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Flavour Quality and Stability of an Encapsulated Meat-Like Process Flavouring Prepared from Soybean Based Acid Hydrolyzed Protein

Hoda H.M. Fadel[1,*], A. Abdel Samad[2], M.I. Kobeasy[2], Magda A. Abdel Mageed[1] and Shereen N. Lotfy[1]

[1]*Chemistry of Flavour and Aroma Department, National Research Centre, Dokki, Cairo, Egypt*

[2]*Faculty of Agriculture, Biochemistry Department, Cairo University, Giza, Egypt*

Abstract: The main objective of this study was to produce meat-like process flavouring by using a model mixture based on acid hydrolyzed soybean protein (a-HVP) in addition to xylose, thiamine, cysteine and taurine . Glutamic was the major amino acid in a-HVP followed by aspartic, glycine and alanine. The meat –like process flavour was encapsulated in gum Arabic and stored at room temperature for 6 months. The changes in quality and flavour stability were followed during storage. The results revealed that the roasty and savoury notes were increased whereas chickeny, beefy and grilled meat notes showed significant ($P< 0.05$) decrease during storage. The gas chromatography-mass spectrometry (GC-MS) analysis showed that 2-methyl-3-furanthiol, the main contributor to beefy note, was the major identified compound in the fresh sample. It showed remarkable decrease after storage for 6 months. The pyrazines and thiazoles showed an opposite trend. A quite agreement was found between the results of the odour profile analysis and those of GC-MS analysis. The results of the present study give important information to the flavourists who are working in field of process flavours, especially meat-like process flavour.

Keywords: Aroma compounds, Aroma profile, Model mixture, Freeze drying, Gas chromatography-mass spectrometry analysis, Thermal process flavour.

INTRODUCTION

Thermally generated imitation meat flavours are often described in the patent literatures as "process flavouring". A wide rang of imitation flavour or meat-like flavours had been developed by flavour industry. However, the characteristic flavour profile of targeted meat item (eg. roasted meat) has proven much difficult to achieve. 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. A number of studies have been conducted on role of precursors in generation of process flavour[1-3]. Characterization of the volatile aroma components of the model systems that simulate the cooking of meat is necessary for the development of process flavour with authentic meat-like qualities, especially for species specific flavours such as beef and chicken. The volatile components of cooked meat model systems have been studied by a number of researchers [1, 2, 4-7]. The thiols; 2-methyl-3-furanthiol, 2-furfurylthiol, and 3-mercapto-2-pentanone belong to the most important aroma impact compounds formed during the thermal reaction of ribose and cysteine [4, 8]. These compounds are also found in cooked meat [9, 10] as well as in commercial meat flavourings and contribute significantly to their aroma [11].

Currently, there is an interest to develop the simulated beef flavour to meet the consumer demand for non meat based on vegetarian products. Extensive studies have been published concerning the production of meat-like process flavouring from soybean based enzyme hydrolyzed vegetable protein [12-14]. However, no one has been reported on generation of meat-like flavouring by thermal treatment of acid HVP involved in model system.

From a commercial point of view, the process flavours are very expensive and difficult to be handled and so not sold alone. In food industries, flavourists convert these flavours to final products by blending with other materials that act as carriers to produce products more convenient for food processing.

Therefore, in the present study the acid hydrolyzed soybean protein (a-HVP) with other flavour precursors were used to generate meat-like process flavour (MPF) which was encapsulated in gum Arabic by freeze drying to prepare a more convenient and easily handled product. The relation between the amino acid composition of the hydrolyzed protein and the generated volatile compounds was investigated. To assess the quality of the encapsulated meat-like process flavouring, the changes in its aroma composition and aroma profile were followed during

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[*]Address correspondence to this author at the Chemistry of Flavour and Aroma Department, National Research Centre, Dokki, Cairo, Egypt;
Tel: + 202 33371718; Fax: + 202 33371718-33370931;
E-mail: hodafadel_f@hotmail.com

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storage for 6 months and a correlation between them was established.

MATERIALS AND METHODS

Materials and Chemicals

Defatted soybean meal (48% protein) was obtained from Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. Thiamine, amino acids; cysteine and taurine, authentic compounds and standard n-paraffins (C8-C22) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). All chemicals used in this study were of analytical grade and the solvents were purified and distilled before use.

Production of Acid Hydrolysate

The acidic hydrolysate of soybean meal protein (a-HVP) was prepared by heating soybean meal (100g) with 240 ml of hydrochloric acid (4 M) in a sealed glass bottle at 110ºC for 6 h [15]. After being cooled to room temperature, the mixture was neutralized to pH 6.5 with sodium hydroxide (4 M) and centrifuged; the precipitate was washed with 200 ml of tap water and centrifuged again. The suspension hydrolysate in water was filtered and immediately subjected to analysis for free amino acids, then freeze dried and stored at -10 °C until further analysis.

Free Amino Acids Composition of a-HVP

Analysis of free amino acids of a-HVP was performed in Central Service Unit, National Research Centre, Cairo, Egypt using LC3000 amino acid analyzer (Eppendor-Biotronik, Germany). The technique was based on the separation of amino acids using strong cation exchange chromatography followed by ninhydrine colour reaction and photometric detection at 570nm. The freeze dried hydrolysate was dissolved in a lithium citrate buffer, pH2.2. Twenty µl (in duplicate) of the solution were loaded onto the cation exchange column (pre-equilibrated with the same buffer), then four lithium citrate buffers with pH values of 2.2, 2.8, 3.3 and 3.7, respectively, were successively applied to the column at a flow rate of 0.2 ml/min. The ninhydrine flow rate was 0.2 ml/min and pressure of 0.0-150.0 bar. The pressure of buffer was from 0.0 to 50.0 bar; and reaction temperature was 130°C.

Preparation of Encapsulated Beef-Like Process Flavouring (BPF)

A model mixture of the freeze dried acid hydrolyzed soybean protein (10g) with 1g xylose and 3g of a mixture containing cysteine, taurine and thiamine (at equal molar ratio) was dissolved in phosphate buffer (100ml; 0.5M; pH 5.0) and heated for 1 h in a thermostatic oil bath with magnetic stirring (150rpm) at 140°C under efficient reflux system. The reaction flask was then cooled to room temperature using running cooled water. Arabic gum at a concentration of 10% w/v was dispersed in the meat process model mixture solution, homogenized, then subjected to freeze drier (Snijders Scientific b.v. Model L45 Fm-Ro ,Tilburg-Holand). The encapsulated beef process flavour was divide into four equal samples, one of them was considered as control whereas, the other three samples were packaged separately in brown glass bottles under nitrogen and stored at room temperature (25°C -30°C) until analysis. The changes in the odour profile and headspace volatiles of the encapsulated beef-like process flavouring were followed during storage for 0, 1, 3 and 6 months.

Odour Profile Analysis

Quantitative descriptive sensory analysis was carried out for evaluating the odour profile of the encapsulated beef-like process flavouring [16]. The evaluation was conducted by a well-trained panel consisting of 10 members (6-female, 4-male) drawn from Nutrition and Food Technology Division, National Research Centre, Cairo, Egypt. All panelists had received a training in descriptive sensory analysis (>20h) and possessed experience with sensory evaluation. Before carrying out the quantitative descriptive sensory analysis, the panelists had thoroughly discussed the aroma properties of samples through three preliminary sessions, each spent 2 h, until all of them had agreed to use them as attributes according to the objective of the present work. In total five descriptors including chickeny, roasty, beefy, savoury and grilled meat notes were used for the descriptive analysis. Standards used to define these aroma descriptors were present during training and formal sessions. The individual panelists separately scored the intensities of descriptive odour qualities on a category scale 0.0 (not perceptible) to 10.0 (strongly perceptible). Samples (10g) were evaluated in odour free steel containers with lid and tempered in 20 min prior to evaluation. Each sample was prepared in triplicate.

Isolation of Headspace Volatiles

Each sample (10g) under investigation was placed in a conical flask containing 100 ml distilled water. The mixture solution at 60ºC was stirred using Teflon-coated magnetic bar for 3 min. 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) [17]. The solvents containing volatiles were dried over anhydrous sodium sulphate for 12 h and concentrated with a Vigreux column (25cm) under 40°C to final volume of 100µl. Three extractions were performed for each sample

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

A gas chromatography (Hewlett-Packard model 5890) coupled to a mass spectrometer (Hewlett-Packard-MS 5970) was used for analysis. Volatiles were separated using a fused silica capillary column DB5 (60m x 0.32mm i.d. x 0.25 µm film thickness). 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 of 1.1 ml/min. The sample size was 2 µl, split ration 1:10, the injector temperature was 220 °C. Mass spectra in the electron impact mode (EI) were obtained at 70 eV and scan m/z range from 39 to 400 amu. The retention indices (Kovats index) of the separated volatile components were calculated with reference to the retention time of a series of n-alkanes (C_6-C_{20}), run at the same conditions. The isolated peaks were identified by matching with data from the library of mass spectra (National Institute of Standard and Technology, NIST) and comparison with those of authentic compounds and published data [1, 12, 13, 18]. The quantitative determination was carried out based on peak area integration.

Statistical Analysis

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

RESULTS

Free Amino Acids Compound

Analysis of the free amino acids in the acid hydrolyzate of defatted soybean protein (a-HVP) revealed the presence of 16 amino acids with total concentration of 179.28±7.90mg/g (Table **1**). Glutamic acid was the major free amino acid (37.62±0.22 mg/g) followed by aspartic (31.91±0.62 mg/g), glycine (27.26±0.50 mg/g) and alanine (16.86±0.70mg/g). Methionine was the only sulfur containing amino acid identified in the present study. It was present in low concentration (2.96 ± 0.04mg/g).

Table 1: Composition of Free Amino Acids in Acid Hydrolyzed (a-HVP) Soybean Protein

Amino acids	Concentration (mg/g)
Aspartic	31.91±0.65
Threonine	3.51±0.71
Serine	5.64±0.09
Glutamic	37.62±0.22
Proline	0.24±0.82
Glycine	27.26±0.50
Alanine	16.86±0.70
Valine	6.66±0.63
Methionine	2.96±0.04
Isoleucine	1.60±0.09
Leucine	7.72±0.83
Tyrosine	4.14±0.10
Phenylalanine	4.68±0.09
Histidine	12.08±0.05
Lysine	9.08±0.91
Arginine	7.32±0.72
Total	179.28±7.90

Volatile Compounds in the Encapsulated MPF Sample

A total of 49 volatile compounds were identified in the headspace volatiles of the investigated samples; comprising over 97% of the total volatiles. These compounds were those which gave significant peaks in the gas chromatograms together with minor compounds which had been reported in previous studies among the potent odorants of beef-like process flavour. The sulfur containing compounds were the dominant chemical class (27), followed by ketones (4), Strecker aldehydes (4), pyrazines (4), thiazoles(5) and furans(5). 2-Methyl-3-furanthiol was the major identified component in the fresh sample (47.78%) followed by 2-methyl thiophene (12.40%) and 2,3-butanedione (11.10%). As shown in Figure **1**, storage of the MPF sample revealed noticeable qualitative and quantitative variations among the volatile compounds. The thiol containing compounds showed gradual decrease during storage whereas, the pyrazins and thiazoles showed an opposite trend.

Aroma Profile Analysis

Mean value of the intensity of each selected attribute was followed during storage for 6 months, as shown in Figure **2.** The roasty note showed significant ($p<0.05$) increase being 1.8 fold higher than its value in fresh sample. The savoury not showed the same behavior whereas, the chickeny, beefy and grilled meat notes showed an opposite trend.

DISCUSSION

Free Amino Acids Composition

The amino acids composition of the acid hydrolyzed soybean protein in the present study is in agreement with that found by solina *et al.*[19] who reported that the major amino acids present in the acid hydrolyzate of soybean protein were glutamic, aspartic and alanine. The free amino acids may play important roles in process flavourings. Methionine was present in low concentration (Table **1**). However, it may have an important influence on Maillard type flavours since its breakdown via Strecker degradation yields numerous sulfur-containing compounds, such as dimethyl disulfide, dimethyl trisulfide and methanthiol [20]. Addition of glutamic acid to a model system contains cysteine and xylose enhances the generation of 2-methyl-3-furanthiol, the potent odorant of meat aroma [21]. Acetaldehyde is the main Strecker aldehyde of alanine [22]; however, it may undergo further condensation reaction to produce important intermediate products that can act as precursors of other volatiles. 2-Methyl propanal, 2-methylbutanal, 3-methylbutanal are strecker aldehydes of valine, leucine and isoleucine, respectively [23, 24].

Volatile Compounds in the Encapsulated MPF Sample

The identified volatile compounds listed in Table **2** were previously identified in the volatiles of model reaction mixtures containing xylose, cysteine and thiamine as well as in cooked meat [25, 26]. 2-Methyl-3-furanthiol (**20**), the odour impact compound of beef aroma [2], was the major identified compound in the fresh encapsulated MPE. It is the key aroma compound of boiled and stewed beef [9, 27]. It was reported that, 2-methyl-3-furanthiol can be formed via thermal degradation of thiamine [28] or by Maillard reaction between ribose and cysteine [4]. Thiamine degradation was proposed as the primary formation path way of 2-methyl-3-furanthiol [29], which results from direct cyclization of 5-hydroxy-3-mercaptopentan-2-one.This compound have been recently reported as the major compound in the volatiles of an encapsulated beef-like process flavouring prepared from enzymatically hydrolyzed soybean protein and flavour precursors like those used in the present study [14]. 2-Furfurylthiol (**27**) was the second major thiol containing compound identified in the present study. This compound was described as having sulfurous coffee like aroma [30], it was the predominant compound in the coffee substitute volatiles [31]. The pathways for the formation of thiols were demonstrated in the previous studies [32], in which 2-methy-3-furanthiol (**20**) was formed from the reaction between 4-hydroxy-5-methyl-3(2H)-furanone through 1,4-dioxy-2,3-diketose from ribose with hydrogen sulfide, an intermediate which formed from cysteine by Strecker degradation. While compound **27** could be formed from the reaction between 2-furanmethanol with hydrogen sulfide [33]. Compounds **20**, **27** were shown to be important odorants in commercial meat flavourings, as well as cooked beef and chicken broth [2, 6, 10, 34].

Among the six identified thiazoles, 4, 5-dimethylthiazole (**25**) was detected in considerable concentration (1.60%). It was known to have roasted or grilled note [35]. The acyl thiazole has been reported in a model system containing cysteine and ribose [36]. According to Vernin and Parkanyi [37], 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 [38]. It has been demonstrated that thiazoles can be formed from mixtures of hydroxyketones, hydrogen sulphide, ammonia and aldehydes [39].

Eight thiophene compounds (**13, 21, 23, 36, 40, 45, 46, 48** and **49**) could be detected in the fresh encapsulated MPF sample. Among these compounds, 2-methylthiophene (**13**) was the second major compound, it comprised 12.40% of the total volatiles (Table **2**). Thiophenes have been suggested as being responsible for the mild sulphurous odour of cooked meat [35].

3-Methylbutanal (**16**), 2-methylbutanal (**17**) and methional (**30**) are the Strecker aldehydes identified in

Table 2. Volatile Compounds Identified in Headspace Volatiles of the Encapsulated Meat-like Process Flavouring (MPF) During Storage for 6 Months

Peak No	Compounds[a]	RI[b]	Period of Storage (months)				Method of Identification[c]
			Zero	1	3	6	
1	Methanthiol	<600	0.09[*]	---	---	---	MS,KI
2	Dimethylsulfide	<600	0.37	5.81	3.70	20.19	MS,KI
3	2,3-Butanedione	613	11.10	12.95	7.99	12.46	MS,KI,St
4	2-Butanone	627	---	5.70	2.03	10.29	MS,KI,St
5	2-Methylpropanal	630	---	0.09	---	---	MS,KI
6	3-Methylbutanal	649	1.19	4.38	2.75	4.25	MS,KI
7	2-Methylbutanal	672	1.04	0.08	---	---	MS,KI
8	2-Pentanone	709	0.07	0.19	0.18	1.55	MS,KI,St
9	2,3-Pentanedione	720	1.81	0.42	0.11	0.20	MS,KI
10	Pyrazine	731	---	0.14	0.05	0.14	MS,KI
11	Methyl-2-pentanone	741	---	0.89	1.73	1.18	MS,KI
12	Dimethyl disulfide	758	0.25	4.56	1.51	5.27	MS,KI
13	2-Methyl thiophene	774	12.40	7.88	0.69	5.35	MS,KI
14	4,5-Dihydro-2-methyl-3(2H) furanone	801	0.15	0.11	0.16	0.11	MS,KI
15	3-Mercapto-2-butanone	812	0.42	---	---	---	MS,KI
16	2-Methyl pyrazine	820	---	---	0.05	2.22	MS,KI
17	2-Furfural	827	---	---	0.05	0.82	MS,KI,St
18	2-Methyl thiazole	832	0.18	---	---	1.95	MS,KI
19	2,4-Dimethylfuran	850	---	---	---	0.87	MS,KI
20	2-Methyl-3-furanthiol	870	47.78	45.5	26.14	7.73	MS,KI
21	2-Ethyl thiophene	875	---	----	8.28	0.04	MS,KI
22	4-Hydroxy-5-methyl-3(2H) furanone	881	---	0.96	3.52	0.11	MS,KI
23	2,5-Dimethylthiophene	885	0.03	0.07	0.02	0.11	MS,KI
24	3-Mercapto-2-pentanone	899	0.02	0.03	0.07	---	MS,KI
25	4,5-Dimethyl thiazole	910	1.60	0.31	0.17	0.20	MS,KI
26	2-Mercapto-3-pentanone	914	---	0.01	0.01	---	MS,KI
27	2-Furfurylthiol	911	3.13	0.09	0.07	---	MS,KI
28	2 or 4- Methyl-2-furfural	922	0.01	---	---	---	MS,KI
29	Dimethyl sulphone	927	0.01	---	---	----	MS,KI
30	Methional	932	0.11	0.18	0.11	0.45	MS,KI
31	2,3-Dimethyl pyrazine	940	---	0.06	---	---	MS,KI,St
32	5-Methyl-2-furfural	944	0.01	---	---	---	MS,KI,St
33	1(2-furyl)-2-propanone	953	0.10	0.04		---	MS,KI
34	Dimethyl trisulfide	968	0.88	0.07	0.04	0.11	MS,KI
35	2-Methyl-4,5-dihydro-3-furanthiol	975	0.01	0.06	0.04	---	MS,KI
36	2or3-Thiophenethiol	978	---	0.09	2.32	0.03	MS,KI
37	4,5-Dihydro-2-methyl-3(2H)thiophene	990	0.05	0.09	0.51	---	MS,KI
38	3,4,5-Trimethyl thiazole	996	---	0.09	0.07	---	MS,KI
39	4-Ethyl-5-methylthiazole	1000	9.12	3.99	8.98	13.15	MS,KI

Table 2 Continued….

Peak No	Compounds[a]	RI[b]	Period of Storage (Months)				Method of Identification[c]
			Zero	1	3	6	
40	Formyl thiophene	1012	1.13	0.40	16.41	4.78	MS,KI
41	2-Acetyl thiazole	1020	4.16	2.86	6.78	2.78	MS,KI
42	2-Methyl-3-thiophenethiol	1056	---	0.08	4.36	---	MS,KI
43	5-Ethyl-2,4-dimethylthiazole	1072	0.68	0.15	---	---	MS,KI
44	2,5-Dimethyl -2-ethylpyrazine	1080	0.02	0.12	---	---	MS,KI
45	2-Acetyl thiophene	1096	0.10	0.27	---	---	MS,KI
46	2-Formyl-5-methylthiophene	1118	0.43	0.02	---	0.11	MS,KI
47	2-Methyl-3-(methyldithio)furan	1170	0.05	0.21	1.08	1.48	MS,KI
48	2-Propionyl thiophene	1175	0.18	---	---	---	MS,KI
49	3-Ethyl-2-formyl thiophene	1181	0.26	0.02	---	---	MS,KI
Total			98.85	98.97	98.98	97.73	

* Value expressed as relative area percentages to total identified compounds. [a]Compounds listed according to their elution on DB5 column. [b]Retention index. [c]Compound identified by GC-MS(MS) and / or by Kovats index on DB5 (KI) and/ or by comparison of MS and KI of standard compounds run under similar GC-MS conditions.

the fresh volatiles of the encapsulated MPF with total concentration (2.34%). The low yield of these compounds may be due to the fact that, the Strecker aldehydes could undergo further reaction to give nonvolatile products including coloured melanoidins [15]. The two identified diketones, 2, 3-butanedione (**3**) and 2,3-pentanedione (**9**)(Table **2**) are Strecker degradation products [6], having a buttery aroma. They are important intermediates in the formation of other volatiles and can react with hydrogen sulfide leading to the formation of mercaptoketones which contribute to the meat-like volatiles [36].

Storage of MPF sample for 6 months revealed remarkable variations in the composition of its volatiles. As shown in Table **2**, 2-butanone (**4**), which was absent in headspace volatiles of the fresh sample, showed noticeable increase after storage for one month followed by a gradual increase during the rest of storage period. This component is the major volatile ketone formed in the acid hydrolyzed soybean protein [39] and may be derived from lipid oxidation or thermal decomposition of glucose [40].

Figure **1** illustrates the effect of storage on the main chemical classes that influence on odour quality of

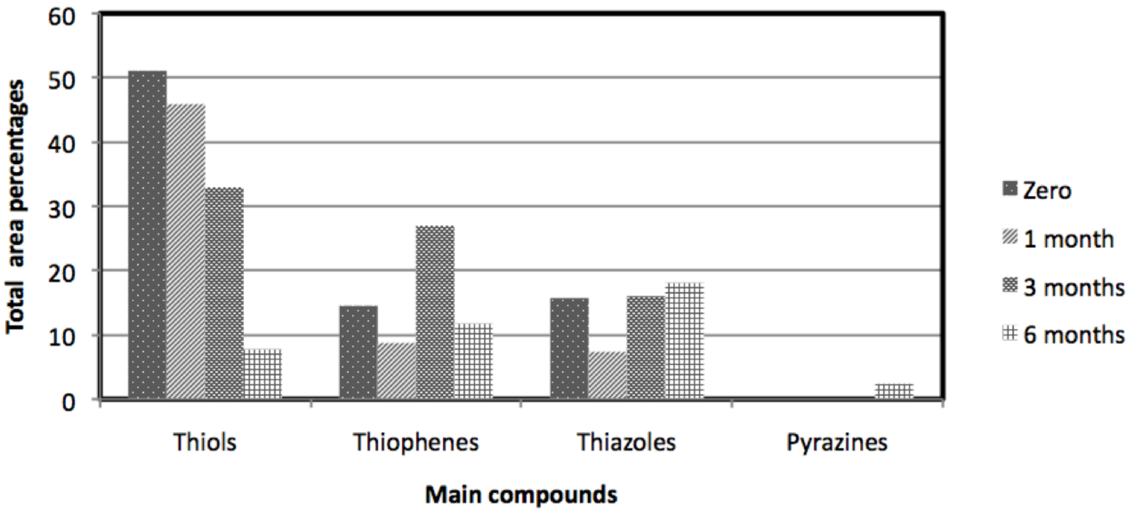

Figure 1: The total yields of the main chemical classes in volatiles of the encapsulated meat-like process flavouring during storage for 6 months.

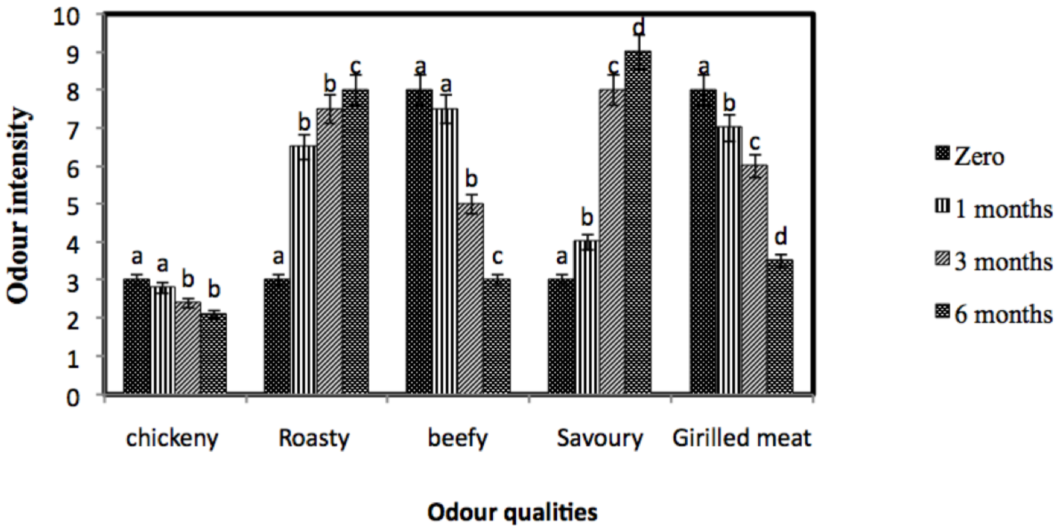

Figure 2: Aroma profile of the encapsulated meat-like process flavouring during storage for 6 months.

MPF. The noticeable decrease in total yield of the thiol containing compounds (**1, 20, 27, 25, 36** and **42**) may be attributed to their oxidation to the corresponding disulfides [41], the interaction with the sulfhydryl group from cysteine [42] and/or the formation of hydrogen bonds with the hydroxyl groups of Arabic gum polysaccharide [43] which used in the present study as encapsulate material for the meat-like process flavouring. On contrary, the total yields of pyrazines (**10, 16, 31** and **44**) and thiazoles (**18, 25, 38, 39, 41** and **43**) which are contributors to roasty flavor [44] showed remarkable increase after storage for 6 months. The total content of thiophene compounds (**13, 21, 23, 37, 40, 45, 46, 48** and **49**) showed no linear behavior during storage (Figure **1**).

Aroma Profile of the Encapsulated MPF

The aforementioned results revealed noticeable variations in composition of the encapsulated MPF volatiles during storage. To confirm these results, odour profile analysis was conducted to explore the effect of storage on the main sensory attributes of this sample (Figure **2**). The intensities of the selected sensory attributes were followed during storage for 6 months. The significant ($P<0.05$) decrease in the chickeny and beefy notes is mainly correlated to the decrease in the thiol containing compounds. The significant ($P< 0.05$) increase in the sensory scores of roasty note is consistent with the increase in pyrazines and thiazoles (Figure **1**), which are the most contributors to roasty note in meat volatiles [35]. While the significant increase in the savoury note may be correlated to the increase in the disulfides [3].

CONCLUSION

The acid hydrolyzed soybean protein can be used, as a base ingredient, with other appropriate flavour precursors for the production of thermal process flavour which have meat-like aroma. Encapsulation of this flavour in gum Arabic produces flavouring having higher odour quality in addition, it is convenient to be used in many food industries. As far as the authors are aware, this is the first study concerned with evaluation of the effect of storage on the meat-like flavours, particularly the encapsulated form. This study may provide some useful information to the food and flavour companies who produce commercial process flavourings.

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