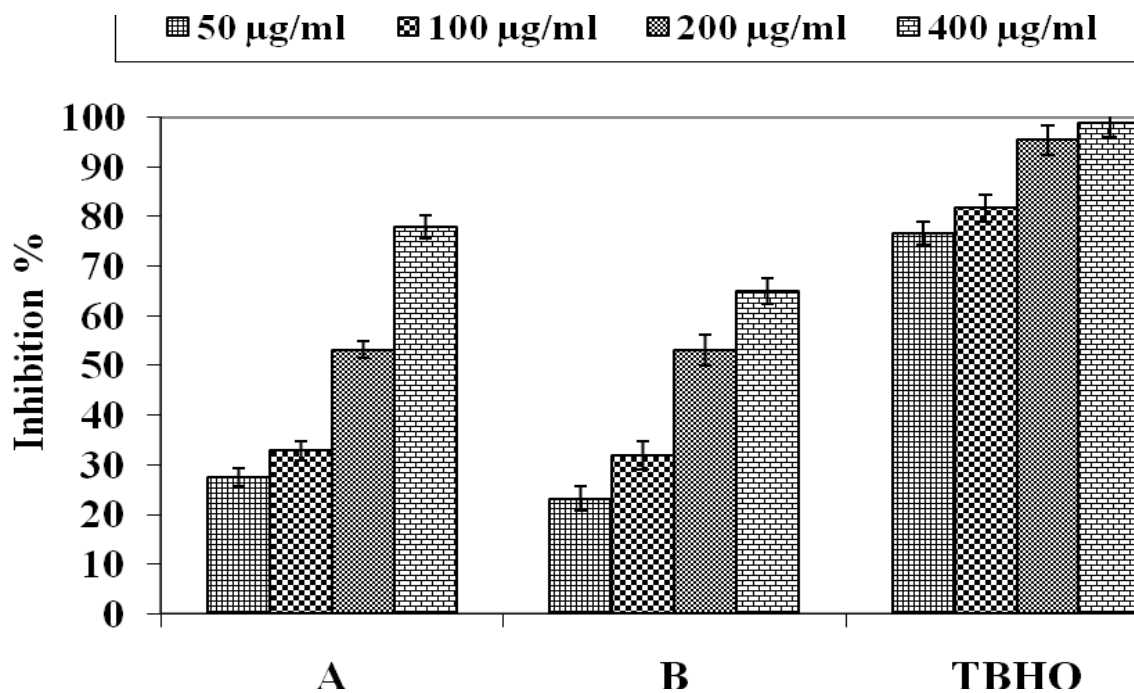

Fig. 2. Aroma profile of B model system.

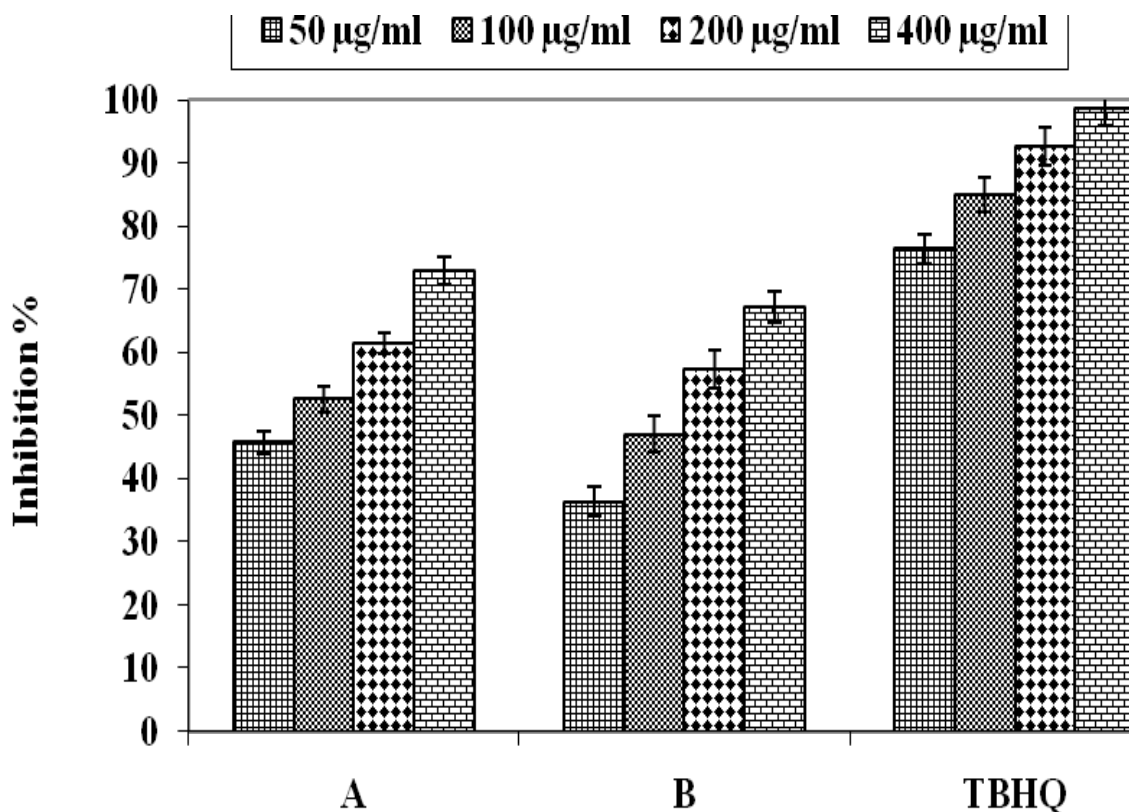**Fig. 3. Antioxidant activity of A and B model systems by dpph method.**

Fig. 4. Antioxidant activity of A and B model systems by β-carotene method.

relatively less expensive products based on fat as a natural source of carbonyl groups instead of sugar, through MR.

● The present work indicated that the meaty-like flavor consists of a variety of compounds having different aroma prosperities.

● Numerous aroma-active compounds were identified especially sulphur and nitrogen containing compounds as well as (aldehydes, ketones, phenols, organic acids, among others) as responsible for the meat like aroma.

● Bovine fat by-product was used to generated naturally meaty aroma has antioxidant activity and highly sensory evaluation.

● The observed clear result for some classes of compounds to be formed in fat/ cysteine model system in presence of water rather than fat/ cysteine in absence of water such as pyrazine, thiazole, thiophene and disulphide derivatives which are responsible for roasted note while boiled note was attributed to thiol derivatives.

● Recently, new aroma active compounds are still being discovered, and moreover investigations are vital to identify them.

● More studies should be concentrate in reconstitution of the food components, to understand the role of each flavor compound specified in the cooked meat and their possible effect in consumer acceptability.

References

1. Brunton, N. P., Cronin, D. A. and Monahan, F. J. Volatile components associated with freshly cooked and oxidized off-Flavors in turkey breast meat. *Flavor Fragr. J.*, **17**, 327–334 (2002).

2. Reineccius, G. *Flavor and aroma chemistry, in Quality Attributes and Their Measurement in Meat, Poultry and Fish Products,* Vol 9 of Advances in Meat Research Series, Ed by Pearson AM and Dutson TR. Blackie Academic & Professional, London, 184-199 (1994).

3. Jayasena D. D., Ahn D. U., Nam K.C. and Jo C. Flavor chemistry of chicken meat: A review. *Asian Australas. J. Anim. Sci.* **26** (5), 732-742 (2013).

4. Dashdorj D., Tripathi V.K., Cho S., Kim Y. and Hwang I. Dry aging of beef; Review. *Journal of Animal Science and Technology.* **58**, 20 (2016).

5. Mottram, D.S. *Some Aspects Of The Chemistry Of Meat Flavor, In Flavor Of Meat And Meat Products,* Ed by Shahidi F. Blackie Academic & Professional, London, 211-230 (1994).

6. Shana L. C., Brúnb A. D., Henchionc M., Lid C., Murrina C., Walla P. G. and Monahan F. J. Consumer evaluations of processed meat products reformulated to be healthier – A conjoint analysis study. *Meat Science* **131**, 82–89 (2017).

7. Carmack, C.F., Kastner, C.L., Dikeman, M.E., Schwenke, J.R. and Garcia Zepeda, C.M. Sensory evaluation of beef-Flavor intensity, tenderness and juiciness among major muscles. *Meat Sci.*, **39**, 143-147 (1995).

8. Kosowska M., Majcher M. A, Fortuna T. Volatile compounds in meat and meat products. *Food Sci. Technol.*, *Campinas*, **37** (1), 1-7 (2017).

9. Rowe, D. High impact aroma chemicals. Part 2: the good, the bad, and the ugly. *Perfumer and Flavorist.* **27**, 24-29 (2008).

10. Cerny, C. The aroma side of the maillard reaction. *Ann. N.Y. Acad. Sci.* 1126, 66–71(2008).

11. Imadon, G. I. and Spanier, A.M. Unravelling the secret of meat Flavor. *Trends Food Sci. Technol.*, **5**, 315-321 (1994).

12. Spanier, A.M., McMillin, K.W., and Miller, J.A. Enzyme activity levels in beef: effect of post-mortem ageing and end-point cooking temperature. *J. Food Sci.* **55**, 318-326 (1990).

13. Ishii, K., Nishimura, T., Okitani A, Tamura Y, Hatae K and Shimada A. Role of peptides in the taste of cooked beef. *J. Home Econ Jpn* **46**, 307-312 (1995).

14. Tamura, M., Ishii, K., Hatae, K. and Shimada, A. Effect of the peptides fraction in cooked beef on taste. *J. Home Econ. Jpn.* **48**, 607-612 (1997).

15. Manley, C.H. and Ahmedi, S. The development of process flavors. *Trends Food Sci. Technol.,* **6**, 46-51 (1995).

16. MacLeod, G., and Ames, J.M. Capillary gas chromatography-masspectrometric analysis of cooked beef aroma. *J. Food Sci.*, **51**, 1427-1434 (1986).

17. Ohloff, G., Flament, I., Pickenhagen, W. Flavor chemistry (Review). *Food Rev. Int.*, **1**, 99–148 (1985).

18. Jayasena D. D., Dong Uk Ahn, Ki Chang Nam and

Cheorun Jo. Flavor chemistry of chicken meat: A review. *Asian Australas. J. Anim. Sci.* **26**(**5**), 732 – 742 May (2013).

19. Belitz, H.D.; Grosch, W.; Schieberle, P. *Food Chemistry;* Springer: Berlin, Germany, (2009).

20. Sullivan, G.A. Naturally cured meats: Quality, safety, and chemistry. *Ph.D Thesis*. Iowa State University Ames, Iowa (2011).

21. 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).

22. Davied, F. *Official Methods and Recommended Practices of the American Oil Chemists Society,* Fifth Edition includes all changes 1993-1997, Edition Analytical methods, Champaigen-Illionois, March. (1998).

23. AOCS. *Official Method Ce 1k ‑ 07, Direct Methylation of Lipids for the Determination of Total Fat, Saturated, cis-Monounsaturated, cis ‑ Polyunsaturated, and trans Fatty Acids by Chromatography. Official Method and Recommended Practices of the American Oil Chemists' Society,* 5th ed., American Oil Chemists Society, USA (2007).

24. IUPAC. *International Union of Pure and Applied Chemistry. Standard Methods for the Analysis of Oils and Fats and Derivatives,* 7th revised and enlarged edn., edited by C. Paquot and A. Haut Fenne, Black Scientific, London (1987).

25. Hassanien M.M.M., Abdel-Razek A. G., Rudzińska M., Siger A., Ratusz K. and Przybylski R. Phytochemical contents and oxidative stability of oils from non-traditional sources. *Eur. J. Lipid Sci. Technol.*, **116**, 1563–1571 (2014).

26. Fatemi, S.H., E.G. Hammond. Analysis of oleate, linoleate and inolenate hydroperoxides in oxidized ester mixtures. *Lipids*, **15**, 379-385 (1980).

27. 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).

28. Matthus, B. Antioxidant activity of extracts obtained from residues of different oilseeds. *J. Agric. Food. Chem.,* **50**, 3444-3452 (2002).

29. Jayathilakan, K. and Sharma, G.K. Role of sugar-amino acids interaction products as antioxidants in a methyl linoleate model system. *Food Chem.,* **95**, 620-26 (2006).

30. El-Massry, K., Farouk, A., and El-Gorab, A. Volatile constituents of glutathione/ ribose model system and its antioxidant activity. *Amino Acid*, **24**, 171-177 (2003).

31. Mendez, E., J. Sanhueza, H. Speisky, A. Valenzuela. Validation of the rancimat test for the assessment of the relative stability of fish oils. *J. Am. Oil Chem. Soc.*, **73**, 1033-1037 (1996).

32. Abdel-Razek A.G., Hassanein M.M.M., Rudzinska M. and Elmallah M.M.H. Role of minor constituents and balanced fatty acids in upgrading the low stability of cooking oils blended with palm super olein. *Asian Journal of Scientific Research.* **3** (10), 150-159 (2017).

33. Virginia, C., Resconi, Ana Escudero, and María, M. Campo. *The Development of Aromas in Ruminant Meat Molecules.* **18**, 6748-6781 (2013).

34. Elmore, J. S., Mottram, D. S., Enser, M., and Wood, J.D. Effects of the polyunsaturated fatty acids composition of beef muscle on the profile of aroma volatiles. *J. Agric. Food. Chem.,* **47**, 1619-1625 (1999).

35. Mottram, D. S., Whitfield, F.B. Maillard-fat interaction in non-aqueous systems: volatiles from the reaction of cysteine and ribose with phosphatidylcholine. *J. Agric. Food Chem.*, **43**, 1302-1306 (1995).

36. MacLeod, G., Seyyedain–Ardebili, M. Natural and simulated meat flavors (with particular reference to beef). *Crit. Rev. Food Sci. Nutr.*, **14**, 309- 437 (1981).

37. Ishii, K., Tsuchida, M., Nishimura, T., Okitani, A., Nakagawa, A., Hatae, K. and Shimada, A. Changes in the taste and taste components of beef during heating at a low temperature for a long time. *J. Home Econ. Jpn.*, **46**, 229-234 (1995).

38. Shahidi, F.; Rubin, L.J. Meat flavor volatiles: A review of the composition, techniques of analysis, and sensory evaluation. *CRC Crit. Revs. Food Sci. and Nutri.* **24**, 141-243 (1986).

39. Guth, H. and Grosch, W. Identification of the character impact odorants of stewed beef juice by instrumental analysis and sensory studies. *J. Agric. Food Chem.*, **42**, 2862-2866 (1994).

40. Mottram, D.S. Flavor compounds formed during the Maillard reaction. *In Thermally Generated Flavors: Maillard, Microwave, And Extrusion Processes;* Parliament, T.H., Morello, M.J., McGorrin, R.J., ACS Symposium Series, 543, ACS: Washington

DC, 104-126 (1994).

41. 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**, 305-310 (1995).

42. El-Ghorab A. H., Khaled, F. El-massry, Atef, F. El-Hadadd, Saeid A. Shedied, Mohamed, S. Shaheen and Gad, M. A. Chemical composition and antiradical activity of the volatile compounds from reaction of cysteine/ ribose and beef fat. *World J. Dairy and Food Sci.*, **4** (2), 164-175 (2009).

43. 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.* Eds. T. H. Parliament, R, J, McGorrin and C.-T. Ho, ACS, Washington, DC, 229-241 (1989).

44. Farmer, L.J. and Patterson, R.L.S. Compounds contributing to meat flavor, *Food Chem.* **40**, 201-205 (1991).

45. Adams, R.P. *Identification Of Essential Oil Components By Gas Chromatography/ Mass Spectroscopy.* Allured Publishing Corporation, USA (1995).

46. Matthus, B. Antioxidant activity of extracts obtained from residues of different oilseeds. *J. Agric. Food. Chem.*, **50**, 3444-3452 (2002).

تحضير مكسبات طعم ورائحة اللحوم الطبيعية مع خصائص قوية كمضاد للأكسدة اعتمادًا على منتج ثانوي من الدهن البقري وذلك عن طريق تفاعل ميلارد

عادل جبر عبدالرازق١ ، الشحات حسن ناشي٢، احمد حسن الغراب٣،٤ و خالد فاروق المصري٣،٤
١قسم الزيوت والدهون،٢قسم كيمياء المـواد الدابـغة وتكنـولـوجيا الجلـود،٣قسم مكسبات الطعم والرائحة - المركز القومي للبحوث- الدقي- القاهرة - مصر و
٤قسم الكيمياء – كلية العلوم -جامعة الجوف- المملكة العربية السعودية.

تعد مخلفات الدهون الطبيعية سلاح ذو حدين حيث إن لم يتم إستغلالها الإستغلال الأمثل اصبحت عبء بيئي ثقيل ومصدرا من مصادر التلوث الكبري والتي يترتب على آثارها مشاكل بيئية وصحية خطيرة . لذلك فإن هذا البحث يهدف إلى إستغلال أحد المنتجات الثانوية من الدهون الطبيعية (دهن بقري) بهدف تحضير مكسبات طعم ورائحة اللحوم الطبيعية مع خصائص قوية كمضاد للأكسدة وذلك عن طريق تفاعل السيستين مع دهن البقر في اثنين من أنظمة نموذجية تحت مسمى تفاعل ميلارد . وحيث ان الأحماض الدهنية تلعب دورا كبيرا كمصدر هام من مكونات النكهة فإن المعالجة الحرارية بين السيستين و دهن البقر مع أو بدون الماء قد أعطت العديد من المركبات ذات المجموعات الوظيفية الكيميائية المختلفة (خمس وثلاثون مركب) مثل مركبات الثيول ومركبات ثنائي الكبريتيد ومركبات الفيوران ومركبات البيرول ومركبات الكربونيل ومشتقاتهم نذكر منها على سبيل المثال ٢-ميثيل-٣-فيوران ثيول، ٢-فيوريل ميثيل ثيول، وميثيل-داي هيدروفيوران ثيول، ٢-ميثيل ٣-فيوريل-٣-ميثيل-٣-ثينيل داي سلفيد، ثنائي (ثنائي ميثيل-٣-فيوريل)ثنائي سلفيد، ٢-ميثيل-٣-فيوريل-ميثيل-٢-بروبيل داي سلفيد. وتعد مركبات الفيوران والمركبات المحتوية على الكبريت ذات أهمية قصوى في تكوين الروائح المميزة لمكسبات الطعم المرغوبة. وقد تمت إختبارات عديدة لتقييم مركبات النكهة الناتجة فضلا عن التقييم الحسى طبقا لطريقة المنظمة الدولية للقياس (الأيزو). وقد أوضحت النتائج أن وجود المواد المتطايرة التي تكونت في وجود الماء يعزى ظهورها إلى إنتاج مركبات الثيول (٢٢,٣٣٪)، ورائحة الشواء تعود الى وجود محتوى عالى من مركبات البيرازين (١,٢٨٪)، ٢-ميثيل ثيازول (٠,٤ ٪)، فيورفيورال (٣,٠٪) وميثيل فيورانول (٣١,٤٧٪). ومن الجدير بالذكر أن النموذج المحتوى على الماء أعطى نشاط مضاد للأكسدة عالى نسبيا (٧٨,٠ ٪)، بالمقارنة مع ثلاثي بيوتيل هيدروكينون (TBHQ). وقد أجريت التحاليل اللازمة على دهن البقر المستخدم قبل وبعد إجراء تفاعل ميلارد ودراسة خواصه وكذلك المركبات الكيميائية المشتقة منه والناتجة من التفاعل وقد تم استخدام جهاز المطياف اللوني، وكروماتوجرافيا الغاز (GC)/ مطياف الكتلة (GC/MS) لتحديد المركبات المتطايرة الناتجة من التفاعل. وقد دلت النتائج التي تم الحصول عليها أن الدهن البقري يمكن استغلاله في تحضير مكسبات طعم ورائحة مماثلة لرائحة اللحوم الطبيعية مع كونه مضاد للأكسدة قوى وبالتالي يمكن تطبيقها كإضافات غذائية.