CHANGES IN MEAT FLAVOUR VOLATILE PROFILES OF BEEF BONE HYDROLYSATES DURING MAILLARD REACTION

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I. INTRODUCTION

Proteins from meat, milk, wheat, and soy are commonly used as ingredients in the food industry. Meat proteins obtained from meaty beef bones have limited food applications due to their high viscosity and weak flavour. There is interest in converting these low-value meat products into high-value functional food ingredients through enzymatic hydrolysis and Maillard reaction (MR), as well as to improve the utilisation of these ingredients into useful materials. The objective of this study was to investigate the effects of enzymatic hydrolysis treatments on the volatile profiles of beef bone hydrolysates during MR. The changes in meat flavour volatile profiles of bone hydrolysates were evaluated using gas chromatography-mass spectrophometry (GC-MS).

II. MATERIALS AND METHODS

Enzymatic hydrolysis of beef bone extract

Beef bone extract (BE, Degree of hydrolysis: 0.33±0.01%) (Taranaki Bio Extracts Ltd, New Zealand) at pH 6.68, was hydrolysed in a shaking incubator (INFORS HT, Switzerland) for 120 min at 150 rpm using enzyme systems as shown in **Table 1**. Hydrolysis was terminated by placing the hydrolysates in an 85°C water bath for 15 min. The DH was determined using the *ortho*-phthaldialdehyde (OPA) method (Nielsen *et al.,* 2001).

Table 1 Enzyme systems used in the hydrolysis of beef bone extract. Results were expressed as mean and standard deviation.

Sample description	Enzyme system [x]	Conditions for enzymatic hydrolysis [y]	Degree of hydrolysis (%)
P	Protamex® (Novozymes, Denmark)	Temperature: 40°C E/S: 1.10% w/w	4.13±0.99[c]
F	Flavourzyme® (Novozymes, Denmark)	Temperature: 50°C E/S: 4.70% w/w	12.24±0.83[b]
P+F	Protamex® + Flavourzyme®	Temperature: 50°C E/S: 1.10% w/w (P), 4.70% w/w (F)	14.65±1.03[a]

[x] "P+F" represent simultaneous hydrolysis using Protamex® with Flavourzyme®.
[y] Enzyme-substrate (E/S) ratio is based on enzyme weight to protein weight.
All samples had DH different from BE. Values bearing different letters in the same column were significantly different ($p \leq 0.05$).

Preparation of Maillard reaction products (MRPs)

Beef bone hydrolysates and D-ribose (Amtrade NZ Ltd, New Zealand) was mixed by weight at a protein to reducing sugar ratio of 1:0.068. The mixtures were adjusted to pH 6.5 with 1 M NaOH and heated at 10 psi (113°C) for 10 min in a pressure cooker to produce MRPs, namely P-MRP, F-MRP and P+F-MRP. A mixture of non-hydrolysed BE and ribose was used as a control (BE-MRP).

Volatile components analysis using Gas Chromatography-Mass Spectrometry (GC-MS)

Volatile extraction was carried out at 60°C (2 min equilibrium time with agitation) by exposing a 50/30 µm DVB/CAR/PDMS fibre (Supelco, USA) for 45 min in static headspace mode. The compounds were then thermally desorbed at 240°C for 5 min in spitless mode. Analyses were performed using the GC (Agilent 6890N) equipped with a mass spectrometer (Agilent 5975B VL MSD). The chromatographic separation was

performed on a ZB-WAX capillary column (Phenomenex, USA) with helium at a flow rate of 1.0 mL/min. The oven temperature was held at 40°C for 5 min, increased at a rate of 4°C/min to 210°C, and then at 10°C/min up to 240°C, at which the temperature was held for 5 min. The transfer line to the MS was set to 230°C, and the quadrupole was set to 150°C. The MS was operated at a scan speed of 5.1 scans/sec, and mass spectra were recorded in the range of 30-300 m/z. Volatile compounds were identified by comparing the detector data (Retention Indices, RI) of published literature and NIST14 database.

Data analysis

Data were analysed using Minitab® 16.2.1 statistical software (Minitab, USA). Statistical analyses of observed differences among means consisted of Tukey's pairwise comparison of means ($p \leq 0.05$).

III. RESULTS AND DISCUSSION

Table 2 Volatile compounds of beef bone extract and four MRPs analysed by GC-MS. Results were expressed as mean and standard deviation.

Compounds	RI	Concentration based on peak area (10^6)				
		BE	BE-MRP	P-MRP	F-MRP	P+F-MRP
Pyrazines						
2-Methylpyrazine	1311	ND	57.8±2.1^{c}	74.0±2.0^{b}	117.6±5.6^{a}	112.3±5.5^{a}
2,5- and 2,6-Dimethylpyrazine (coeluting)	1378	147.7±14.6^{d}	249.4±0.9^{c}	547.0±23.6^{b}	741.2±31.9^{a}	794.4±50.6^{a}
2-Ethyl-6-methylpyrazine	1441	6.6±1.1^{d}	19.0±1.9^{c}	23.1±1.2^{b}	40.7±1.5^{a}	42.4±1.3^{a}
2-Ethyl-5-methylpyrazine	1447	75.2±3.3^{e}	60.4±0.5^{d}	106.6±1.9^{c}	145.3±2.0^{b}	168.2±3.2^{a}
Trimethylpyrazine	1470	35.9±2.0^{e}	60.2±0.6^{d}	95.5±2.9^{c}	158.3±3.6^{b}	171.0±3.8^{a}
3-Ethyl-2,5-dimethylpyrazine	1506	134.5±10.0^{d}	363.3±15.1^{c}	451.9±18.7^{b}	512.8±18.5^{a}	533.8±14.8^{a}
3,5-Diethyl-2-methylpyrazine	1579	10.7±0.1^{c}	68.4±1.0^{b}	81.8±4.4^{a}	81.0±3.1^{a}	84.9±1.5^{a}
2,5-Dimethyl-3-isopentylpyrazine	1723	ND	ND	ND	64.7±2.2^{b}	76.3±0.6^{a}
2-Acetyl-3,5-dimethylpyrazine	1766	ND	ND	ND	7.5±0.2^{a}	8.3±0.6^{a}
Thioethers						
Dimethyl disulfide	1043	ND	23.6±1.6^{c}	31.6±5.5^{bc}	52.5±7.5^{a}	47.2±7.5^{ab}
Dimethyl trisulfide	1428	ND	23.5±1.2^{b}	28.8±2.1^{b}	87.6±5.9^{a}	87.9±8.9^{a}

Values bearing different letters in the same row were significantly different ($p \leq 0.05$).

Ten pyrazines and two thioethers (**Table 2**) were identified in four MRPs of BE and hydrolysates during MR (van Boekel, 2006). Some of them were the major volatile components, and others were intermediates in the formation of volatiles. The content of pyrazines and thioethers generally increased with increasing DH. For example, 2,5- and 2,6-dimethylpyrazine (coeluting), heterocyclic compounds associated with meaty flavour, increased by 437.8% when BE was hydrolysed with P+F. This showed that enzymatic hydrolysis and MR played an essential role in enhancing the volatile profiles in the MRPs.

IV. CONCLUSION

GC-MS results showed that the volatile compounds concentration increased with increasing DH. The changes in volatile profiles of bone hydrolysates during MR indicated enzymatic hydrolysis and MR could be used as an effective approach to increase the flavour quality of bone extract as a natural meat flavour enhancer.

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