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EVALUATION OF AROMA QUALITY AND STABILITY OF AN ENCAPSULATED BEEF-LIKE PROCESS FLAVOURING PREPARED FROM FLAVOUR PRECURSORS AND ENZYMATICALLY HYDROLYZED SOYBEAN PROTEIN

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

[1]Department of Chemistry of Flavour and Aroma, National Research Centre, Dokki, Cairo, Egypt,
[2]Department of Biochemistry, Cairo University, Giza, Egypt.

*Corresponding Author: hodafadel_f@hotmail.com

ABSTRACT

The primary objective of the present study was to prepare beef-like process flavouring(BPF) from a thermal reaction model system of enzymatically hydrolyzed soybean protein (E-HVP)/ amino acids /thiamine / xylose. Effect of storage on the quality of BPF encapsulated in gum Arabic by freeze drying was evaluated. Alanine was the major free amino acid inE-HVP followed by leucine, lysine, valine and isoleucine. The gas chromatography- mass spectrometry (GC-MS) analysis revealed that 2-Methyl-3-furanthiol, the key compound of beef flavour, was the most abundant compound in the volatiles of the encapsulated sample followed by dimethyl disulphide. The changes in odour profile and headspace volatiles of the encapsulated beef-like process flavouring were followed during storage for 6 months. The results revealed a significant ($P< 0.05$) increase in the beefy, roasty and savoury attributes. This finding confirmed the results of the GC-MS analysis of the headspace volatiles that revealed a remarkable increase in the thiol containing compounds (the important meaty flavour compounds). The pyrazines, thiazoles and thiazolines, which contribute to roasty-beefy note, were also increased during storage.

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 generation of process flavours(Wu and Cadwallader, 2002; Song and Xia 2008; Song et al., 2010). 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, 1994).

Thermally generated imitation meat flavours are often described in the patent literatures as "process flavourings". The flavour industry has developed a range of imitation flavour or meat-like flavours, although the specific flavour profile of targeted meat item (eg. roasted meat) has proven much difficult to achieve. Knowledge of the identities of the volatile aroma components of the 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 and chicken. The volatile components of

cooked meat model systems have been studied by a number of researchers (Farmer et al.,1989;Hofman and Schieberle 1995; Elmore et al., 2002; Song and Xia, 2008; Song et al., 2010; Song et al.,2012; Song et al., 2013). Ribose and cysteine are important precursors for formation of meat aroma and are consequently employed in the production of process meat flavour (Wu and Cadwallader, 2002; Song et al., 2010). However, ribose is frequently replaced by its less expensive isomer xylose (Cerny and Davidek, 2003). Among other compounds, 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(Farmer et al., 1989 ; Hofman and Schieberile, 1995). These compounds are also found in cooked meat (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

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materials to produce meat-like savoury flavourings, including hydrolyzed vegetable proteins (Lieske and Konrad, 1994). However, during acid hydrolysis of proteins, undesirable byproducts may be formed, including 1-chloro-2, 3-propanediol and 1, 3-dichloro-2-propanol from vegetable proteins and creatinine in the hydrolysate of meat protein. Accordingly, enzymatic hydrolysis of vegetable proteins has the potential to provide a suitable means for preparation of flavourings (Ho, 1989; Wu et al., 2000; Baek et al., 2001; Wu and Cadwallader, 2002).

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 enzymatically hydrolyzed soybean protein with other flavour precursors were used to generate beef-like process flavour(BPF) which was encapsulated in gum Arabic by freeze drying to prepare a product more convenient and easy to be handled. 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 beef-like process flavouring, the changes in its aroma composition and aroma profile were followed during 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 and amino acids; cysteine and taurine, 1,2 dichlorobenzene (99%, spectrophotometric grade), authentic compounds and standard n-paraffins (C8-C22) were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Flavourzyme (from *Aspergillus oryzae* EC232-752-2) and Alcalase (from *Bacillus Licheniformis* EC232-752-2) were obtained from Novo Nordisk A/S (Bagsvaerd, Denmark). All chemicals used in this study were of analytical grade and the solvents were purified and distilled before use.

PRODUCTION OF ENZYMATIC HYDROLYSATE

` The enzymatic hydrolysate (HVP) was prepared according to Aaslyng (1998a). Defatted soybean meal (150 g) was mixed with 825 g of tap water and pasteurized at 85 °C for 5 min. After cooling the mixture to 50 °C, the pH was adjusted to pH 7.0 with 4M NaOH. Flavourzyme (0.78g) and Alcalase (0.75 g) were added, and the mixture was allowed to stand without pH adjustment at 50 °C. After 5 h the pH was adjusted to pH 5.0 with 4M HCl and 14.6 g of NaCl and 0.39 g of Flavourzyme were added. The hydrolysis continued without pH adjustment at 50 °C

for a total of 24 h. The enzymes were deactivated at 85 °C for 5 min. After cooling the mixture to 50 °C, the pH was adjusted to pH 6.5 with 4M NaOH. After centrifugation the precipitate was washed with 300 ml of tap water and centrifuged again. The combined hydrolysate in water was filtered, freeze-dried (Snijders Scientific b.v. Model L45 Fm-Ro, Tilburg-Holand) and stored immediately in closed glass bottles at -10 °C until further analysis.

FREE AMINO ACIDS COMPOSITION OF E-HVP

Analysis of free amino acids of E-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 bars; and reaction temperature was 130°C.

PREPARATION OF ENCAPSULATED BEEF-LIKE PROCESS FLAVOURING

A model mixture of the freeze dried enzymatically 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 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 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 (Fadel and Farouk, 2002). The evaluation was conducted by a well-trained panel consisting of 10 members (6-female, 4-male) drawn from Nutrition and Food Technology Division,

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National Research Centre, Cairo, Egypt. All panelists had received a training in descriptive sensory analysis (>20h) and possessed experience with sensory evaluation. Prior to the quantitative descriptive sensory analysis, the panelists had thoroughly discussed 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 four descriptors including beefy, roasty, savoury and chickney 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. 10 µl of dichlorobenzene, internal standard (0.50 µ g /µl in methanol), were added to each sample. The mixture solution at 60ºC was stirred using Teflon-coated magnetic bar for 3 min. The volatiles were purged with purified nitrogen (grade of N_2 >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 (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 (Adams, 1995, Baek et al., 2001, Wu and Cadwallader, 2002, Song and Xia, 2008). The calculated

amount of the volatile compounds was approximated by comparison of their peak areas with that of 1, 2-dichlororobenzene (internal standard), obtained from the total ion chromatograms using a response factor of 1.

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

Analysis of the free amino acids in the enzyme hydrolyzed soybean protein (E-HVP) revealed the presence of 16 amino acids with total concentration of 192.7±7.9 mg/g (Table 1). Alanine was the most abundant free amino acid in E-HVP (22.0 ± 0.7 mg/g) followed by leucine(20.4±0.8 mg/g). Methoinine was present in less concentration (5.0± 0.0 mg/g).

Table1.Composition of free amino acids of the enzyme hydrolyzed soybean protein (E-HVP)

No	Amino acid	Concentration(mg/g)
1	Aspartic	13.3±0.7
2	Threonine	12.5±0.7
3	Serine	6.9±0.1
4	Glutamic	9.7±0.2
5	Proline	13.0±0.8
6	Glycine	12.5±0.5
7	Alanine	22.0±0.7
8	Valine	17.9±0.6
9	Methionine	5.0±0.0
10	Isoleucine	10.4±0.1
11	Leucine	20.4±0.8
12	Tyrosine	5.2±0.1
13	Phenylalanine	9.0±0.1
14	Histidine	3.9±0.1
15	Lysine	13.5±0.9
16	Arginine	17.5±0.7
	Total	192.7±7.9

A total of 42 compounds were identified in the headspace volatiles of the investigated samples; comprising over 95% of the total volatiles (Table 2). 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 identified compounds were related to different chemical classes such as sulfur containing compounds (6),Strecker aldehydes (4), ketones (3),alcohols (5),furans(5), pyrazines(8)and pyrrole(1). As shown in Table 2 the thiol containing compounds were increased during storage for 6 months whereas the pyrazines and thiazoles showed an opposite

trend. The effect of storage for 6 months on the odour profile of the encapsulated BPF is shown in Fig. 1. The

beefy, roasty and savoury notes were increased decrease during storage.

Table 2.Volatile compounds identified in headspace of the encapsulated beef-likeprocess flavouring during storage for 6 months

Volatile Compounds[a]	RI[b]	Period of storage(months)				Methods of identification
		Control (zero)	1	3	6	
Methanthiol	<600	20.9±1.5	27.1±1.9	22.8±1.6	18.6±1.3	MS,KI
2-Methylpropanal	<600	10.8±0.8	12.5±0.9	5.1±0.4	0.5±0.0	MS,KI
2,3-Butanedione	612	71.4±5.0	37.4±2.6	38.9±2.7	73.8±5.1	MS,KI,St
2-Butanone	623	1.1±0.1	1.6 ±0.1	3.4±0.2	---	MS,KI,St
2-Methyl-1-propanol	640	10.2±0.7	37.0±3.1	57.4±4.0	89.4±6.2	MS,KI
3-Methylbutanal	647	5.4±0.4	3.2±0.2	0.8±0.1	---	MS,KI
2-Methylbutanal	671	3.2±0.2	26.5±1.9	53.3±3.7	7.2±0.5	MS,KI
2-Pentanone	699	1.6±0.1	4.6±0.3	9.6±0.7	7.2±0.5	MS,KI,St
2,3-Pentanedione	721	4.2±0.3	3.24±0.2	3.1±0.2	3.6±0.3	MS,KI
Pyrazine	730	0.7±0.1	1.0±0.1	0.6±0.0	3.0±0.2	MS,KI,St
2-Methyl-3-buten-1-ol	739	1.8±0.1	16.9 ±1.2	13.7±1.0	1.3±0.1	MS,KI
3-Methyl-1-butanol	751	1.3±0.1	2.6±0.2	5.5±0.4	1.2±0.1	MS,KI
2-Methyl-1-butanol	755	10.8±0.8	10.3±0.7	31.7±2.2	8.4±0.6	MS,KI
Dimethyldisulfide	768	174.5±12.1	23.5±1.6	54.4±3.8	8.4±0.6	MS,KI
2-Methyl pyrazine	825	2.0±0.1	4.2±0.3	0.9±0.1	---	MS,KI,St
2-Furfural	847	3.2±0.2	4.2±0.3	0. 9±0.1	---	MS,KI,St
2-Methyl-3-furanthiol	869	201.1±13.9	250.5±19.0	240.6±13.5	270.3±18.1	MS,KI
2-Ethyl-6-methylpyrazine	873	1.7±0.1	0.8±0.1	0.8±0.1	1.3±0.1	MS,KI
4-Hydroxy-5-methyl-3(2H)furanone	883	1.86±0.1	---	---	---	MS,KI
3-Mercapto-2-pentanone	897	1.9±0.1	1.2±0.1	---	---	MS,KI
2-Furfurylthiol	911	2.8±0.2	1.0±0.1	1.4±0.1	---	MS,KI,St
2-Acetyl-1-pyrroline	928	2.9±0.2	2.0±0.1	1.8±0.1	2.5±0.2	MS,KI,St
2-Ethylpyrazine	933	1.1±0.1	0.5±0.0	0.1±00	2.9±0.2	MS,KI,St
2-Methyl-3-(methylthio)furan	938	10.8±0.8	4.1±0.3	3.7±0.3	2.3±0.2	MS,KI
Dimethyltrisulfide	964	0.1±0.0	0.1±0.0	0.2±0.0	0.7±0.1	MS,KI
2-Methyl-2-acetylfuran	982	0.2±0.0	0.2±0.0	0.9±0.1	2.0±0.1	MS,KI
2-Methyltetrahydrothiophene-3-one	990	1.6±0.1	2.4±0.1	2.4±0.1	1.3±0.1	MS,KI
3,4,5-Trimethylpyrazine	1004	10.6±0.7	43.2±0.9	37.2±2.6	41.2±2.7	MS,KI,St
Formylthiophene	1012	2.9±0.2	0.1±0.0	0.5±0.0	1.5±0.1	MS,KI
2-Acetyl thiazole	1020	3.6±0.3	10.0±0.7	19.2±1.3	20.9±1.5	MS,KI
2-Ethyl-1-hexanol	1037	16.8±1.2	31.2±2.2	18.1±1.3	28.7±2.0	MS,KI
Phenylacetaldehyde	1049	1.3±0.1	1.7±0.1	3.7±0.3	---	MS,KI
2-Methyl-3-thiophenethiol	1067	0.7±0.1	0.7±0.1	4.7±0.3	---	MS,KI
2-Ethyl-3,5-dimethylpyrazine	1074	4.2±0.1	2.4±0.2	3.7±0.3	2.5±0.2	MS,KI,St
2,5-Dimethyl-3-ethylpyrazine	1094	0.5±0.0	0.8±0.1	0.3±0.0	2.1±0.2	MS,KI,St
2-Thenylmercaptan	1107	1.1±0.1	0.7±0.1	0.8±0.1	---	MS,KI
2-Acetyl-2-thiazoline	1120	0.9±0.1	1.3±0.1	1.68±0.1	5.2±0.4	MS,KI
3-Methyl-2-formylthiophene	1148	1.7±0.4	---	---	---	MS,KI
3,5-Diethyl-2-methylpyrazine	1153	0.8±0.1	---	---	---	MS,KI,St
2-Methyl-3-(methyldithio)furan	1168	4.3±0.2	0.7±0.1	1.0±0.1	---	MS,KI
2-Propionyl-2-thiazoline	1197	0.2±0.0	0.1±0.0	0.1±0.0	---	MS,KI
Dimethyltetrasulfide	1220	1.7±0.1	0.6±0.1	0.9±0.1	---	MS,KI
Main chemical classes affected the odour profile of BPF						
Thiols		225.5±17.1	279.3±21.2	269.5±20.0	288.9±22.0	

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Thiophenes		6.2±0.5	2.5 ±0.2	2.9±0.2	2.8±0.2	
Pyrazines		21.6±1.6	52.9±4.0	43.6±3.3	53.0±4.0	
Thiazole and thiazolines		4.7±0.4	11.4±0.9	21.0±1.6	26.1±2.0	

Values are approximate concentration of volatiles (µg/10g BPF) in the encapsulated meat process flavouring (BPF), reported as mean values of triplicate analysis with standard deviation.

[a] Compound listed according to their elution on DB5 column.   [b]Retention indices.

[c] Compound identified by GC-MS(MS) and /or by Kovat index on DB5(KI) and /or by comparison of MS and KI of standard compounds run under similar GC-MS conditions.
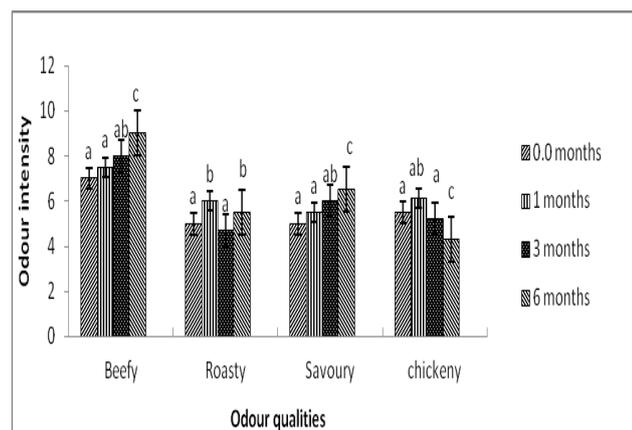
--Not detected

Figure1. Effect of storage on the aroma profile of the encapsulated meat process flavouring

Different superscript letters (a, b, c) indicate significant differences (P< 0.05) between stored samples for each odour sensory quality

DISCUSSION

FREE AMINO ACIDS COMPOSITION

The free amino acids may play important roles in process flavourings. In the present study the free amino acids composition of enzyme hydrolyzed soybean protein (E-HVP) is in agreement with the previous studies carried out by Solina et al. (2007); Wu and Cadwallader(2002). As shown in Table 1, alanine was the major free amino acid (22.0±0.7). Acetaldehyde is the main strecker aldehyde of alanine (Shu, 1998). However, this aldehyde may undergo further condensation reaction to produce important intermediate products that can act as precursors of other volatiles. Among the free amino acids present in Table 1, lysine (13.5±0.9mg/g)can undergo thermal reaction leading to formation of alkylpyrazines and 2-acetyl-1-pyrroline (Griffith and Hammond, 1989). 3-Methylbutanal and phenylacetaldehyde are Strecker degradation of leucine and phenylalanine, respectively (Coleman et al., 1994; Hofmann et al., 2000).   Methionine was present in low concentration in the enzyme hydrolyzed soybean proteins (Table 1). However, methionine 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(Griffith and Hammond,1989).

VOLATILE COMPOUNDS IN ENCAPSULATED MEAT PROCESS FLAVOURING

The aroma of a process flavouring is composed of a highly complex mixture of volatile compounds, most being formed via sugar - amino acids (or peptide) interaction or by the thermal degradation of specific components (Manley, 1994; Song et al., 2012;Song et al., 2013). The identified volatile compounds listed in Table 2 were previously detected in the model reaction mixtures containing xylose, cysteine and thiamine as well as in cooked meat (Elmore et al., 1994; Cerny, 2007).

The major compound in the fresh BPF sample was 2-Methy-3-furanthiol (201.1±13.9 µg/10g) (Table 2).This compound has been identified as the odour impact compound of beef aroma (Gasser and Grosch 1988; Song et al., 2010) and other related model systems for preparing meat-like flavours (Madruga and Mottram, 1995; Moon et al., 2006;Xu et al., 2008). It was reported that, 2-methyl-3-furanthiol can be formed via thermal degradation of thiamine (Grosch and Zeiler-Hilgar, 1992) or by Maillard reaction between ribose and cysteine (Hofman and Schieberle, 1995). Bolton et al. (1994) have proposed that thiamine degradation was the primary formation path way of 2-methyl-3-furanthiol, which results from direct cyclization of 5-hydroxy-3-mercaptopentan-2-one. Dimethyl disulfide, the Strecker degradation or thermal degradation product of methionine or cysteine (Vernin and Parkanyi, 1982), was the second major compound (174.5±12.1 µg/10g) in fresh encapsulated BPF sample (Table 2). This compound was previously found among the major identified compounds in meat-like flavour (Guo et al., 2010). Also it was identified in the aroma of E-HVP (Aaslyng et al., 1998b), its content may be increased during generation of the beef-like process flavour in the present study. 3-Mercapto-2-pentanone and 2-Furfurylthiol were identified at much less concentration (1.9±0.1 and 2.8±0.2 µg/10g, respectively). The first compound was thought to give undesirable aroma note (spicy, catty) to the beef-like process flavour generated from extruded enzyme-hydrolyzed soybean protein with flavour precursors(Baek et al., 2001) like those used in the present study.

Hofmann and Schieberle(1998) reported that, degradation of pentoses may results 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 or furan-2-aldehyde as the major products. The reaction of furan-2-aldehyde with hydrogen sulfide, which can be liberated from cysteine, was shown to generate furfurylthiol very effectively (Münch et al., 1997). This compound was described as having sulfurous coffee like aroma (Wu and Cradwallader, 2002), it was the predominant compound in the coffee substitute volatiles (Fadel et al., 2008). 2-Methyl-3-furanthiol and 2-furfurylthiol were shown to be important odorants in commercial meat flavourings, as well as cooked beef and chicken broth (Gasser and Grosch 1990a, b; Ruther and Baltes, 1994; Song et al., 2010, 2012). 2-Furfurythiol was the dominate compound followed by 2-methyl-3-furanthiol and 3-mercapto-2-pentanone in the aroma of meat like process flavour from soybean based enzyme hydrolyzed vegetable protein with cysteine and ribose (Wu et al.,2002). Among the identified sulfur containing compounds (Table 2), 2-methyl-3-(methylthio)furan and 2-methyl-3-(methyldithio) furan were detected in low concentrations (10.8 ± 0.8 and 4.3 ± 0.2 µg/10g, respectively). These compounds had been previously reported among the active compounds in the volatiles of the extruded enzyme hydrolyzed soybean protein (Baek et al., 2001) and were proposed to be formed via Maillard reaction of ribose and cysteine, involving the reaction of 2-methyl-3-furanthiol with methanthiol (Mottram and Whitfield, 1995).

Eight pyrazines were detected in the fresh BPF sample (Table 2) with total concentration (21.6 ± 1.6µg/10g). In addition to the sulfur containing compounds, thermally generated pyrazines were thought to impart a beef like flavour to extruded E-HVP with added precursors (Baek et al., 2001). 2-Acetyl -1-pyrroline, that possesses a popcorn-like note, was the only identified pyrrole in the present study. It was demonstrated that 2-acetyl-1-pyrroline can be generated from the thermal reaction of lysine or proline with certain carbonyls (Griffith and Hammond, 1989). These two amino acids comprised 13.5 ± 0.9 and 13.0 ± 0.8 mg/g, respectively in the enzyme hydrolyzed soybean protein (Table 1).

Methylpropanal, 2/3-methybutanal and phenylacetaldehyde are Strecker degradation products of valine, leucine, isoleucine and phenylalanine, respectively (Coleman et al., 1994; Hofmann et al., 2000). The total amount of these volatile compounds in the fresh sample was 20.7 µg/10g. The three first mentioned compounds possessing chocolate-like odour note whereas, the later one has been described as floral honey note (Wu and Cadwallader, 2002). The low content of the Strecker aldehydes may be due to the fact that they could undergo further reaction to give nonvolatile products including coloured melanoidins (Aaslyng et al., 1998b).

The short chain ketones reported in Table 2 are Maillard reaction and Strecker degradation products (Madruga and Mottran, 1995). The diketones 2, 3-butanedione and 2, 3-pentanedione are important intermediates in the formation of other volatiles. These two compounds can react with hydrogen sulfide leading to the formation of mercaptoketones such as 3-mercapto-2-pentanone (Table 2) that had been reported in the aroma of boiled meat and chicken broth (Gasser and Grosch, 1990a; Zhang and Ho, 1991).

To illustrate the effect of storage on the volatiles of BPF sample, the total contents of the main chemical classes that influence on the odour quality were calculated and cited in Table 2. The thiol containing compounds were increased by more than 28% compared with fresh sample. This increase was mainly attributed to the increase in 2-methyl-3-furanthiol from 201.1 ± 13.9 to 270.3 ± 18.1 µg/10g after storage for 6 months. The pyrazines showed remarkable increase being more than 2 fold higher than their content in the fresh sample. The thiazole and thiazolines showed the same behaviors being more than 6 fold higher than their total content in fresh sample after storage for 6 months. 2-Acetyl-2-thiazoline with its roasty, popcorn-like odour has been indicated as a potent odorant in several processed meat products (Cerny and Grosch, 1992; Guth and Grosch, 1994). Thiazoles are thermal degradation products of cysteine or thiamine either alone or in the presence of reducing sugar (Vernin and Parkanyi, 1982). On contrary, the thiophenes were decreased from 6.2 ± 0.5 to 2.8 ± 0.2 after storage for 6 months compared with the fresh sample. 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 involve the reaction of furfural and furanones with hydrogen sulfide or condensation of mercapto acetaldehyde with α and β unsaturated aldehydes (Vernin and Parkanyi, 1982).The variations in behavior between the chemical classes of the volatiles in the encapsulated BPF during storage may be correlated to the fact that; release of the volatiles from encapsulated samples is affected by independent variables. Several mechanisms can take place such as component/component interaction and component/capsule material interaction. Furthermore molecular weight and volatility of the compounds can affect their migration from the wall material of the capsule.

ODOUR PROFILE ANALYSIS

According to the results of GC analysis the total yield of the beefy note related compounds showed remarkable increase during storage (Table 2). To confirm these results, odour profile analysis was conducted to explore the effect of storage on the main sensory attributes of the encapsulated BPF sample. The aroma sensory attributes beefy, roasty, savoury and chickeny were selected and scored by panelists. The intensities of these attributes were followed during storage for 6 months. As shown in Fig.1, storage for 6 months resulted in a

significant ($P<0.05$) increase in the sensory score of beefy note. This finding is mainly attributed to the increase in 2-methyl-3-furanthiol, the most potent odorant of beef aroma. The considerable increase in roasty note is consistent with the increase in pyrazines and thiazoles (Table 2), which are the main contributors to roasty note (Guo et al., 2010). The savoury note showed gradual ($P< 0.05$) increase during storage. The significant ($P< 0.05$) decrease in chickeny attribute during storage for 6 months confirms the results of GC analysis (Table 2) that showed considerable decrease of some volatile compounds which may probably contribute to this attribute.

CONCLUSION

The encapsulated beef-like process flavouring prepared in the present study showed high odour quality in addition it is convenient to be used in many food industries. Storage for 6 months gave rise to a significant increase in the intensities of the beefy, roasty and savoury notes. This finding was confirmed by GC-MS analysis of the volatile compounds in the encapsulated samples that revealed a significant ($P<0.05$) increase in the most potent odorants of beef aroma, particularly 2-methyl-3-furanthiol.As far as the authors are aware, this is the first study concerned with evaluation of the effect of storage on the meat- like process 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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EVALUATION OF AROMA QUALITY AND STABILITY OF AN ENCAPSULATED BEEF-LIKE PROCESS FLAVOURING PREPARED FROM FLAVOUR PRECURSORS AND ENZYMATICALLY HYDROLYZED SOYBEAN PROTEIN

Hoda H.M.Fadel, A.Abdel Samad, MI.Kobeasy; Magda A.Abdel Mageed and Shereen N.Lotfy

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