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GENERATION OF MEAT-LIKE PROCESS FLAVOURINGS FROM SOYBEAN-BASED ACID HYDROLYZED VEGETABLE PROTEIN. EFFECT OF HYDROLYSIS TIME ON GENERATED VOLATILES.

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ABSTRACT

Hydrolyzed vegetable protein (HVP) was prepared by acid hydrolysis of defatted soybean at different time intervals 2,4 and 6h. Glutamic acid was the most abundant free amino acid after 6 h hydrolysis followed by aspartic acid and glycine. The total colour change of the hydrolyzed protein showed gradual increases by increasing hydrolysis time. The hydrolyzed soybean protein samples were involved in three model mixtures (MHSM2, MHSM4 and MHSM6) with xylose, thiamine and sulfur containing amino acids to develop meat-like process flavouring. The freeze dried flavouring samples were encapsulated in gum Arabic and subjected to detailed sensory evaluation. The most important odorants of meat-like process flavouring, 2-methyl-3-furfurylthiol and 2-furfurylthiol, showed the highest yield in MHSM6. The presence of 2, 4-dimethylthiazole in considerable concentration in sample MHSM6 confirms the high score of grilled attribute.

Key words: HVP, Flavourings agent, meat aroma, Maillard reaction, sensory evaluation

INTRODUCTION

Process flavour is defined as a group of flavours or flavouring ingredients that are produced from precursors via some type of processing techniques such as thermal processing. Precursors play an

important role in the generation of process flavours. The Maillard reaction between reducing sugars and amino acids is known to generate flavours similar to those of cooked foods. Meat flavour can be produced through process flavour technology (Manley, 1994a).

The Maillard reaction plays a substantial role in flavour generation during the cooking of meat. Besides thiamine, the precursors ribose and cysteine are important for meat aroma and are consequently employed in the production of process flavours (Morton *et al.*, 1960). Ribose is frequently replaced by its less expensive isomer xylose. Among other compounds, the thiols 2-methyl-3-furanthiol (2MFT), 2-furfurylthiol (FFT), and 3-mercapto-2-pentanone belong to the most important aroma impact compounds formed during the thermal reaction of ribose and cysteine. These molecules are also found in cooked meat (Evers *et al.*, 1976; Gasser and Grosch, 1988, 1990a), as well as in commercial meat flavourings and contribute significantly to their aroma (Gasser and Grosch, 1990b).

There is an ongoing interest to develop a simulated beef flavour to meet the consumer demand for non-meat based on vegetarian products. In particular, different types of protein hydrolysates were used as materials to produce meat-like savoury flavourings, including hydrolysed vegetable proteins produced from soy (Aaslyng *et al.*, 1998a), thermally treated yeast extracts (Munch *et al.*, 1997), extruded enzyme hydrolysed soybean protein (Baek *et al.*, 2001) and soybean-based enzyme-hydrolysed vegetable protein (Wu and Cadwallader, 2002).

Hydrolyzed vegetable protein (HVP) has been used for more than 100 years to impart meat-like flavour to prepared foods and represents one of the earliest forms of process flavour. HVP is primarily composed of amino acids liberated by the hydrolysis of vegetable protein with HCl at 110-140 °C (Manley, 1994b).

Thermally generated imitation meat flavours are often described in the patent literature as “process flavouring”. Knowledge of the identities of the volatile aroma components of model systems that simulate the cooking of meat is important for developing process flavours with authentic meat-like qualities, especially for species specific flavours such as beef, chicken, and pork. The volatile components of cooked meat model systems have been studied by a number of researchers (Mussinán and Katz, 1973; Farmer *et al.*, 1989 and Hofmann and Schieberle, 1995).

The volatile compounds of a cysteine and ribose reaction mixture were dominated by sulfur-containing heterocyclic compounds such as 2-methyl-3-furanthiol, 2-furanmethanethiol, 2-thiophenethiol and 3-mercapto-2-pentanone as mentioned by Farmer *et al.*(1989).

Hofmann and Schieberle (1995) studied the aroma components of a thermally treated aqueous solution of cysteine/ribose and found that 2-furanmethanethiol, 2-methyl-3-furanthiol, 2-thenylmercaptan, and ethylmercaptan were the main contributors to the overall roasty, meat-like aroma.

Many studies have been found concerning the production of meat-like process flavouring from soybean based enzyme hydrolyzed vegetable protein (Wu *et al.*, 2000 and Baek *et al.*, 2001) however very little have been reported on generation of meat-like flavouring by thermal treatment of acid HVP involved in model system (Misharina *et al.*, 1987).

The main objectives of the present study were to generate meat-like process flavourings by thermal treatment of acid hydrolyzed soybean protein involved in model mixtures with xylose, thiamine and other precursors of meat aroma in addition, effect of time of hydrolysis on the free amino acid composition of hydrolyzed protein. The study was extended to find a mathematical correlation between the composition of free amino acids, sensory aroma profile and the generated volatiles of each model mixture.

MATERIALS AND METHODS

Chemicals:

Defatted soybean (48 % protein) was obtained from Food Technology Research Institute, Agric. Res. Center, Giza Egypt. Thiamine and amino acids cysteine and taurine were purchased from Sigma Aldrich Chemical Co.(St. Louis, MN, USA).

Authentic compounds and standard n-paraffins(C8-C22) were purchased from Sigma Aldrich Chemical Co.(St. Louis, MN, USA) and E. Merck. All chemicals were of analytical grade

Production of hydrolysates

The acidic hydrolysate (A-HSM) of soybean protein was prepared by heating defatted soybean (100g) with 240 ml of hydrochloric acid (4 M) in a sealed glass bottle at 110°C for 6 h (Aaslyng *et al.*, 1998b). After being cooled to room temperature, the

mixture was neutralized to pH 6.5 with 4M sodium hydroxide and centrifuged. The hydrolyzate was subjected to analysis of free amino acids, total colour changes and then freeze dried and stored at -10°C until analysis.

Content of free amino acids

Composition of free amino acids of each hydrolysate was determined by using an amino acid analyzer, Eppendorf- Germany LC3000 Amino acid analyzer under the condition: Flow rate: 0.2me/min, pressure of buffer solution 50bar, pressure of reagent to 0-150 bar, Reaction Temperature:123°C.

Preparation of the thermal process flavourings:

Three model mixtures MAHSM2, MAHSM4 and MAHSM6 were prepared by mixing 10g of the freeze dried hydrolyzed soybean protein (obtained after 2, 4 and 6 h hydrolysis, respectively) with 1g xylose and 3g of the mixture containing cysteine, taurine and thiamine(at equal molar ratio). Each of the three model mixtures was dissolved in phosphate buffer (100ml; 0.5M; pH 5.0) and refluxed for 1 h, then the reaction flasks were cooled to room temperature using running cooled water.

Preparation of encapsulated thermal processed flavourings:

Arabic gum at concentration 10% w/v was dispersed in the aqueous solution of each of the three thermal processed model mixtures MAHSM2, MAHSM4 and MAHSM6, homogenized, then subjected to freeze drier (Snijders Scientific b.v.Model L45 Fm-Ro, Tilburg-Holand). The freeze dried materials were subjected to sensory analysis, isolation and identification of volatiles.

Sensory evaluation

I- Flavour acceptability

The flavour of three prepared beef processed flavourings was evaluated for sensory acceptability by 20 untrained panelist who were staffs and graduate students at Food Technology and Nutrition Research Division, National Research Center. Soup samples containing 2 % (w/v) of each encapsulated flavouring, sample were prepared. The panelists were asked to evaluate the acceptability of each sample as beef-like flavouring interms of odour, flavour and overall acceptability. A point scale was used for the evaluation with

9=extremely acceptable, 1 extremely unacceptable. The samples were three coded and randomly offered to the panelists (Larmond, 1977).

II-Aroma profile analysis:

A comparative odour profile analysis was carried out for the three prepared beef process flavourings ; MAHSM2, MAHSM4 and MAHSM6. The sensory test of odour attributes was investigated according to the International Standards ISO .The panel was carried out by 10 experienced assessors. They were asked to identify the various smelling attributes of each sample under investigation. The attributes that were to be used to describe the olfactory sensations for the samples were selected. The individual panelists separately scored, the intensities of the selected odour qualities on a category scale of 0.0 (not perceptible) to 10.0 (strongly perceptible) in increments of 0.5.

Colour measurements

L (lightness), a (red to green colour dimension) and b (yellow to blue colour dimension) values of each sample (MAHSM2, MAHSM4 and MAHSM6) were measured with a tristimulus colour analyzer (Hunter Lab scan XE- Reston VA; USA).The instrument was calibrated using a standard white reflector plate: (L=98.25; a=0.007; b=1.111). .Normalized L values were calculated from the following equation

$$\text{Normalized L} = (L - L_{\min}) / (L_{\max} - L_{\min}).$$

The total colour change (ΔE) was used to evaluate browning potential as mentioned by Ozoglu and Baymdirh (2002) as follows:

$$\Delta E = [(L_t - L_{t_0})^2 + (a_t - a_{t_0})^2 + (b_t - b_{t_0})^2]^{0.5}$$

Where t and t_0 correspond to any time during the experiments and the initial time, respectively.

Isolation of headspace volatiles

About 50 g of each sample were placed in conical flask containing 500 ml distilled water. The mixture solution at 60°C was stirred using teflon-coated magnetic bar. The volatiles were purged with purified nitrogen (grade of N₂>99.99%), at flow rate 100ml/min for 5h to three cooling traps at low temperature (ice-water/ice-acetone/dry ice-acetone). Volatile chemicals collected in each trap were recovered with diethyl ether-pentane (1:1, v/v) (Fadel *et al.*,

2006). The solvents containing volatiles were dried over anhydrous sodium sulphate for 12 h and concentrated with a Vigreux column (25 cm) to final volume of 100 μ l.

Gas chromatographic (GC) analysis:

GC analysis was performed by using Hewlett-Packard model 5890 equipped with flame ionization detector (FID). A fused silica capillary column DB5 (60m x 0.32mm i.d.) was used. The oven temperature was maintained initially at 50°C for 5 min, then programmed from 50 to 250°C at a rate of 4°C/min. Helium was used as the carrier gas, at flow rate 1.1 ml/min. The injector and detector temperatures were 220 and 250°C, respectively. The retention indices (Kovats index) of the separated volatile components were calculated with hydrocarbons (C8 – C22, Aldrich Chemical Co.) as references.

Gas chromatographic-mass spectrometric (GC-MS) analysis

The analysis was carried out using a coupled gas chromatography Hewlett-Packard (5890)/mass spectrometry Hewlett-Packard-MS (5970). The ionization voltage was 70 eV, mass range m/z 39-400 amu. The GC condition carried out as mentioned above. The isolated peaks were identified by matching with data from the library of mass spectra (NIST) and comparison with those of authentic compounds and published data (Adams, 1995). The quantitative determination was carried out based on peak area integration.

Statistical analysis

Data were analyzed using the analysis of variance (ANOVA) by the statistical package (Statistical Graphics Corporation, 1993, Manugistics Inc., USA). The multiple range test L.S.D (Duncan multiple range test), with significant level at $p < 0.05$, was applied to the results to test the significant difference.

RESULTS AND DISCUSSION

Amino acid composition and total colour change:

Analysis of amino acids present in defatted soybean meal before and after acid hydrolysis for a period of 0, 2, 4 and 6 h revealed that the defatted soybean protein was principally made up of 16 amino acids. As shown in Table (1) only aspartic acid and glutamic acid were the free amino acid determined in the defatted soybean meal before hydrolysis. Aspartic acid (287.2mg/l) was the most abundant

free amino acid in the sample MAHSM2 comprising over 287.2 mg/l of the total free amino acids followed by glutamic acid (259.2mg/l), glycine (258mg/l) and alanine(201.8mg/l), whereas glutamic acid (376.2mg/l) comprised the highest concentration in sample MAHSM6 followed by asparatic acid(319.4mg/l), glycine(272.6mg/l) and alanine(168.6mg/l). This finding is in accordance with that found in previous study (Solina *et al.*, 2007). Methionene was the only sulfur containing amino acid detected in the present study (Table1). Its concentration was decreased from 61.2mg/l in sample MAHSM2 to29.6mg/l in sample MAHSM6. Despite its low concentration, methionene may have an important influence on Maillard type flavour since its breakdown via Strecker degradation yields numerous sulfur-containing compounds such as dimethyl disulfide, dimethyl trisulfide and methanthiol that have an important role in cooked meat like flavor as mentioned by Griffith and Hammond (1989) and Wu and Cadwallader (2002).

Table (1): Amino acid composition (mg/l) of defatted soybean meal (DSM) and acid hydrolyzed soybean meal (A-HSM)

Amino acid	DSM	(A-HSM)		
	Zero	Time of hydrolysis		
		(A-HSM) 2h	(A-HSM) 4h	(A-HSM) 6h
Aspartic	122.2	287.2	405.6	319.4
Threonine	nd	43.8	32.4	36.2
Serine	nd	104.4	93.2	56.4
Glutamic	30.0	259.2	328.4	376.2
Proline	nd	2.6	0.00	2.4
Glycine	" "	258	247.2	272.6
Alanine	" "	201.8	129.4	168.6
Valine	" "	38.2	55.6	66.6
Methionene	" "	61.2	23.4	29.6
Isoleucine	" "	55.6	19.4	16
Leucine	" "	40	71.8	77.2
Tyrosine	" "	75.2	32.8	41.4
Phenylalanine	" "	133.6	35.2	46.8
Histidine	" "	53.6	102.4	120.8
Lysine	" "	69.2	77.6	90.8
Arginine	" "	53.2	76.6	73.2
Ammonia	" "	263	269.2	205.8

nd: not detected

As expected the total colour change ΔE showed significant ($p < 0.05$) increase with increasing time of hydrolysis (Table 2). This increase is mainly attributed to the formation of coloured melanoidins according to Aaslyng *et al.* (1998b).

Table (2): Total colour change in MAHSM2, MAHSM4 and MAHSM6.

Samples	Total colour change ΔE
A-HSM 2h	8 ± 0.60^c
A-HSM 4h	13 ± 0.80^b
A-HSM 6h	17 ± 1.0^a

a,b,c significant difference $p < 0.05$.

Volatile compounds:

A total of 49 volatile compounds were identified in the headspace volatiles of the three model mixtures, containing acid-hydrolyzed soybean protein (A-HSM), cysteine and ribose (MAHSM2, MAHSM4 and MAHSM6). The identified compounds including compounds represented in high concentrations with some selected compounds, present in lower than 1.0% of the total volatiles that was of sensory significance. Some of these volatile compounds may be generated via Maillard reaction in hydrolyzed soybean protein during hydrolysis (Solina *et al.*, 2005).

The volatile compounds identified in the present study were qualitatively and quantitatively dominated by sulfur containing compounds such as thiol containing furans and thiophenes, alkylsulphide, mercapto compounds, in addition to furans, pyrazines, ketones and Strecker aldehydes.

The obtained data are in agreement with those of related systems of Firmenich (2007) who detected the compounds 2-methylthiophene (12), 4,5-dihydro-2-methyl-3(2H) furanone (13), 3-Mercapto-2-butanone (14), 2-furfural (16), 2-Methyl-3-furanthiol (19), 3-Mercapto-2-pentanone (23), 2-Methyl-4,5-dihydro-3-furanthiol (35) in the headspace volatiles of the thermal reaction mixture containing xylose, cysteine and thiamine also Fadel *et al.* (2001) found most of the identified compounds in the volatiles of chicken flavour model system.

Methanthiol (1) and dimethyl sulfide (2) are the predominant volatile compounds in sample MAHSM2 and MAHSM4, respectively.

Methanethiol was described as having rotten/sulfurous aroma note (Wu and Cadwallader, 2002). It was reported in code (Milo and Grosch, 1995) and stewed beef juice, with odour detection threshold values 0.2 ppb in water (Guth and Grosch, 1994). Methanethiol may be present in the unheated model mixture and increased during heating (Wu and Cadwallader, 2002). Dimethyl sulfide had a corn-like note and was identified in code along with methanethiol (Milo and Grosch, 1995). 2-Methyl propanal (4), 3-methylbutanal (5), 2-methylbutanal (6) and methional (30) are Strecker degradation products of valine, leucine, isoleucine and methionene respectively. Sample MAHSM4 contained the highest yield of these compounds (7.08, 2.96, 3.68 and 1.27% respectively) followed by MAHSM2 (8.63, 0.03, 1.06 and 0.94 % respectively.) whereas MAHSM6 comprised only (0, 1.04, 1.19 and 0.11% respectively) of these compounds. Dimethyl disulphide (11) derived from the Strecker aldehyde or thermal degradation of cysteine or methionene (Vernin and Parkanyi, 1982). MAHSM2 comprised the highest yield of this compound (10.03%) followed by MAHSM4 (5.43%), it was identified at much less concentration (0.25%) in MAHSM6. Methional (3-methyl thiopropanal) is typically described as potato-like (Guadagni *et al.*, 1972), meat-like (Furia and Bellanca, 1971) or bouillon/onion-like (Arclander, 1969), boiled food (Aaslyng *et al.*, 1998b). It is important in aroma of heated beef and chicken (Gasser and Grosch, 1988, 1990a and Munch *et al.*, 1997). It has been found in HVP heated with mixture of glucose and xylose (Umano *et al.*, 1995).

The decrease in Strecker aldehydes content in MHSM6 may be due to the fact that Strecker aldehydes could undergo further reactions to give nonvolatile products including coloured melanoidins. This result proves the results of total colour changes (Table 2).

The presence of the short chain ketones (2,3-butandione(3),2-pentanone (7),3-pentanone (8), 2,3-pentandione (9)) can be explained by Maillard type reaction and Strecker degradation which can lead to their formation. The diketones, 2,3-butanedione and 2,3-pentadione are important intermediate in the formation of the other volatiles. They can react with hydrogen sulfide leading to the formation of mercapto ketones which can produce important meat-like volatiles (Madruga and Mottram, 1998). 2, 3-Butandione and 2,3-pentadione have a buttery aroma (Wu and Cadwallader, 2002). 2, 3-Butandione with a threshold of 2.6ppb in water (Fors, 1983) is a characteristic

component of cooked foods and is thermally generated through the Maillard reaction (Hodge, 1967).

2-Methyl-3-furanthiol (19) is considered to play an important role in the flavour of meat (Wu and Cadwallader, 2002). It possesses meaty, roasty and boiled note (Madruga and Mottram, 1995). As shown in Table (3) this compound comprised more than 47% of the total volatiles in headspace of sample MAHSM6. Hofmann and Schieberle (1998) reported that, dehydration of pentoses may result in the formation of either the 1-or 3-deoxypentosone. Their subsequent cyclization and elimination of water lead to the formation of either 4-hydroxy-5-methyl-3(2H)furanone (21) or furan-2-aldehyde as the major products. However, it is yet unknown whether both deoxyosones are formed in equal amounts. The reaction of furan-2-aldehyde with hydrogen sulfide, which can be liberated from cysteine, was shown to generate the furfurylthiol (27) very effectively (Munch *et al.*, 1997). It might, therefore, be assumed that also the 3-deoxypentosone could act as a precursor of this flavour compound. As shown in Table (3) FFT (27) showed the highest yield (3.13%) in sample MAHSM6. It was previously described as sulfurous/coffee like and was dominated in coffee substitute aroma (Fadel *et al.*, 2008). Furfurylthiol was described as one of the primary odorants of chicken broth and several commercial meat flavourings (Gasser and Grosch, 1990a and Rulher and Baltes, 1994). Two thiophenethiols, 2 or 3-thiophenethiol (36) and 2-methylthiophenethiol (42) were only detected in MAHSM2 and MAHSM4 in small amount (0.09 and 0.30%, respectively) and (0.20 and 0.53%, respectively). Madruga and Mottram (1998) postulated that 2 or 3-thiophenethiol may be probably formed from the thermal degradation of cysteine, also 2-methylthiophenethiol may be formed from α -dicarbonyls or furanones by reaction with hydrogen sulfide. Three mercaptoketones (3-Mercapto-2-butanone (14), 3-Mercapto-2-pentanone (23), and 2-Mercapto-2-pentanone (26)) were identified in the present study, their highest yields were found in volatiles of MAHSM4. These compounds were probably formed from the reaction of the corresponding alkanedione with hydrogen sulfide (i.e./2, 3-butandione/H₂S or 2,3-pentanedione/H₂S) and they has been reported in the aroma of boiled meat and chicken broth (Gasser and Grosch, 1990a; Zhang and Ho, 1991).

Nine thiophenes (2-methylthiophene (12), 2-ethylthiophene (20), 2,5-dimethylthiophene (22), 4,5-dihydro-2-methyl-3(2H)thiophene (37), formyl thiophene (40), 2-acetylthiophene (45), 2-formyl-5-methylthiophene (46), 2-propionylthiophene(48) and 3-ethyl-2-formyl thiophene(49)) are reported in Table (3). Thiophenes constitute a class which has been reported in volatiles from a number of foods and related model systems (Xu *et al.*, 2008), and have been suggested as being responsible for the mild sulphurous odour of cooked meat (Mottram, 1991). The main route for their formation involves the reaction of furfural and furanones with hydrogen sulphide (Vernin and Parkanyi, 1982) or the condensation of mercapto acetaldehyde with α,β -unsaturated aldehydes. Recently Guo *et al.* (2010) found dimethyl disulphide among the major identified compounds in meat like flavours. In present study this compound was determined in considerable amount in MAHSM2 (10.03%) and MAHSM4 (5.43%). It may be generated during the acid hydrolysis of soybean protein (Aaslyng *et al.*, 1998b).

Six thiazoles (2-methylthiazole (17), 4,5-dimethylthiazole (25), 2,4,5-trimethylthiazole (38), 4-ethyl-5-methylthiazole (39), 2-acetylthiazole (41) and 5-ethyl-2,4-dimethylthiazole (43)) were identified in the present study. MAHSM6 comprised the highest amount of these compounds (15.74%). 4,5-Dimethylthiazole (25) which was found in considerable concentration (1.6%) in MAHSM6 is known to have roasted or grilled notes (Mottram, 1998). The acyl thiazole has been reported in a model system containing cysteine and ribose (Madruga and Mottram, 1998). According to Vernin and Parkanyi (1982), the thermal degradation of cysteine, either alone or in the presence of reducing sugars such as ribose, is a source of thiazoles, also they can be formed by heat degradation of thiamine. Alkyl thiazole having low odour threshold values, have been found in meat and bread (Elmore and Mottram, 1997). It has been demonstrated that thiazoles can be formed from mixtures of hydroxyketone, hydrogen sulphide, ammonia and an aldehyde (Elmore *et al.*, 1997).

Four alkyl pyrazine (pyrazine (10), 2-methylpyrazine (15), 2,3-dimethylpyrazine (31) and 2,5-dimethyl-2-ethylpyrazine (44)) were found in the present study (Table 3). The probable route by which alkyl pyrazine might be formed is from the condensation of two α -aminoketone molecules produced in the Strecker degradation of amino

Table (3): Volatile compounds identified in headspace volatiles of process flavouring samples MAHSM2, MAHSM4 and MAHSM6:

Peak No	Compounds	RI ^a	Relative area%			Methods of ^b identification
			MAHSM2	MAHSM4	MAHSM6	
1	Methanthiol	<600	21.50 ^c	13.09	0.09	KI,MS
2	Dimethyl sulfide	<600	30.10	33.56	0.37	KI,MS
3	2,3-Butandione	613	3.00	4.11	12.13	KI,MS
4	2-Methylpropanal	630	8.63	7.08	---	KI,MS
5	3-Methyl butanal	649	1.06	3.68	1.19	KI,MS
6	2-Methyl butanal	672	0.03	2.96	1.04	KI,MS
7	2-Pentanone	693	0.11	1.58	0.07	KI,MS
8	3-pentanone	709	2.12	0.15	0.07	KI,MS
9	2,3-pentandione	720	0.79	0.40	1.81	KI,MS
10	Pyrazine	731	0.50	0.24	---	KI,MS,St
11	Dimethyldisulfide	758	10.03	5.43	0.25	KI,MS
12	2-Methylthiophene	774	12.65	9.8	12.36	KI,MS
13	4,5-Dihydro-2-methyl-3(2H) furanone	801	0.19	0.05	0.15	KI,MS
14	3-Mercapto-2-butanone	812	0.84	1.93	0.42	KI,MS
15	2-Methyl pyrazine	820	1.68	1.20	---	KI,MS,St
16	2-Furfural	827	0.02	0.03	---	KI,MS,St
17	2-Methyl thiazole	832	0.16	0.13	0.18	KI,MS,St
18	2,4-Dimethylfuran	850	---	0.11	---	KI,MS,St
19	2-Methyl-3-furanthiol	870	0.08	0.96	47.78	KI,MS
20	2-Ethylthiophene	875	0.03	0.07	---	KI,MS
21	4-Hydroxy-5-methyl-3(2H) furanone	881	0.92	0.36	---	KI,MS
22	2,5-Dimethylthiophene	885	0.09	0.02	0.03	KI,MS
23	3-Mercapto-2-pentanone	899	0.84	1.5	0.02	KI,MS
24	2-Acetyl furan	906	0.03	0.08	---	KI,MS,St
25	4,5-Dimethylthiazole	910	0.05	0.09	1.6	KI,MS
26	2-Mercapto-2-pentanone	914	0.01	1.11	---	KI,MS
27	2-Furylmethanthiol	918	0.31	1.93	3.13	KI,MS,St
28	2 or 4- Methyl-2furfural	922	0.02	0.18	0.01	KI,MS
29	Dimethyl sulphone	927	0.03	---	0.01	KI,MS
30	Methional	932	0.94	1.27	0.11	KI,MS
31	2,3-Dimethyl pyrazine	940	0.49	0.72	---	KI,MS,St
32	5-Methyl-2-furfural	944	0.04	0.09	0.01	KI,MS,St
33	1(2-furyl)-2-propanone	953	0.11	0.20	0.1	KI,MS

Continue

34	Dimehyl trisulfide	968	0.16	0.19	0.88	KI,MS
35	2-Methyl-4,5-dihydro-3-furanthiol	975	0.12	0.3	0.01	KI,MS
36	2or3-Thiophenthiol	978	0.09	0.20	0.01	KI,MS
37	4,5-Dihydro-2-methyl-3(2H)thiophene	990	0.02	0.04	0.05	KI,MS
38	2,4,5-Trimethylthiazole	996	0.20	0.70	---	KI,MS
39	4-Ethyl-5-methylthiazole	1000	0.06	1.20	9.12	KI,MS
40	formyl thiophene	1012	0.23	0.31	1.13	KI,MS
41	2-Acetyl thiazole	1020	0.23	0.31	4.16	KI,MS.St
42	2-Methyl-3-thiophenthiol	1056	0.30	0.53	---	KI,MS
43	5-Ethyl-2,4-dimethylthiazole	1072	0.90	0.90	0.68	KI,MS
44	2,5-Dimethyl -2-ethylpyrazine	1080	---	0.29	0.02	KI,MS
45	2-Acetyl thiophene	1096	0.24	0.64	0.10	KI,MS
46	2-Formyl-5-methylthiophene	1118	0.09	0.10	0.43	KI,MS
47	2-Methyl-3-(methyldithio)furan	1170	---	0.40	0.05	KI,MS
48	2-Propionyl thiophene	1175	---	0.17	0.18	KI,MS
49	3-Ethyl-2-formyl thiophene	1181	---	---	0.26	KI,MS

Compounds listed according to their elution on DB5 column.

a:Kovats index

b:Compound identified by GC-MS(MS) and /or by kovats index on DB5(KI) and or by comparison of MS and KI of standard compound (St) run under similar GC-MS condition

c:Values are the average of two experiments.

acids by dicarbonyl compounds (Guo *et al.*, 2010). It is well known that the roasted flavour is usually associated with the presence of heterocyclic compounds particularly pyrazine and thiazole. Many of these compounds have been found in meat volatiles (Mottram, 1998). The presence of alkyl pyrazines in present study mainly correlated to the amino acids involved in the model mixtures other than cysteine which inhibit their formation (Madruga and Mottram, 1998). The identified pyrazines may be produced during acid hydrolysis in present study. Pyrazines accounted for 59% of the heterocyclic compounds derived from Maillard reaction found in A-HVP (Solina *et al.*, 2005).

Sensory evaluation

-Flavour acceptability:

As shown in Table (4) sample MAHSM6 possessed the highest acceptability as beef process flavouring. There were significance differences ($p < 0.05$) among all samples for all examined attributes. The highest acceptability of sample MAHSM6 may be correlated to the fact that the increase in hydrolysis time of defatted soybean protein is associated with an increase in generation of the intermediate volatile compounds that can undergo further condensation reactions to produce some potent odorants of beef aroma (Aaslyng *et al.*, 1998b).

Table (4): Sensory evaluation of beef-like process flavourings MAHSM2, MAHSM4 and MAHSM6.

Samples	odour	taste	overall
A-HSM-2h	3.11±0.63c	4.20±0.98c	3.7±0.82c
A-HSM-4h	6.3±0.82b	6.3±0.52b	6.0±0.76b
A-HSM -6h	8.5±0.54a	8.5±0.55a	8.5±0.84a

a,b,c significant difference ($p < 0.05$)

Aroma profile analysis:

A detail sensory profile analysis was carried out with the three process flavouring samples MAHSM2, MAHSM4 and MAHSM6. A trained panel using a scaling procedure qualified each of the six selected attributes. Three-fold repetition and use of standards gave reproducibility of the test. Mean values were then calculated and displayed as a spider web diagram as shown in Fig.(1). It is obvious that MAHSM6 was very strong in grilled beef note followed by roasted and to a lesser extent cooked meat note. Chicken broth note was the highest scored attribute in sample MAHSM4 followed by roasted and cooked meat note. Whereas sample MAHSM2 was strong in sulfury-eggy note. Savoury note showed approximately similar intensity in all samples. The above mentioned results confirm those of GC-MS analysis of headspace volatiles (Table 3). It was reported that 2-methyl-3-furanthiol, the predominate component in MAHSM6, possesses meaty note (Wu and Cadwallader, 2002). It is an important beef characteristic note compound with extremely low odour threshold (Hofmann and Schieberle, 1998).

3-Mercapto-2-pentanone with 2-methyl-3-furanthiol were proposed to contribute significantly to chicken broth flavour (Zhang *et*

al.,1997). The eggy-like note was probably due to the presence of hydrogen sulfide which is formed during thermal degradation of cysteine, it is a precursors of various compounds associated with meat aroma (Shibamoto, 1980).

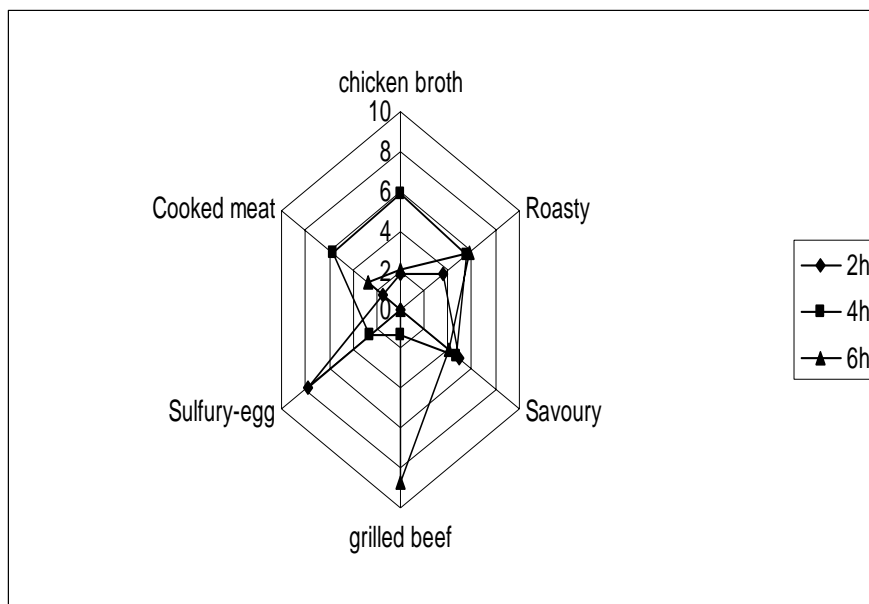


Fig (1): Aroma profile of MAHSM2, MAHSM4 and MAHSM6.

Conclusion

The time of acid hydrolysis highly influenced the composition of the free amino acids of soybean protein and consequently affected the volatiles generated by thermal treatment of the model mixtures containing the acid hydrolyzed soybean protein with other precursors of meat aroma. Beef-like flavour was more pronounced in flavouring produced by thermal processing of the model mixture containing soybean protein hydrolyzed for 6 h .The sensory profile analysis confirmed this result.

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توليد مكسبات نهكه اللحوم من بروتين فول الصويا المحلل . تأثير زمن التحلل على المواد الطيارة المتولده

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تم تحضير بروتين فول الصويا المحلل بواسطة التحليل الحامضى لدقيق فول الصويا منزوع الدهن وذلك لفترات زمنيه مختلفه 2و4و6 ساعات . وجد أن حامض الجلوتامك اكثر الاحماض الامينيه الحره بعد 6 ساعات من التحليل الحامضى يليه الاسبارتك ثم الجليسين. و أظهر التغير اللوني الكلى للبروتين المحلل زياده تدريجيه بزياده وقت التحلل. تم عمل مخلوط من بروتين فول الصويا المحلل عند فترات زمنيه 2و4و6 كل على حده مع سكر الريبوز والثيامين و احماض أمينيه تحتوى على عنصر الكبريت وذلك لتوليد نكهه اللحوم. و لقد تم كبسله نكهات اللحوم المحضره باستخدام الصمغ العربى كماده حامله و تلى ذلك تجفيفها و عمل التقييم الحسى لها . ولقد وجد ان المركبات المسئوله عن نكهه اللحوم 2-ميثيل-3- فيوريل-ثيول و 2-فيوريل ثيول أظهرت أعلى تركيز فى العينه المحضره بعد 6 ساعات تحلل. كما أن وجود المركب 2و4 داي ميثيل ثيازول فى العينه المحضره بعد 6 ساعات تحلل بتركيز محسوس يتفق مع التقييم الحسى حيث وجد أن صفه نكهه الشواء لها درجات مرتفعه.