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Enhancing effect of enzymatically hydrolyzed soybean protein isolate on acceptability and aroma compounds in headspace of real beef soup sample.

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Abstract: Evaluation of the enhancing effect of enzymatically hydrolyzed soybean protein isolate HSPI, as a potential source of glutamate, on the acceptability and headspace volatiles of real beef soup sample was carried out in comparison with monosodium glutamate MSG, the most widely used enhancer in meat products. Real beef soup samples were supplemented separately with MSG, HSPI and combination of them MSG+HSPI. The headspace volatiles and flavour palatability of each soup sample, S-MSG, S-HSPI and S-MSG+HSPI were compared with those of control sample SC (unsupplemented with enhancer). Among the 68 identified compounds only 34 were found in the aroma of SC sample. Sulfur containing compounds were quantitatively the predominant compounds. The results revealed that all investigated enhancers favoured the release of the thiol containing compounds, the most important volatile compounds in meat aroma. Disulfides and diketones showed the same behavior whereas lipid degradation products showed opposite trend. The results of sensory evaluation confirmed those of gas chromatography-mass spectrometry analysis of the aroma compounds in headspace of all beef soup samples. No significant differences ($p>0.05$) was found among the three samples S-MSG, S-HSPI and S-MSG+HSPI, however, the slight increase in degree of liking of the later sample may be attributed to its highest content of the most potent odourants of beef aroma.
Keywords: meat flavour; glutamate; soybean protein isolate monosodium glutamate MSG; sensory evaluation; enhancers

Introduction

Glutamate is the most important contributor to food flavour, it is the most studied flavour enhancer and umami substance [1]. Monosodium glutamate (MSG), sodium salt of the nonessential amino acid glutamic acid, provides umami taste and is characterized as flavour enhancer [2]. It is common ingredient in meat products due to its contribution to flavour [3]. The taste of pure MSG is described as broth-like, salty like and meaty. Addition of MSG to foods at low concentrations increases the palatability and pleasantness of the foods [4], this effect depends on food composition and on the characteristics of odour and flavour notes [5, 6].

Numerous studies have been carried out on umami taste and glutamate and their relation to food palatability and flavour acceptance [5, 7, 8,9,10]. The umami taste substances are contained abundantly in various foods, including vegetables such as soybean, seafood, meat and cheese [11]. Glutamate liberates as a by-product

during preparation of the hydrolyzed vegetable protein (HVP), which are widely used as seasonings and flavouring agents in canned, dry mixes, sauces and other manufactured products [4].

Although glutamate is naturally occurring in many foods, it is frequently added as flavour enhancers [12]. Added glutamate to foods provides a flavouring function similar to naturally occurring free glutamate [13], increases their umami taste quality, and increases their acceptability and their consumption [14]. Therefore, it is used to enhance the natural flavours of meats, poultry, seafood, snacks, soups and stews [15]. The optimum amount of added glutamate to enhance the taste of food is 0.1-0.8% by weight [16] which is the same as that occurring in general foods.

Meat flavour is one of the most important factors affecting the preferences of meat consumers [17]. A great number of volatile compounds have been reported in cooked meat products [18]. However, only limited compounds are considered as potent odourants of meat aroma, depending on their odour threshold and concentration in the food [19]. Perception of aroma not only depends on nature and concentration of volatile compounds but also on their availability that can be modulated by physicochemical interaction with other food components [20]. So, changes in the composition or constituents of the food product affect the volatility of aroma compounds.

Most of the reported studies, dealing with the effect of umami compounds on food flavour, had been concerned with their effect on palatability and pleasantness of simple food model systems [6]. Ventanas et al. [21] designed simple model systems including meat broth and two meat odour active compounds (1-octen-3-ol and 2, 6 dimethyl pyrazine) and evaluated the effect of added umami compounds (MSG and ribonucleotides) on the perceived odour and flavour also their effect on the perception of the odour active compounds. The effect of raising the added amount of umami compounds on the palatability of various food products had been evaluated [5]. Recently, Nishimura et al.[10] examined the influence of umami compounds including MSG on the retronasal aroma sensation from model chicken soups however they could not clarify the mechanism of enhancing aroma.

The aforementioned studies used pure chemical umami compounds however; meat products are complex systems including non-volatile compounds such as protein, fat and carbohydrates in addition to endogenous umami compounds. During processing several interactions can take place between these compounds and the endogenous volatile compounds, which are generated during processing and affect their perception in beef products. Therefore, the results of simple beef model systems do not give accurate indication for the release and perception of the volatile compounds from a real beef product [22].

Evaluation of the enhancing effect of umami compounds, such as MSG and 5-nucleotides on palatability of foods is usually carried out by using soups [23] therefore; the main objective of the present study was to evaluate the enhancing effect of HSBI as a potential source of glutamate in comparison with pure MSG on aroma compounds and flavour acceptability of real beef soup. Synergy effect between MSG and HSPI on the released volatiles and acceptability of beef soup was also evaluated.

Materials and Methods

Materials

Beef meat was purchased from local market on the day of slaughter. SPI (5.5% moisture, 90.5% protein, 0.5% fat, 4% ash and 0.5% crude fiber) was purchased from Miro for Export & Import Co. (Giza, Egypt). The glutamic acid comprised the highest content 20.40 g/100g of SPI. Monosodium glutamate (MSG) was purchased from (s.d fine chem. Ind). Authentic volatile compounds and standard n-paraffin ($C_6 - C_{22}$) were purchased from Sigma-Aldrich Co.s (st. Louis Mo, USA). All other chemicals were at least of analytical grade.

Preparation of enzymatic SPI hydrolyzate

The enzymatic hydrolysate was prepared according to Aaslyng et al. [24]. SPI (150 g) was mixed with 825 g of tap water and pasteurized at 85 °C for 5 min. After the mixture cooled 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 the mixture cooled 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, then 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.

Preparation of beef soup

Beef (1 kg of minced beef with 1% fat content), was used for preparing the soup. Sodium chloride (10 g) and Onion (50 g) were added, with no other addition to avoid possible uncontrolled variability which might affect the palatability of the samples. The soup was cooked (40 min. in 3L boiling water), cooled down and filtered twice in order to remove meat solids and stored at 4°C (overnight) until preparation of the samples supplemented with enhancers. The soup was let to reach room temperature (30 °C) then divided into four equal samples, the first considered as control (SC, unsupplemented with enhancer) sample. The investigated umami compounds MSG, HSPI and mixture of MSG + HSPI were added separately to the other three beef soup samples as shown in Table 1, then stirred with magnetic stir. All samples were subjected to sensory analysis as well as isolation and gas chromatography – mass spectrometry (GC – MS) analysis of the headspace volatiles.

Table 1. Composition of the studied samples

	Beef soup (g)	MSG (g)	HSBI (g)
S-SC	100	----	----
S-MSG	100	0.5	----
S-HSPI	100	----	0.5
S-MSG+HSPI	100	0.25	0.25

Sample cods: SC, unsupplemented beef soup, S-MSG, beef soup supplemented with pure chemical monosodium glutamate, S-HSPI, beef soup supplemented with enzymatically hydrolyzed soybean protein isolate HSPI, S-MSG+HSPI, beef soup supplemented with MSG + HSPI.

Isolation of meat soup volatiles

Each sample (100 ml) under investigation was placed in a conical flask and stirred at 60°C by using Teflon-coated magnetic bar. The volatiles were isolated according to Fadel et al [25]. The collected volatiles were recovered with diethyl ether - pentane (1:1, v/v). The solvents containing volatiles were dried over anhydrous sodium sulphate for 1 h and concentrated with a Vigreux column (25 cm) under 40°C to final volume of 100 µl. 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 (60 m × 0.32 mm i.d. × 0.25 µm film thickness). The oven temperature was maintained initially at 50 °C for 5 min, and 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 alkanes (C6–C20) as external standard 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 [26, 27, 28]. The quantitative determination was carried out based on peak area integration.

Sensory analysis

The investigated soup samples (supplemented with enhancers) as well as the control sample (unsupplemented with enhancers) were warmed up to 70 °C before evaluation. Each sample (20 ml) were put in coded warmed-up glass bakers, covered by Petri dishes and kept warm until tested [5]. The method of hedonic ranking was applied to assess the relative degree of liking against control sample. The evaluation was conducted by 20 members (12- female, 8- mail) drawn from Food Technology and Nutrition Division, National Research Center, Cairo, Egypt. They were participated occasionally in hedonic tests. The panelists were asked to rank the four samples according the degree of liking on bar diagrams expressed in the range of 1 (least like) - 9 (most like). The sensory evaluations were carried out according to ISO [29]. Each sample was prepared in triplicate.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) and least significant difference (LSD) was performed to determine any significant difference among various treatments that were used to compare the means. Differences were considered to be significant at $p < 0.05$.

Results and Discussion

Volatile compounds

Maillard reaction and lipid degradation are the main routes for the generation of the volatiles of meat products[30] the main volatile compounds identified in the headspace volatiles of beef soup samples (S-MSG, S-HSPI and S-MSG+HSPI) and those identified in the headspace of the control sample SC (unsupplemented with umami compounds) are presented in Table 2. A total of 68 compounds were positively identified in the investigated samples among them only 34 compounds could be identified in control sample. Whereas, 56, 59 and 60 compounds were found in the headspace of the samples supplemented with enhancers, S-MSG, S-HSPI and S-MSG+HSPI, respectively. Most of these compounds were identified in the volatiles of cooked and boiled meat [18, 27, 31, 32].

Table 2. Volatile compounds in headspace of real beef soup samples supplemented and unsupplemented (control) with enhancers.

No.	RI [a]	Volatile compounds [b]	Relative peak area (%) [c]				ID [d]
			SC	S-MSG	S-HSPI	S-MSG+HSPI	
1	>600	Methanthiol	4.06 ± 0.55	4.75 ± 0.64	10.38 ± 1.41	2.60 ± 0.35	B
2	>600	Dimethyl sulfide	6.83 ± 0.93	2.72 ± 0.37	1.15 ± 0.16	0.89 ± 0.12	B
3	611	2,3-Butandione	19.20 ± 2.60	20.42 ± 2.77	20.75 ± 2.81	19.27 ± 2.61	A
4	635	2-Methyl-1-propanol	-----	0.02 ± 0.00	0.24 ± 0.03	2.78 ± 0.38	B
5	643	3-Methylbutanal	-----	0.06 ± 0.00	3.50 ±0.47	0.06 ± 0.00	B
6	664	2-Methylbutanal	0.22 ± 0.03	0.07 ± 0.00	0.08 ± 0.01	0.16 ± 0.02	B
7	683	Ethyl furan	-----	0.11 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	B
8	696	2-Pentanone	-----	0.07 ± 0.00	0.05 ± 0.00	0.06 ± 0.00	B
9	718	2,3-Pentandione	-----	0.97 ± 0.13	1.02 ± 0.14	0.89 ± 0.12	B
10	735	3-Hydroxy-2-butanone	0.33 ± 0.04	0.85 ± 0.12	1.40 ± 0.19	1.81 ± 0.25	B
11	745	2-Methyl-2-pentanone	-----	0.14 ± 0.02	0.08 ± 0.01	0.03 ± 0.00	B
12	753	Dimethyl disulfide	-----	0.09 ± 0.01	0.26 ± 0.04	0.06 ± 0.00	B
13	802	Hexanal	3.25 ± 0.44	1.16 ± 0.16	1.78 ± 0.24	0.23± 0.03	A
14	817	3-Mercapto-2-butanone	29.28 ± 3.67	50.02 ± 6.78	40.91 ± 5.54	49.78 ± 6.74	B
15	820	2-Methyl pyrazine	-----	0.90 ± 0.12	-----	-----	A
16	835	2-Furfural	0.54 ± 0.07	0.11 ± 0.01	-----	-----	A
17	839	2-Methylthiazol	-----	3.02 ± 0.41	1.49 ± 0.20	4.08 ± 0.55	B
18	854	2,4-Dimethyl furan	-----	0.07 ± 0.00	0.03 ± 0.00	0.07 ± 0.00	B
19	861	2-Methyl-3-furanthiol	-----	0.19 ± 0.03	0.36 ± 0.05	0.32 ± 0.04	B
20	872	4-Hydroxy-5-methyl-3-(2H)furanone	0.33 ± 0.04	0.07 ± 0.00	0.06 ± 0.00	0.06 ± 0.00	B
21	899	3-Mercapto-2-pentanone	-----	0.17 ± 0.02	0.13 ± 0.02	0.12 ± 0.01	B
22	901	Heptanal	-----	0.80 ± 0.11	0.09 ± 0.01	0.01 ± 0.00	A
23	904	3 (Methylthio) propanal	-----	-----	0.35 ± 0.05	0.88 ± 0.12	B
24	916	2-Furan methanthiol	-----	1.68 ± 0.23	1.53 ± 0.21	1.76 ±0.24	A
25	919	2-Acytylpyrroline	-----	0.33 ± 0.04	0.10 ± 0.01	0.14 ± 0.02	A
26	930	2-Methyl-3(methylthio)furan	-----	2.90 ± 0.39	0.69 ± 0.09	0.72 ± 0.10	B

27	956	Dimethyl trisulfide	-----	0.27 ± 0.04	0.05 ± 0.00	0.45 ± 0.06	B
28	962	Benzaldehyde	2.06 ± 0.28	0.02 ± 0.00	0.09 ± 0.01	0.07 ± 0.00	A
29	983	1-Octene-3-ol	0.43 ± 0.06	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	B
30	992	2-Pentyl furan	2.93 ± 0.40	0.49 ± 0.07	0.01 ± 0.00	0.01 ± 0.00	B
31	1002	1-Octene-3-one	-----	0.02 ± 0.00	0.14 ± 0.02	0.10 ± 0.01	B
32	1005	Octanal	-----	0.04 ± 0.00	0.26 ± 0.04	0.04 ± 0.00	A
33	1027	Acetyl thiazole	0.43 ± 0.06	0.28 ± 0.04	1.01 ±0.14	0.96 ± 0.13	A
34	1031	2-Ethyl-1-hexanol	0.98 ± 0.13	0.13 ± 0.02	0.03 ± 0.00	0.04 ± 0.00	B
35	1051	Phenyl acetaldehyde	-----	0.53 ± 0.07	0.15 ± 0.02	0.39 ± 0.05	A
36	1058	(E)-2-Octenal	-----	0.05 ± 0.00	0.12 ± 0.02	0.03 ± 0.00	B
37	1063	2-Methyl-3-thiophenethiol	-----	1.18 ± 0.53	1.06 ± 0.14	1.03 ± 0.14	B
38	1072	Formyl thiophene	-----	0.12 ± 0.02	0.32 ± 0.04	0.18 ± 0.02	B
39	1080	1-Octanol	-----	0.11 ± 0.01	-----	0.04 ± 0.00	B
40	1084	1-Nonene-3-one	9.54 ± 1.29	1.01 ± 0.14	0.26 ± 0.04	2.35 ± 0.32	B
41	1096	2-Acetyl thiophene	-----	0.02 ± 0.00	0.03 ± 0.00	1.30 ± 0.18	B
42	1102	Nonanal	0.98 ± 0.13	0.03 ± 0.00	0.08 ± 0.01	0.04 ± 0.00	A
43	1104	2-Thenyl mercaptane	-----	0.04 ± 0.00	0.46 ± 0.06	0.22 ± 0.03	B
44	1114	2-Formyl-5-methyl thiophene	-----	0.04 ± 0.00	0.05 ± 0.00	0.85 ± 0.12	B
45	1160	4-Hydroxy-2,5-dimethyl furanone	-----	0.30 ± 0.04	0.14 ± 0.02	0.55 ± 0.07	A
46	1187	Decanone	0.33 ± 0.04	-----	-----	0.03 ± 0.00	B
47	1205	Decanal	0.22 ± 0.03	0.01 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	A
48	1226	2,4-Nonadienal	1.74 ± 0.24	0.03 ± 0.00	-----	-----	A
49	1255	Benzo thiazole	1.95 ± 0.26	0.04 ± 0.00	0.09 ± 0.01	0.06 ± 0.00	B
50	1266	(E)-2-Decanal	0.76 ± 0.10	-----	-----	0.07 ± 0.00	B
51	1267	2-Acetyl-2,5-dimethyl thiophene	0.43 ± 0.06	-----	-----	0.06 ± 0.00	B
52	1274	Decanol	0.65 ± 0.09	0.02 ± 0.00	-----	0.03 ± 0.00	B
53	1292	Undecanal	0.54 ± 0.07	-----	1.08 ± 0.15	-----	B
54	1306	(E, Z) 2,4-Decadienal	-----	-----	0.22 ± 0.03	0.03 ± 0.00	A
55	1323	(E, E) 2,4-Decadienal	-----	-----	0.14 ± 0.02	0.06 ± 0.00	A
56	1344	Dipropyl trisulfide	-----	-----	0.04 ± 0.00	-----	B
57	1357	(E)-2-Undecanal	0.22 ± 0.03	0.07 ± 0.00	0.04 ± 0.00	-----	B
58	1376	Undecanol	0.43 ± 0.06	-----	0.08 ± 0.01	0.01 ± 0.00	B
59	1402	Dodecanal	-----	0.02 ± 0.00	0.35 ± 0.04	0.06 ± 0.00	B
60	1475	1-Dodecanol	0.98 ± 0.13	0.02 ± 0.00	0.03 ± 0.00	0.07 ± 0.00	B
61	1483	2-[(Methyltrithio) methyl] furan	1.30 ± 0.18	0.07 ± 0.00	2.81 ± 0.05	0.72 ± 0.10	B
62	1498	Tridecanone	0.54 ± 0.07	-----	0.09 ± 0.01	-----	B
63	1510	Tridecanal	5.10 ± 0.69	-----	0.03 ± 0.00	0.12 ± 0.02	B
64	1527	Benzyl furan	0.76 ± 0.10	-----	-----	-----	B
65	1540	Bis (2-Methyl-3-furyl) disulfide	0.33 ± 0.04	1.50 ± 0.20	1.45 ± 0.20	0.63 ± 0.09	B
66	1556	1 (2-Furylmethyldithio) propanone	1.30 ± 0.18	0.17 ± 0.02	0.65 ± 0.09	0.50 ± 0.07	B
67	1594	2 (2-furylmethyldithio) -2-butanone	0.43 ± 0.06	0.22 ± 0.03	0.20 ± 0.03	1.59 ±0.22	B
68	1609	Tetradecanal	-----	0.28 ± 0.04	0.99 ± 0.13	0.13 ± 0.02	B

Sample cods: SC, unsupplemented beef soup, S-MSG, beef soup supplemented with pure chemical monosodium glutamate, S-HSPI, beef soup supplemented with enzymatically hydrolyzed soybean protein isolate HSPI, S-MSG+HSPI, beef soup supplemented with MSG + HSPI. --- = not detected.

[A] Retention indices.

[B] Compounds listed according to their elution on DB5 column.

[C] Values expressed as relative area percentages to total identified compounds. ± standard deviation.

[D] Volatile compound identification was performed as follows: (A) Mass spectrum and retention index were consistent with those of an authentic standard. (B) Mass spectrum was identical with that of NIST mass spectrum database, and retention index was consistent with that of the literature [26, 27, 28].

As shown in Table 2, the compounds identified in all investigated samples were quantitatively dominated with sulfur containing compounds. These compounds are important in cooked beef flavour[33]. They could be generated either from thermal degradation of cysteine or cystine [33] through the interaction between carbonyl compounds and sulfur containing amino acids [35] or as a result of thermal decomposition of thiamine[36]. 3-Mercapto-2-butanone comprised the highest concentration in the volatiles of all investigated samples however, it showed 70.83%, 39.72% and 70.01% increase in samples S-MSG, S-HSPI and S-MSG+HSPI, respectively compared with control sample SC. It was described to have cooked rise meat aroma note[27] and considered as one of the impact compounds of process flavourings prepared from enzymatic hydrolyzed beef protein[27], hydrolyzed soybean protein[28, 37, 38, 39] with cysteine, thiamine and taurine. Mercaptoketones that produce important meat like volatiles, can be prepared by the reaction of dicarbonyls, 2, 3-butandione (**3**) and 2, 3-pentandione (**9**) (sugar degradation product) with hydrogen sulfide (Strecker degradation products of cysteine) [40].

As shown in Table 2, the percentage of methanthiol (**1**) released from beef soup sample S-HSPI was more than twice that released from control sample SC. However, insignificance (p>0.05) differences were found between its release from control sample and that from S-MSG and S-MSG+HSPI samples. Methanthiol, with its cooked cabbage note [41] was found in cooked ground beef [42] and boiled beef [41]. It can be perceived at very low concentration due to its low threshold (0.2 µg/ kg). This compound was reported as one of the character impact odour of stewed beef juice [43]. Compound (**1**) and dimethyl sulfide (**2**) were recognized as the potent odourants in boiled beef aroma [44].

The two furanthiols compounds, 2-methyl-3-furanthiol (**19**) and 2-furan methanthiol (**24**), the most potent odourants of beef aroma, could not be detected in SC sample. However, they were detected in considerable total concentrations 2.27%, 1.79% and 1.98%, in S-MSG, S-HSPI and S-MSG+HSPI samples, respectively. The same trend was found for 2-methyl-3-thiophenethiol (**37**). Furans and thiophenethiols have been reported as important volatile compounds in cooked meat [45]. 2-Methyl-3(methylthio) furan (**26**), showed the highest concentration in the headspace of S-MSG followed by S-MSG+HSPI and S-HSPI beef soup samples (Table 2). This compound was proposed to be formed via either Maillard reaction of ribose and cysteine involving the reaction with methanthiol (**1**), or the reaction of 2-methyl-3-furanthiol (**19**) with methanthiol (**1**) [46]. 3 (Methylthio) propanal (**23**), Strecker aldehyde of methionine [47], was found only in headspace of S-HSPI and S-MSG+HSPI. It was described to have soy sauce note [26].

In addition to the above mentioned compounds other sulfur containing compounds such as dimethyl trisulfide (**27**), 2-acetyl thiophene (**41**), 2-thenyl mercaptane (**43**), 2-formyl-5-methyl thiophene (**44**) and dipropyl trisulfide (**56**) could not be detected in the control sample. Three thiazoles were reported in the present study namely, 2-methylthiazol (**17**), acetyl thiazole (**33**) and benzo thiazole (**49**). Compound (**17**) was absent in headspace of SC sample, whereas it was found at considerable concentration in the samples supplemented with the investigated umami compounds (enhancers). These compounds were reported among the dominant sulfur containing compounds in cooked beef meat [48]. They are thermal degradation products of cysteine either alone or in the presence of reducing sugar such as ribose or xylose [49] also can be formed by heat degradation of thiamine[50]

Four disulfide compounds were identified in the present study namely, dimethyl disulfide (**12**), bis (2-methyl-3-furyl) disulfide (**65**), 1 (2- furylmethyldithio) propanone (**66**) and 2 (2- furylmethyldithio) 2-butanone (**67**). Compound **12**, could not be identified in SC sample, it is a degradation product of methionine and responsible for onion and cabbage like odours [51]. Whereas, compounds **65 – 67** possess savoury aromatic note and are generally formed by the dehydrogenation among furanthiol, thiophenthiol and α- mercaptoketones[52]. It is well known that compound **19** is easily oxidized to compound **65**.

Three furans, 2-furfural (**16**), 4-hydroxy-5-methyl-3-(2H) furanone (**20**) and 4-hydroxy-2, 5-dimethyl furanone (**45**) were detected at low concentrations (Table 2) in the headspace of the investigated samples. These compounds considered as the secondary intermediates for the production of the thiol containing volatile compounds[53]. Compound **20** could be generated from the degradation of ribonucleotides such as ribose -5-phosphate or from sugar degradation [41]. Compound **16** (sugar degradation product) can react with hydrogen sulfide to form compound **24,** whereas, compound **20** could be involved in a reaction with hydrogen sulfide to

form compound **19** [54]. Compound **44** with compounds **19** and **24** were reported as the key aroma compounds in boiled beef[55].

Forty four lipid degradation products could be identified in the present study including, 16 aldehydes, 7 ketones, 7alcohols and 3 alkyl furans. Most of these compounds were identified in boiled [41] and cooked [31, 48] beef. Among the identified aldehydes only hexanal (**13**), benzaldehyde (**28**), nonanal (**42**), decanal (**47**), 2, 4–nonadienal (**48**), (E)-2-decanal (**50**), undecanal (**53**), (E)-2-undecenal (**57**), tridecanal (**63**) were presented in headspace of SC sample. E, E-2, 4-Decadienal (**55**), which considered one of the potent odourants of cooked beef aroma [31], was absent in SC and S-MSG samples. Aldehydes have two functions, as contributors to the aroma of beef as well as intermediate compounds to produce flavour through amino carbonyl reaction [56]. 3-Hydroxy-2-butanone (**10**), 1-nonene-3-one (**40**) and decanone (**46**) were the only ketones found in headspace volatiles of control beef soup. These compounds have been implicated in the buttery aroma of cooked meats[57]. Five alcohols could be identified in SC sample namely 1-octene-3-ol (**29**), 2-ethyl-1-hexanol (**34**), decanol (**52**), undecanol (**58**) and 1-dodecanol (**60**). Compound (**31**) and 1-nonene-3-one (**40**) have been reported among the active compounds in the extract of beef flavouring[27]. 2-Pentyl furan (**30**) comprised considerable concentration (2.93%) in headspace of SC sample; whereas it was detected in much less concentration in beef soup samples supplemented with enhancers (Table 2). It was identified in various beef products[41, 42, 58, 59] and may be generated by auto oxidation of linoleic acid [27].

Salting out of certain volatile compounds by addition of glutamate is expected. In general salts bind water molecules to build hydration shells during solubilization, this phenomenon leads to increased mobility and release of flavour compounds caused by the decreased availability of water molecules for the solubilization of flavour compounds [60].

Previous studies confirmed the ability of glutamate to enhance the flavour of some model food products[5, 10, 21]. MSG alone as a taste stimulus is described as broth-like, salty-like and meaty[61, 62]. Bellisle[4] reported that addition of low concentration of MSG to appropriate foods increase the palatability and pleasantness of food, however this effect depends on food composition and on the characteristics of odour and flavour notes[5,6].

Salting-out phenomenon of NaCl has been demonstrated using sensory techniques for model system containing odour active compounds typically present in meat products[21]. In present study, NaCl was added at constant concentration to all investigated samples. Previous studies showed that MSG, IMP and GMP enhance the perception of other taste active compounds including NaCl[4, 61, 63]. In contrast, Ventanas et al.[21] reported that the combination of NaCl with umami compounds revealed modulator effect rather than potentiator effect. Recently Nishimura et al.[10] reported that the aroma intensity of 0.4% NaCl solution containing aroma chicken model with added glutamic acid was significantly higher (p<0.05) than that of control sample containing only 0.4% NaCl with aroma chicken model.

To illustrate the enhancing effect of the investigated enhancers on the release or retention of the headspace volatiles of real beef soup samples, the GC peaks of the key odourants of beef products aroma were selected and grouped according to their chemical structures to be used as indicator peaks such as:

Thiols: Thiols are the most important group of volatile compounds in the aroma of meat products[18, 27, 64, 65] . As shown in Table 2 and Fig. 1, supplementation of real beef soup with each of the investigated enhancers, MSG, HSPI and MSG+HSPI revealed significant (p<0.05) release of the thiols. The enhancement of the palatability of a model chicken broth by MSG is attributed to the sodium and glutamate ions [66]. Nishimura et al.[10] suggested that addition of umami compounds (glutamic acid and MSG) to an aqueous solution containing aroma compounds increases the amount of aroma released from the solution. However, the authors reported that this suggestion needs to further investigation.

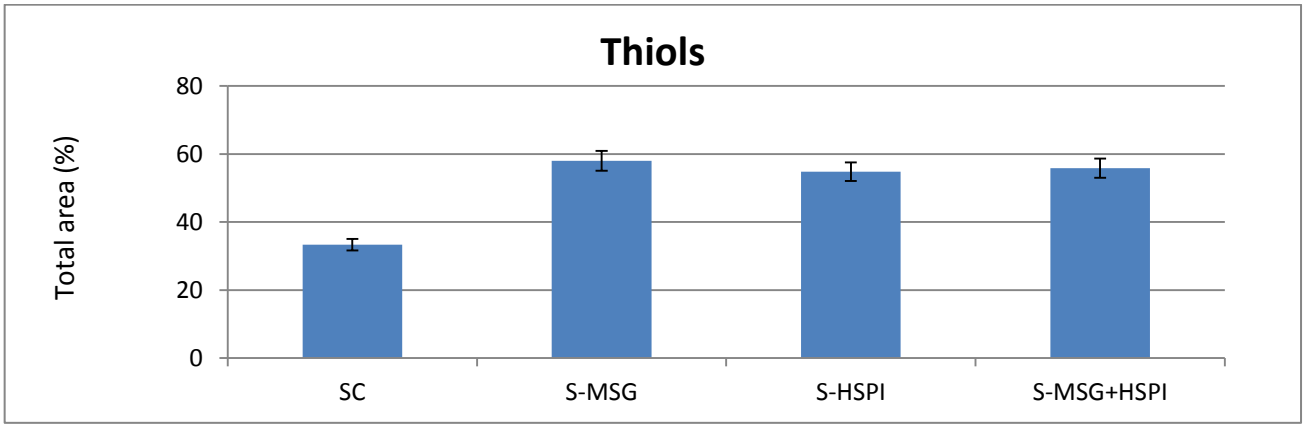

Fig.1. Total thiol containing compounds in headspace of investigated beef soup samples.

The significant release of thiol containing compounds in sample S-HSPI and S-MSG+HSPI may be correlated to the high content of glutamic acid in HSPI. During enzymatic hydrolysis glutamic acid changes to MSG [4]. Synergy between MSG and HSPI revealed the higher release of thiols in headspace of S-MSG+HSPI compared with S-HSPI.

Disulfides: As shown in Fig. 2 supplementation of real beef soup with the investigated enhancers gave rise to considerable increase in the total disulfides compounds, dimethyl disulfide (**12**), bis (2-methyl-3-furyl) disulfide (**65**), 1 (2- furylmethyldithio) propanone (**66**) and 2 (2- furylmethyldithio) 2-butanone (**67**). However, the synergy effect between MSG and HSPI revealed significant release of these compounds in headspace of sample S-MSG+HSPI.

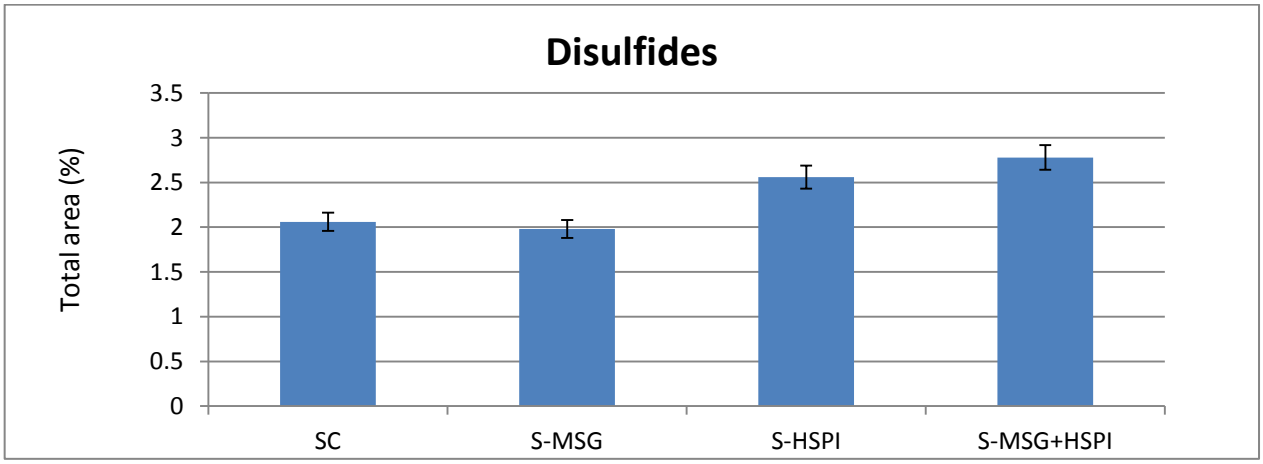

Fig. 2. Total disulfide compounds in headspace of investigated beef soup samples.

Diketones: In the present study two diketones could be identified namely 2, 3-butandione (**3**) and 2,3-pentandione (**9**) (Table 2). They are degradation products of sugar and considered as important intermediate in the formation of the two mercaptoketones compounds 3-mercapto-2-butanone (**14**) and 3-mercapto-2-pentanone (**21**) by the interaction with hydrogen sulfide [40]. These diketones were reported among the meat odour active compounds and described to have a buttery note [67]. As shown in Fig. 3 all the investigated enhancers MSG, HSPI and MSG+HSPI favoured the release of the diketones in the headspace of the real beef soup.

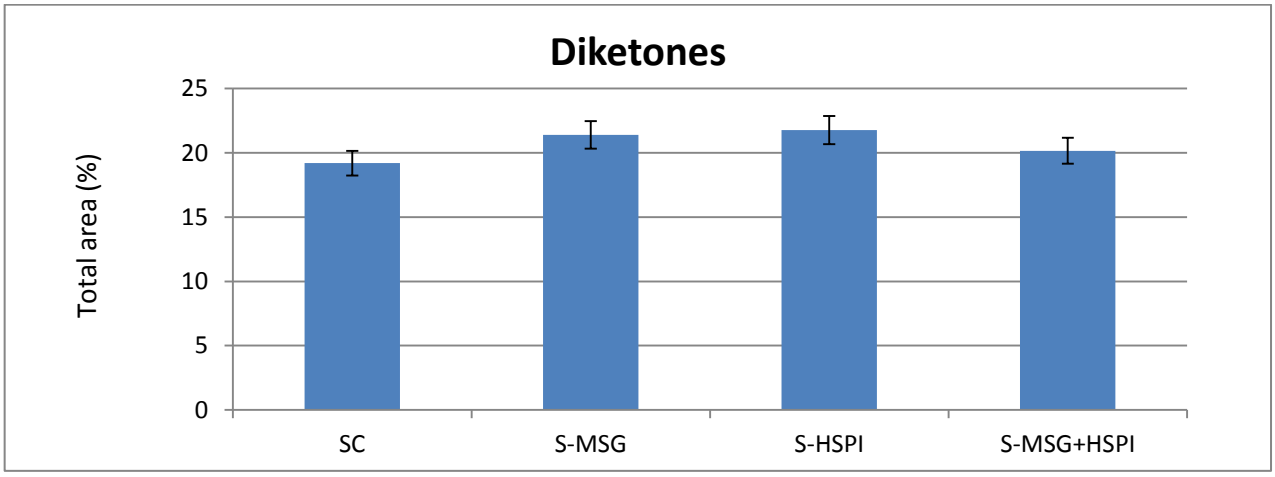

Fig. 3. Total diketone compounds in headspace of investigated beef soup samples.

Lipid degradation products: It is obvious that supplementation of real beef soup with the investigated enhancers, in the present study, revealed dramatic decreases (p<0.05) in the lipid degradation products in the headspace of all beef soup samples S-MSG, S-HSPI and S-MSG+HSPI (Fig. 4). This finding may be attributed to the interaction between the matrix contents of the beef soup such as protein, fat and carbohydrates and the lipid degradation products (aldehydes, ketones, alcohols and alkyl furans). Voilley and Etiévant[20] reported that the physicochemical properties of aroma compounds and their interaction with other food components affect flavour release and perception. On the other hand the hydrophobic bonds between proteins in soup matrix and volatile compounds such as aldehyde, ketone and alcohol may take place[68].

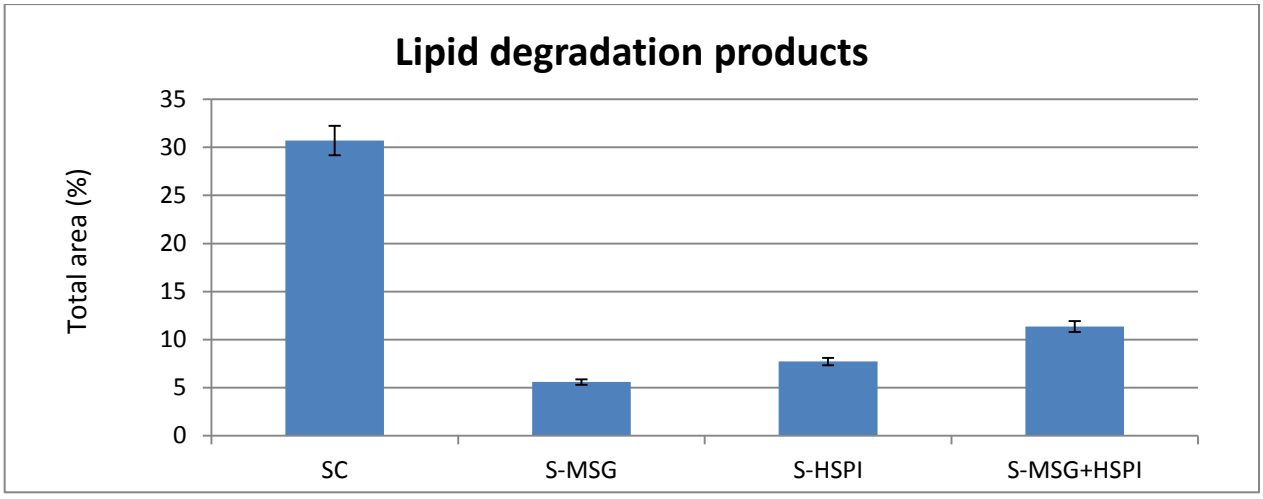

Fig. 4. Total lipid degradation products in headspace of investigated beef soup samples.

Sensory analysis

The present study was designed to evaluate the effect of HSPI on palatability enhancement of real beef soup in comparison with pure MSG. Also to evaluate the synergy effect between MSG and HSPI. As expected, supplementation of beef soup with the investigated glutamate enhancers (MSG, HSPI and MSG+HSPI) revealed significant (p<0.05) increase in palatability of beef soup compared with control sample SC Fig. 5. This finding is in agreement with previous studies dealing with effect of MSG on odour perception of meat soup model systems [5]. Ventanas et al.[21] reported that flavour enhancing properties of umami compounds such as MSG could increase the perception of beef broth like flavour and saltiness intensities. As shown in Fig 5 no significant differences (p>0.05) was found among the three samples S-MSG, S-HSPI and S-MSG+HSPI, however, the slight increase in degree of liking of the later sample may be attributed to its highest content of compounds 2-methyl-3-furanthiol (**19**) and 2-furan methanthiol (**24**) (Table 2), the most potent odourants of boiled beef aroma[18], compared with S-MSG and S-HSPI as well as the disulfide compounds which possess savoury aromatic notes [52].

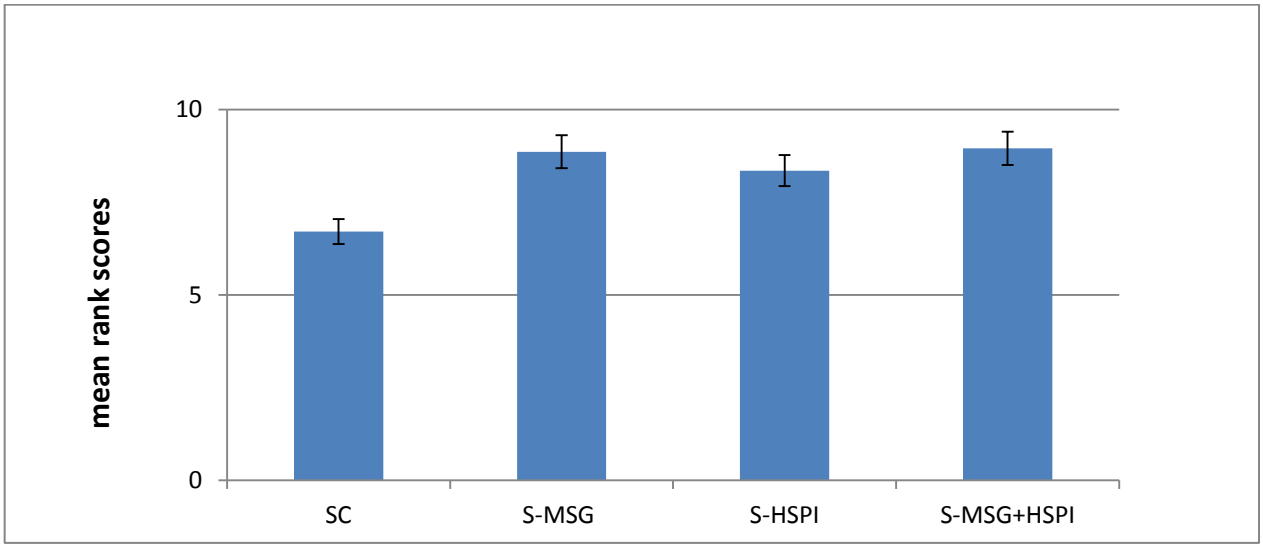

Fig. 5. Change in palatability of beef soup supplemented with different enhancers.

Conclusion

From the above mentioned results it can be concluded that HSPI with its high glutamate content can be used as natural enhancer instead of the pure chemical MSG. The present study is the first that dealing with evaluation of the enhancing effect of glutamate on volatiles and palatability of real beef soup. A high correlation had been found between the degrees of liking and released volatiles in headspace of each investigated sample.

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