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Effect of pH and Water Activity in Generation of Selected Meaty Aroma Compounds in a Meat Model System

[1]V.K. Modi, [2]R. Linforth and [2]A.J. Taylor
[1]Meat Fish and Poultry Technology,
Central Food Technological Research Institute, Mysore, 570020, India
[2]Department of Applied Biochemistry and Food Science,
University of Nottingham Sutton Bonnigton, Nottingham,
LE12 5RD, United Kingdom

Abstract: Longissimus dorsi post rigor minced beef meat was washed repeatedly with 0.02 M phosphate buffer (pH 6.8) to obtain pigment-free muscle fibers. The resultant muscle fiber was freeze dried and used as a meat model system. The results were compared with or without an aqueous model system containing a mixture of cysteine, thiamine and ribose reaction mixture. Gas chromatography-Mass spectrophotometry was used to study the effect of pH and water activity on the generation of volatiles in model systems. Seven sulphur-containing meaty aroma volatiles were identified in the meat fiber model system by odour port analyser and the generated mass spectra. The generation of volatile in reaction mixture as well as in meat fiber model system were strongly influenced by pH. The intensity of 2-methyl tetrahydrothioph-ene-3-one was low ($p \leq 0.05$) at lower pH. The generation of 2-methyl-3-furanthiol and bis-(2-methyl-3-furyl) was more ($p \leq 0.05$) in reaction mixture model system at a pH 5.5 and 6.5 than at lower pH. However, the increase in generation of 2-methyl thiophene in model systems studied was dependent ($p \leq 0.05$) on the increase of pH. The volatiles, 2-methyl-4-5-dihydrothiophene, 2-methyl-3-(methylthio) furan and 2-methyl-3-thiophenethiol were not detected at low pH in a meat fiber model system. However, their generation in reaction mixture and meat fiber containing reaction mixture system was observed. The formation of 2-methyl-3-furanthiol and bis-(2-methyl-3-furyl) was favoured ($p \leq 0.05$) by higher a_w whereas, the formation of thiophene was maximum at a_w 0.80. At higher a_w an inverse relationship between a_w and quantity of thiophene was observed.

Key words: Meat, aroma, cysteine, thiamine, ribose, water activity

INTRODUCTION

Meat is an integral part of diet for the large majority of mankind, not only as a good source of protein but also because of its sensory properties, of which flavour is one of the most important. Raw meat has little desirable flavour but cooked meat has a characteristic flavour attributes to the animal species, breed, sex, age at slaughter, treatment of meat and the temperature and type of method of cooking (Bailey, 1983). The total volatile flavour concentration of cooked chicken, beef and pork by products blends was three times more than that observed for cooked chicken muscle alone (Wettasinghe *et al*., 2001).

The chemistry of the compounds responsible for the flavour of cooked meat and how these flavour compounds are produced during the process of cooking have been the subject of considerable

Corresponding Author: V.K. Modi, Meat Fish and Poultry Technology,
Central Food Technological Research Institute, Mysore, 570020, India
Tel: +091 0821-2514840 Fax: +091 0821-2517233

research. The important reaction, which occurs upon cooking meat, includes degradation of sugar, pyrolysis of protein and amino acids and lipid degradation. The Maillard reaction is an important route to many of the aroma volatiles found in cooked meat (Golovnya *et al*., 1983). An interaction of two or more precursors may also occur as in the Strecker degradation, Maillard reaction (Mottram, 2007) and various protein-lipid oxidations (Moody, 1983; Shahidi *et al*., 1986; Varavinit *et al*., 2000). Apart from amino acids, carbohydrates and fat, thiamine also appears to be an important precursor of meat aroma (MacLeod and Seyyedain-Ardebilli, 1981; Mottram, 2007).

Number of sulphur containing compounds has been detected among the volatile products of Maillard reaction between amino acids and ribose in an aqueous model system (Whitfield *et al*., 1988; Farmer and Mottram, 1990a). Cysteine contributes to meat flavour by its participation in the Maillard reaction, while the thermal degradation of thiamine leads to sulphur containing flavour compounds which contributes to the pleasant aroma of cooked and roasted meat. An attempt to elucidate the contribution of methionine-glucose reaction products to the formation of typical meat aroma has also been made by Barylko-Pikielna (2006).

Werkhoff *et al*. (1990) isolated and characterised the sulphur containing meat flavour components in a model system containing cysteine, thiamine, glutamate and ascorbic acid. In an another meat model system containing thiamine, cysteine, cystine and various carbohydrates studied by Guntert *et al*. (1990) and Werkhoff *et al*. (1990). Misharina *et al*. (1992) found in a model system containing thiamine, bovine plasma and animal fat in aqueous suspension had an intensive odour of cooked meat after heating. Formation of sulphur meaty aroma compounds in reaction mixture containing cysteine and ribose has been investigated by Mottram and Nobrega (2002).

Farmer and Mottram (1990b) examined the formation of selected compounds at pH values over the range pH 4.5 to 6.5. Mottram and Leseigneur (1990), Meynier and Mottram (1995) found that the products of the Maillard reaction between ribose and amino acid in an aqueous systems were strongly influenced by pH. The total quantity of volatile compounds in cooked meat increased as the pH decreased (Madruga and Mottram, 1995). Analysis of the volatile components using GC-odour Port analyser showed sulphur-and nitrogen sulphur containing compounds to be possible contributors to the sulphury aromas detected in extrudates produced at higher temperature (Bredie *et al*., 2002). Precursors of the heterocyclic amines, such as, creatinine, glucose, amino acids, glysine, alanine and phenylalanine are the major contributors in generation of meaty aroma in a model system (Zochling and Murkovie, 2002; Bordas *et al*., 2004). The difference in flavour due to cooking is probably a direct function of temperature in the meat (Heath, 1970). Hartman *et al*. (1984b) reported that the a_w has a significant effect on the generation of volatiles in cooked meat.

The studies so for have been carried out in an aqueous model system using amino acids and sugars as precursors of meat flavour. The literature on the generation of meaty aroma in meat model system under different pH and a_w is rather scanty. Therefore, in the present investigation, the effect of pH and a_w on the formation of selected volatiles were studied using a meat fiber model system and the combined effect of reaction mixture containing cysteine, thiamine and ribose.

MATERIALS AND METHODS

Longissimus dorsi post rigor beef meat was used for the present investigation. Meat (four batches of 1.5 kg each) was obtained from male animal slaughtered at about 18 months of age. The excess fat was trimmed and resultant lean meat was used for further use.

Preparation of Meat Fiber Model System

The minced meat was washed with phosphate buffer (0.02 M, pH 6.8). The meat and buffer in the ratio of 1:3 were homogenized by using hand-operated homogenizer (Brawn, UK) for one minute

and kept at 4°C for 30 min, stirring every ten minutes. The whole mass was centrifuged at 8,000 g (Mistral 6L MSE, UK) for 20 min at 4°C. The supernatant liquid after centrifugation was removed and a portion was filtered for Optical Density (OD) measurement. The homogenisation and centrifugation process was continued seven to eight times, till all pigments were extracted and an OD remains unchanged. After 5th washing the homogenised mass was kept for 15 h at 4°C and for further 6th and 7th washing it was kept for 30 min as mentioned above. A thick white paste of meat fiber was obtained. It was freeze dried and made into powder in a pestle and mortar. The powdered meat fiber was vacuum packed in laminate d mettalised polyester pouches and was stored at 20°C till further use. This meat fiber was selected as a model system for present investigation. The washing of meat with buffer eliminates the interaction of heme pigments or other constituents, which may effect during aroma generation. The preparation of meat fiber was carried out by four separate batches of minced meat served as replicates.

Sample Preparation at Different pH

A reaction mixture solution of a cysteine (1.43 mg mL^{-1}), thiamine (1.43 mg mL^{-1}) and ribose (1.28 mg mL^{-1}) was prepared in 0.2 M pyrophosphate buffer at the chosen pH 4.0, 4.5, 5.5 and 6.5. The reaction mixture solution 3.5 mL was added to meat fiber (0.5 g) and flame sealed in 10 mL Pyrex glass ampoules after flushing with nitrogen for 2 min. following the procedure as described by Farmer and Mottram (1990a). The buffer 3.5 mL was used to get a homogeneous mixture with 0.5 g meat fiber after heat treatment. For aqueous system, the reaction mixture solution containing cystine 5.0 mg mL^{-1}, thiamine 5.0 mg mL^{-1} and ribose 4.5 mg mL^{-1} in 0.2 M pyrophosphate buffer at chosen pH were made and 1 mL of this solution were flame sealed in a glass ampoules. Pyrophosphate buffer was selected because it controlled better pH of the system after heat treatment. Sample (without reaction mixture) of meat fiber (0.5 g) was also flame sealed in glass ampoules after flushing with nitrogen for 2 min. These samples were used for identification of volatiles in model system. Samples were made in four replicates. Total number of samples was fifty two (4xmeat fiber model system + 4×4 pH meat fiber model system 4x4pH aqueous system + 4X4pH meat fiber with reaction mixture).

Sample Preparation at Different a_w

An aqueous solution of a mixture of cysteine (5.0 mg mL^{-1}), thiamine (5.0 mg mL^{-1}) and ribose (4.5 mg mL^{-1}) was prepared in 0.2 M pyrophosphate buffer (pH 5.8). For the present investigation three different a_w were chosen (0.60, 0.80, 0.90). The selected a_w 's of the samples were obtained by mixing buffer and reaction mixture with meat fiber as shown below:

Meat fiber (g)	Buffer (μL) (0.2 M, pH 5.8)	Reaction mixture (μL) (in buffer)	Final a_w
4	400	400	0.60
4	1200	400	0.80
4	3000	400	0.90

The amount of reaction mixture was kept constant to get a uniform amount of reaction mixture in each sample under study at chosen a_w's.

A technique was devised for even distribution of buffer and reaction mixture solution in meat fiber. The meat fiber and required amount of reaction mixture solution and buffer were mixed with 20-30 mL of liquid nitrogen. The contents were ground in frozen condition to get a fine powder. The resultant meat fiber, 0.5 g was immediately poured in 10 mL glass ampoules under frozen conations (for easy pouring) and flame sealed after flushing with nitrogen for 2 min. Samples were made in four replicates. Total number of samples was twelve ($4\times3a_w$).

All the above samples prepared under different pH and a_w were heated at 130°C for 1 h in an autoclave (Harvard-LTE, Bench top-50, UK). After heating, the samples were cooled at room temperature 16±4°Cand stored at 20°C till further use for head space analysis.

Moisture, crude fat and crude protein contents of washed and dried meat fiber were estimated by the methods as described for meat and meat products in AOAC (2004). The pH of muscle and meat fiber was measured using a pH meter (Cyberscan-1000). Ten gram meat fiber was mixed with 90 mL of distilled water. The mixture was stirred at regular intervals and kept for 15 min and pH was measured. The muscle pH was measured by directly inserting the electrode in the meat mince. Water activity was measured directly by using water activity meter (Aqualab Series 3 TE Decagon, USA).

Chemicals

L-cysteine, D(-)ribose, Thiamine hydrochloride were obtained from Sigma Chemical Company (UK). Phosphate buffer was prepared from sodium dihydrogen orthophosphate ($NaH_2PO_4.2H_2O$), (Fisons Chemicals Ltd., UK) and disodium hydrogen orthophosphate ($Na_2HPO_4.12H_2O$), (BDH Chemicals Ltd., UK) in glass distilled water. Pyrophosphate buffer was prepared from tetra-sodium pyrophosphate ($Na_4P_2O_7.10H_2O$), (Fisons Chemicals Ltd., UK) and disodium dihydrogen pyrophosphate ($Na_2H_2P_2O_7$) (BDH Chemicals Ltd., UK). Other chemicals and solvents used were of Analar grade supplied by standard companies.

Head Space Collection

Each glass ampoule-containing sample was cooled in 50-60 mL liquid nitrogen pool by constantly stirring for 30-40 sec to minimise the rate of diffusion of volatiles and then it was smashed in 250 mL glass bottles (Schott, Duran, West Germany) containing 1.5 inch magnetic bar. The sample was diluted to 20 mL in pyrophosphate buffer with same pH as of sample while the samples of different a_w and meat fiber (without reaction mixture) a buffer of pH 5.8 was used for dilution. The sample in glass bottle was held at 30°C. Sample was magnetically stirred by lowest speed during the head space collection procedure. The sample was equilibrated at 30°C for 5 min. before purging the nitrogen. The volatile compounds were swept on to the adsorbent in the glass lined stainless steel trap (154 mm long ×1.6 mm E.D., packed with tenax GC) with a stream of nitrogen (10 mL min^{-1}). The collection was continued for 5 min. A standard mixture 1 μL of C_6-C_{15} n-alkane in ethanol was added to the trap and the solvent was removed by purging with nitrogen for 5 min.

Gas Chromatography-Mass Spectrometry

A Hewlett Packard 5890 series II gas chromatography fitted with a BP-1 fused silica capillary column 25 m long × 0.22 mm ID. was used for all chromatography fractions. The GC was coupled to a MD 800 (Fisons Instruments Ltd. UK) mass spectrometer. The end of the coloum was connected directly into the ion source. The helium carrier gas was set at 18 psi to give a flow of 1 mL min^{-1}. The volatile reaction components were thermally desorbed directly on to the GC column by heating the trap at 240°C while cooling a 40 cm region at the column in liquid nitrogen. The column temperature, after the removal of the coolant, was kept at 30°C for 2 min. In 1st ramp the column temperature was increased by 8°C min^{-1} to 90°C, in 2nd ramp the temperature was increased by 4°C min^{-1} to 140°C. In 3rd ramp the temperature was increased by 10°C min^{-1} to 240°C and was maintained for one min.

The mass spectrometer was operated in the electron impact mode with electron energy of 70 ev and an ion source temperature of 240°C. A continuous scan mode (0.5 sec scan, with a mass range 33-300) was used for the first few samples for identification of compounds and then the samples were run in Selected Ion Recording (SIR) mode. All GC-MS data were monitored, stored and processed using Lab-base system. The Linear Retention Indices (LRI) of selected compounds were calculated using the scan positions of the compounds and the two adjacent n-alkane (C_6-C_{15}) and were identified from their mass spectra data and LRI with those published one. The compounds were confirmed on the basis of one characteristic ion having highest intensity.

Statistical Analysis

The effect of pH and a_w on formation of selected aroma compounds was studied using a completely randomized design with three levels of a_w, four levels of pH and four meat fiber replicates. The data obtained was statistically analysed for significance by employing analysis of variance (ANOVA) technique to evaluate the significant difference between means. Mean separation, wherever significance existed, was accomplished using Duncan's multiple range tests. All the statistical analysis was carried out using the software STATISTICA (Anonymous, 1999).

RESULTS AND DISCUSSION

The minced meat had a pH of 5.46±0.11. The supernatant liquid obtained after centrifugation of each washing was filtered and Optical Density (OD) was and optical density at 525 nm wavelengths was recorded (Table 1). The OD after 7th or 8th washing of meat was unchanged. Since there was no change ($p \leq 0.05$) in OD after 7th washing, it was assumed that the maximum haem pigments have been extracted. The homogeneous meat mass after 7th washing was creamy white in colour. There is a sudden drop in OD (0.56 to 0.25) after 5th washing ($p \leq 0.05$). This could be because after 5th washing (before centrifugation) the content was kept for 15 h at 4°C as compared to half an hour in other washings. Therefore, the extraction of colour pigments from meat was more. Vani *et al*. (2006) reported that the OD of washed solution from chicken meat decreased markedly with number of washings up to 3 washings and the values remained the same thereafter on subsequent washings. Whereas, Kavitha and Modi (2007) reported OD of buffer extract from chicken breast and leg muscles after eight washing were 0.01±0.00 and 0.02±0.00, respectively and found the OD values remained constant after 6th washing.

Characteristics of Meat Fiber

The recoveries of freeze dried meat fiber after eight washing was 6.87±0.98%. The poor recovery of fiber could be due to repeated washing of meat. Most of the sarcoplasmic protein fractions, some myofibrillar proteins, carbohydrates and soluble nitrogen substances might have been washed away.

The dried meat fiber was cream in colour, no detectable odour and a mild sweet in taste was observed. On mixing with hot water it developed a light meaty aroma and while on boiling the fiber in limited quantity of water it coagulated to a loose bounded rubbery mass, when it was boiled in excess of water the fibers were dispersed on shaking in water and developed a cooked appetizing meaty aroma. This indicates the aroma is present throughout the meat. It is not confined to meat juice or to meat proteins only, though the intensity of meaty aroma could be varied. It was also noticed more and more boiling the fiber; distinct meaty flavour was developed in the fiber as well as in aqueous phase.

The dried meat fiber was similar in sensory characteristics to the meat system and allowed easy control of pH and a_w in the system and the biological variation could also be avoided. The gross composition of the freeze dried meat fiber was, crude protein 92.67±1.89%, crude fat 1.08±0.33% and moisture 5.95±0.65%. The pH of meat fiber was 6.99±0.02. The basic composition of meat fiber

Table 1: Optical densities of washings of minced meat with phosphate buffer solution

No. of meat washings (Phosphate buffer, 0.02 M, pH 6.85)	Optical density (525 nm)
1	1.00±01[a]
2	0.97±01[a]
3	0.80±01[b]
4	0.72±01[b]
5	0.56±01[c]
6	0.25±00[d]
7	0.22±00[d]
8	0.22±00[d]

Values are mean±SD (n = 4); Values with different letter(s) (a-d) for optical density differ significantly ($p \leq 0.05$)

prepared from leg and breast chicken meat as reported by Kavitha and Modi (2007) was, crude protein 85.63±0.50, 86.50±0.48%, crude fat 5.70±0.08, 4.83±0.05% moisture 3.50±0.08, 3.25±0.01% and ash contents 2.43±0.05, 2.80±0.22%, respectively. The variation in composition of meat fiber could be because the species muscle variation. The water activity of the meat fiber was 0.26±0.04.

Volatiles in Meat Fiber System

The odour port assessment was made with reaction mixture containing cysteine, thiamine and ribose mixture for few samples to select the compounds with meaty aroma or an intense odour. A typical GC-MS chromatogram of volatiles generated during Maillard reaction between cysteine, thiamine and ribose mixture is presented in Fig. 1. Seven compounds were selected, as listed in Table 2 with odour description, their Linear Retention Indices (LRI) and MS data. The presence of these compounds was also observed in a meat fiber system (without reaction mixture) (Table 3). The detection of compounds was confirmed with generated MS data of chosen compounds in present investigation with the published MS data of respective compounds. The MS data of 2-Methyl

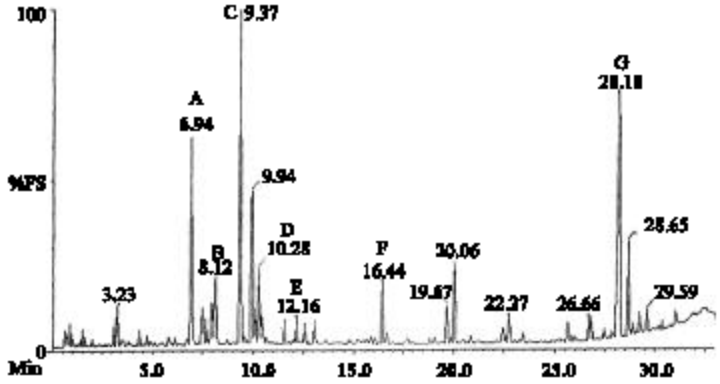

Fig. 1: Typical GC-MS chromatogram of volatiles generated during Maillard reaction between cysteine, thiamine and ribose mixture; A: 2-Methyl thiophene; B: 2-Methyl-4-5-dihydrothiophene; C: 2-Mehtyl-3-furanthiol; D: 2-Methyl-3-(methylthio) furan; E: 2-Methyl-tetrahydrothiophene-3-one; F: 2-Methyl-3-thiophenethiol; G: Bis-(2-methyl-3-furyl)-disulfide

Table 2: Odour description and Ms data of some selected volatiles generated during the Maillard reaction between cysteine ,thiamine and ribose mixture

Compounds identified	Retention time (min)	LRI (BP-1)	Ion for peak area	Odour description	MS data
2-Methyl thiophene	6.94	747	97	Burnt onion	97(100), 98(55), 45(26), 39(13), 53(8), 99(7), 69(6), 58(5).
2-Methyl-4-5 -dihydrothiophene	8.12	812	85	Cabbage, Roasted meat	85(100), 59(78), 100(73), 99(62), 65(30), 45(28), 39(24), 41(12), 53(10), 58(9).
2-Methyl-3-furanthiol	9.37	836	114	Sulphurous, Meaty	114(100), 45(30), 113(29), 43(27), 85(25), 51(22), 53(20), 71(19), 86(17), 52(16).
2-Methyl-3-(methylthio) furan	10.28	915	128	Spicy, Meaty	128(100), 113, 99, 85, 81, 69, 51, 45, 43.
2-Methyl tetrahydro-thiophene-3-one	12.16	936	60	Sulphurous	60(100), 116(34), 45(27), 59(25), 44(17), 58(16), 64(10), 88(7), 48(7).
2-Methyl-3-thiophenethiol	16.44	1016	130	Roasted meat, Cooked meat	130(100), 97(71), 45(58), 129(50), 59(29), 69(24), 85(19), 71(15), 70(10), 132(9).
Bis-(2-methyl-3-furyl) disulphide	28.18	1495	228	Sweet meaty, Burnt meaty	228(100), 113(90), 115(22), 45(18), 164(18), 71(16), 43(14), 230(14), 229(12), 69(10).

Table 3: Peak areas* of selected volatiles generated in washed meat fibre model systems when heated at 130°C for 1 h under different pH

	pH			
Compounds	4.0	5.0	5.5	6.5
2-Methyl thiophene	79.60±6.21[a]	72.40±5.77[b]	91.30±6.36[c]	107.25±9.19[d]
2-Methyl-4-5-dihydrothiophene	nd	nd	nd	1.30±0.21
2-Methyl-3-furanthiol	6.60±1.13[a]	10.80±0.89[b]	16.10±1.38[c]	1.70±0.05[d]
2-Methyl-3-(methylthio) furan	nd	nd	nd	0.60±0.08
2-Methyl tetrahydrothioph-ene-3-one	0.90±0.20[a]	1.80±0.36[b]	1.80±0.29[b]	2.30±0.16[c]
2-Methyl-3-thiophenethiol	nd	nd	nd	0.70±0.04
Bis-(2-methyl-3-furyl) disulfide	385.00±32.61[a]	412.40±59.13[b]	906.10±81.42[c]	198.10±20.19[d]

*Peak Areasx10^3, Mean±SD, nd: not detected. (n = 4); Values with different letters (a,b,c,d) among pH differ significantly ($p \leq 0.05$)

thiophene, 2-Methyl-3-furanthiol, 2-Methyl-3-thiophenthiol and Bis-(2-methyl-3-furyl) disulphide (Guntert *et al*., 1990), 2-Methyl-4-5-dihydrothiophene (Werkhoff *et al*., 1990), 2-Methyl-3-(methylthio) furan (Whitfield *et al*., 1988) and 2-Methyl tetrahydrothioph-ene-3-one (ten Noever de Brauw *et al*., 1980; Hartman *et al*., 1984a) are similar to the present findings. The generation of most characteristics impact meaty aroma compounds 2-methyl-3-furanthiol and bis-(2-methyl-3-furyl) disulphide has been reported by Gasser and Grosch (1988) in a cooked beef volatiles. While Werkhoff *et al*. (1990) reported these compounds are the product of thermal degradation of thiamine. Mottram and Leseigneur (1990) and Farmer and Patterson (1991) found that the Maillard reaction between cysteine and ribose is the major route in the generation of these compounds.

The 2-methyl-3-furanthiol, 2-methyl-3-(methylthio) furan, bis-(2-methyl-3-furyl) disulphide has been recorded with meaty aroma as reported by several researchers (MacLeod and Ames, 1986; Gasser and Grosch, 1988; Farmer and Patterson, 1991; Varavinit *et al*., 2000; Mottram, 2007).

Effect of pH in Generation of Volatiles

It was observed that 2-methyl-4-5-dihydrothiophene, 2-methyl-3-(methylthio) furan and 2-methyl-3-thiophenethiol compounds were not detected at lower pH (4.0 to 5.5) in a control meat fiber system (Table 3). They were only detectable at pH 6.5. The generation of 2-methyl thiophene and 2-methyl tetrahydrothiophene-3-one was more ($p \leq 0.05$) at higher pH and the generation of 2-methyl-3-furanthiol and bis-(2-methyl-3-furyl) disulphide was minimum ($p \leq 0.05$) at pH 6.5. It suddenly increased ($p \leq 0.05$) at pH 5.5 and then constantly decreasing with decreasing pH. Both these compounds are behaving in the same way in the system. This could be because bis-(2-methyl-3-furyl) disulphide is a dimer of 2-methyl-3-furanthiol. The mechanisms of the formation of these compounds are well described by Werkhoff *et al*. (1990).

The products of the thermal degradation of cysteine, thiamine and ribose mixture in aqueous as well as with meat fiber system were strongly influenced by pH (Table 4 and 5). The generation of volatiles were suppressed in meat fiber system containing reaction mixture (Table 5) as compared to liquid system (Table 4). There could be due to the presence of some constraint in meat fiber or intermediate factor, which is effecting the generation of these compounds. The results also indicated that the peak areas of all compounds were increasing with an increase in pH in both the system i.e. an over all picture of decrease or an increase in the generation of volatiles in meat fiber as well as in aqueous system was found in the same pattern. This concludes that the aqueous system was very close to the meat fiber model system.

The peak areas of 2-methyl-3-furanthiol and bis-(2-methyl-3-furyl) disulphide in meat fiber model system containing reaction mixture (Table 5) was maximum ($p \leq 0.05$) at pH 5.5 and then decreased significantly ($p \leq 0.05$) at pH 6.5, as it was observed in control meat fiber model system (Table 3). Mottram and Leseigneur (1990), Meynier and Mottram (1995) reported 2-methyl-3-furanthiol

Table 4: Peak areas* of selected volatiles generated in a Maillard reaction between cysteine, thiamine and ribose reaction mixture, when heated at 130°C for 1 h under different pH

	pH			
Compounds	4.0	5.0	5.5	6.5
2-Methyl thiophene	8.000±0.80^a	58.20±1.60^b	139.30±1.00^c	201.30±6.50^d
2-Methyl-4-5-dihydrothiophene	0.003±0.00^a	0.06±0.00^b	2.30±0.10^c	16.80±0.80^d
2-Methyl-3-furanthiol	37.400±11.30^a	30.00±3.30^a	196.30±20.30^b	193.10±9.70^b
2-Methyl-3-(methylthio) furan	1.300±0.04^a	3.00±0.24^b	51.00±7.00^c	131.00±13.50^d
2-Methyl tetrahydrothioph-ene-3-one	0.030±0.01^a	0.15±0.01b^b	1.33±0.03^c	6.01±0.10^d
2-Methyl-3-thiophenethiol	5.000±0.90^a	18.60±1.70^b	1045.00±131.00^c	2293.00±213.00^d
Bis-(2-methyl-3-furyl) disulfide	5.500±1.40^a	15.30±4.50b^a	141.20±31.30^c	177.00±15.10^d

*Peak Areasx10^3, Mean±SD, nd: not detected. (n = 4); Values with different letters (a,b,c,d) among pH differ significantly ($p \leq 0.05$)

Table 5: Peak areas* of selected volatiles generated in washed meat fibre model systems containing reaction mixture, when heated at 130°C for 1 h under different pH

	pH			
Compounds	4.0	5.0	5.5	6.5
2-Methyl thiophene	15.20±2.20^a	38.00±5.00^b	33.50±4.00^b	126.00±9.00^c
2-Methyl-4-5-dihydrothiophene	0.50±0.10^a	0.30±0.00^b	0.65±0.10^c	6.00±0.40^d
2-Methyl-3-furanthiol	43.00±5.40^a	110.4±26.00^b	141.00±44.10^c	21.00±7.00^d
2-Methyl-3-(methylthio) furan	1.30±0.10^a	1.00±0.04^a	2.03±0.01^b	2.30±0.20^b
2-Methyl tetrahydrothioph-ene-3-one	0.13±0.00^a	0.22±0.00^a	0.40±0.02^b	1.23±0.10^c
2-Methyl-3-thiophenethiol	3.50±0.40^a	3.70±0.40^a	6.80±1.60^b	8.60±0.40^c
Bis-(2-methyl-3-furyl) disulfide	9.10±3.60^a	11.60±1.70^b	27.40±0.80^c	6.20±0.40^d

*Peak Areasx10^3, Mean±SD, nd: not detected. (n = 4); Values with different letters (a,b,c,d) among pH differ significantly ($p \leq 0.05$)

generation was more at lower pH in a cysteine ribose Maillard reaction. Sulphur containing furans, particularly 2-methyl-3-furanthiol and its di and tri sulfides, were formed in much larger amounts in meat containing 5'IMP and initial pH 4.5 (Madruga and Mottram, 1995). Whereas, Guntert *et al.* (1992) found bis-(2-methyl-3-furyl) disulphide in trace levels at pH 7.0, while they were unable to detect at lower pH in a thiamine degradation reaction. In present investigation since cysteine, thiamine and ribose all the three were present along with meat fiber, the concentration of these compounds could be more. Therefore, it was detectable even at lower pH also. The explanation for generation more of these two compounds at pH 5.5 could be given as some intermediate by-product of the reaction is resulting from meat fibers, which could be highly depended on pH, because in aqueous system the generation of these compounds are in a linear function (Table 4). The generation of thiophene was favoured by higher pH in both the systems, which support the findings of Mottram and Leseigneur, (1990). The increase or decrease of certain compounds in either system at a particular pH could only be explained that each step of reaction is strongly controlled by pH (Leahy and Reineccius, 1989).

Effect of Water Activity in Generation of Volatiles

The formation of 2-methyl-3-furanthiol and the corresponding bis-(2-methyl-3-furyl) disulphide was favoured by higher a_w (Table 6) because both compounds posses meaty aroma and have extremely low odour threshold. The presence of greater amount ($p \leq 0.05$) of these two compounds at higher moisture containing system could be due to more release of hydrogen sulphide at higher a_w (Hartman *et al*., 1984b) which mainly takes part in the formation of these compounds in the chemical reaction (Werkhoff *et al*., 1990).

The generation of thiophene at a_w 0.80 was recorded significantly ($p \leq 0.05$) more. Above a_w 0.80 an inverse relationship between a_w and quantity of thiophene evolves. This may be due to a dilute

Table 6: Peak areas* of selected volatiles generated in washed meat fibre model systems when heated at 130°C for 1 h under different water activity

	Water activity		
Compounds	0.60	0.80	0.90
2-Methyl thiophene	26.00±4.00^{a}	956.80±196.60^{b}	327.60±18.60^{c}
2-Methyl-4-5-dihydrothiophene	12.60±0.14^{a}	125.00±12.50^{b}	66.40±2.30^{c}
2-Methyl-3-furanthiol	0.50±0.05^{a}	3.50±0.00^{b}	38.50±6.10^{c}
2-Methyl-3-(methylthio) furan	nd	0.50±0.10^{a}	0.70±1.00^{b}
2-Methyl tetrahydrothioph-ene-3-one	nd	1.10±0.12^{a}	1.10±0.05^{a}
2-Methyl-3-thiophenethiol	nd	nd	nd
Bis-(2-methyl-3-furyl) disulfide	8.90±3.20^{a}	9.10±0.40^{a}	38.20±1.20^{b}

*Peak Areasx10^{3}, Mean±SD, nd: not detected (n = 4); Values with different letters (a,b,c) among a_w differ significantly ($p \leq 0.05$)

effect or inhibition of condensation steps by water (Eichner and Karel, 1972). Compounds 2-methyl-3-(methylthio) furan and 2-methyl tetrahydrothiophene-3-one were not detected at lower a_w. Whereas, 2-methyl-3-thiophenethiol was not detected at all the three studied a_w's. Hartman *et al*. (1984b) reported the maximum amount of volatiles were produced at about a_w 0.72 with a rapid decline to a_w 0.40. Water had a significant effect on the generation of volatile compound from the reaction of cystein, ribose and phospholipid (Mottram and Whitefield, 1995).

CONCLUSIONS

The generation of meaty aroma compounds in a meat fiber model system proved an effective model system. The intensity of aroma compounds were highly influenced by pH and a_w. The 2-methyl-3-furanthiol, 2-methyul-3-(methylthio) furan and bis-(2-methyl-3-furyl) disulphide were found with characteristic meaty aroma compounds, whereas, 2-methyl-4-5-dihydrothiophene and 2-methyul-3-thiphenethiol was recorded as roasted meaty aroma compounds. The 2-methyl-4-5-dihydrothiophene, 2-methyl-3-(methylthio) furan and 2-methyl-3-thiphenethiol were not detected at lower pH. The formation of 2-methyl-3-furanthiol and bis-(2-methyl-3-furyl) was favoured by higher a_w.

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