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A novel method for beef bone protein extraction by lipase-pretreatment and its application in the Maillard reaction

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ABSTRACT

Five beef bone hydrolysates were obtained by different enzyme treatment schemes, including papain (M), combination of porcine pancreatic lipase and papain (Z + M, combination of lipase and papain (Y + M), Protamex (F), combination of porcine pancreatic lipase and Protamex (Z + F). The degree of hydrolysis (DH), free amino acids and molecular weight distribution of these hydrolysates were evaluated. To further explore the differences between these five hydrolysates, Maillard reaction products (MRPs) were prepared using a xylose/cysteine/hydrolysate model. It was found that the DH, content of low molecular weight peptides and amino acids of hydrolysates increased significantly after lipase pre-treatment. GC–MS showed that the total content of furans, pyrroles and thioethers in MRPs Y + M increased by 78.0% compared with MRPs M, while in MRPs Z + F, pyrazines increased by 44.1% compared with MRPs F. Examining the sensory characteristics of the MRPs, the MRP from the hydrolysate of Y + M had the best mouthful, umami and meaty characteristics. The correlation analysis further confirmed that an appropriate lipase pre-treatment could improve the flavour of MRPs.

1. Introduction

Approximately 48.28 million cattle were slaughtered in China in 2013 (State Statistical Bureau, 2014). Most of this beef is deboned in plant and the meat is sold as packaged beef. During this process, as much as 6–12% (based on carcase weight) of bone was left. This could result in approximately 4.43 million tonnes of bone (almost no meat attached to the bone) disposed of or sold at a lower price as inedible by-products (Wang, Yu, Han, & Yu, 2015). Beef bone contains a notable amount of muscle, connective tissue and fat, and therefore represents a valuable source of proteins, containing about 47% moisture, 21% protein (collagen), 15% fat, and 15% ash. Non-utilisation or underutilisation of animal by-products not only leads to loss of potential revenues but also leads to a higher cost of disposal of these products. For that reason, industries have begun to develop various technologies to make use of this waste, mainly in the form of value-added products, at the same time reducing the cost derived from its disposal.

Continuous efforts have been made to improve the functional and nutritional value of bone. Boles, Rathgeber, and Shand (2000) used different solutions (4% sodium chloride, 4% sodium chloride with either 0.3 M sodium tripolyphosphate, tetrasodium pyrophosphate or 0.05 M NaOH to effectively extract proteins from beef bones. These proteins could be used to manufacture finely comminuted sausage products with similar texture to sausages made with commercially available proteins. Nikolaev et al. (2008) stated that a functional meat protein could be obtained by the fermentation of meat-bone broiler residues. Recently, enzymatic hydrolysis was employed to extract proteins and produce peptides (Morimura et al., 2002), which formed an effective way to recover proteins from the by-products of animal processing. Unlike acidic or alkaline hydrolysis, enzymatic proteolysis is mild and controllable, which helps to improve the quality and functional properties of protein (Kristinsson & Rasco, 2000). Linder, Fanni, Parmentier, Sergent, and Phan-tan-luu (1995) reported that

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the utilisation of bone mainly focused on the enzymatic extraction of nutrients. After hydrolysis, bone could be developed into value-added products. Linder et al. (1997) also described that the enzymatic hydrolysate of veal bone contained a large amount of glycine and proline, whose nutritional value was much higher than the hydrolysate treated by acid or alkali. It was found that the hydrolysates were useful for soups, sauces and gravies. Dong et al. (2014) used hot-pressure combined with enzymolysis to extract protein from chicken bone; these hydrolysates demonstrated a new kind of potential suitable nutritional supplement in various foods. Other researchers also found that the hydrolysed protein was an important flavouring agent (Lafarga & Hayes, 2014; Lieske & Konrad, 1994; Zhan, Tian, Zhang, & Wang, 2013), which could give Maillard reaction products (MRPs) with lifelike meat flavour (Pommer, 1995).

The water-soluble meat flavour precursors consist of free amino acids, peptides, and reducing sugars (Khan, Jo, & Tariq, 2015). Madruga, Elmore, Oruna-Concha, Balagiannis, and Mottram (2010) reported that by controlling the degree of hydrolysis (DH), different constituents of these precursors could generate different flavours. In general, the lower the DH is, the fewer the precursors. It is well-known that beef bone is usually surrounded by adipose tissue, which may prevent the combination of protein and protease, leading to a low degree of hydrolysis. Linder et al. (1997) hydrolysed veal bone using Neutrase only, with an unsatisfactory DH. Therefore, it is necessary to develop an effective way for better utilisation of beef bone by-product. Since lipase could hydrolyse redundant adipose tissue during lean meat processing, this enzyme was chosen to pre-treat the beef bone.

The objective of present study is to develop a new method for the preparation of protein from beef bone, and compare it with other methods. The study includes (A) analysis of the degree of hydrolysis, free amino acids and molecular weight of five bone hydrolysates hydrolysed by different treatment schemes, including papain, combination of porcine pancreatic lipase and papain, combination of lipase and papain, Protamex, combination of porcine pancreatic lipase and Protamex; (B) Comparison of the sensory characteristics and the volatile compounds of the MRPs prepared from the five hydrolysates. (C) Study of the relationship between free amino acids, molecular weight distribution of hydrolysates and the sensory characteristics of MRPs. Through the above analyses, the influence of the lipase pre-treatment on beef bone hydrolysate and MRP was investigated.

2. Materials and methods

2.1. Chemicals and materials

Beef bone was purchased from Shanghai Tesco Supermarket (Shanghai, China). Xylose and cysteine were purchased from Sigma China Co., Ltd. (Beijing, China). Papain (2000 U/mg) and Protamex (31.4 U/mg) were obtained from Novo Co., Ltd. (Novozyme Nordisk, Bagsvaerd, Denmark). Porcine pancreatic lipase (20 U/mg) was purchased from Shanghai Source Poly Biological Technology Co., Ltd. (Shanghai, China). Lipase (Yiming, 20 U/mg) was purchased from Yiming Biological Products Co., Ltd (Jiangsu, China). Formaldehyde and NaOH were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The other chemical reagents were purchased from National Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Preparation of bone hydrolysates hydrolysed by different enzymes

Beef bones were first cleaned of meat, fat and bone marrow and heated for 4 h at 121 °C, 0.1 MPa a in pressure vapour steriliser (Shanghai Shenan Medical Devices Co., Ltd., Shanghai, China). Then it was dried at 60 °C for 4 h before grinding into powder (80 mesh size) by high-speed grinding machine (Tianjin Instrument Co., Ltd., Tianjin, China). Bone powder was mixed with deionised water in a certain proportion. Then the mixture was hydrolysed by different enzymes. The preparations of five different beef bone hydrolysates are listed in Table 1. All five hydrolysates were prepared at the optimal conditions of the enzymes. The composite enzymatic hydrolysates were hydrolysed by porcine pancreatic lipase or lipase for 3 h, followed by papain or Protamex for 3 h. After the enzyme deactivation at 90 °C for 10 min, these five hydrolysates (designated M, F, Z + M, Z + F, Y + M) were centrifuged (Scientific Instrument Co., Ltd., Shanghai, China) at 4000*g* for 20 min. The supernatants were kept at 4 °C until used.

2.3. Determination of DH

DH of the hydrolysates was measured according to Song et al. (2013).

2.4. Free amino acid analysis

A pre-treatment of the sample was needed before the amino acid analysis. For the determination of free amino acids, 5 mL sample were added to a volumetric flask (25 mL), and 5% trichloroacetic acid (TCA) was added to volume, to precipitate peptides or proteins (Song et al., 2013). The solution was filtered through Whatman filter paper No. 4 after incubation for 2 h at room temperature. Then the filtrate was centrifuged (Scientific Instrument Co., Ltd., Shanghai, China) at 12,300*g* for 10 min and stored at 4 °C.

Amino acids in bone hydrolysates were analysed (Liu et al., 2012; Song et al., 2013). The sample (20 μL) was injected into an automated online derivatisation system with and analyzed by an Agilent 1100 HPLC with UV detector operated at 338 nm/262 nm

Table 1
Preparation of five different beef bone hydrolysates.

Hydrolysates	Optimum conditions of lipase pretreatment	Optimum conditions of protein treatment	Degree of hydrolysis (%)
M	—	Papain temperature: 60 °C; pH = 6.0; time: 3 h; enzyme/substrate ratio: 1.0% (w/w)	$15.43^{b} \pm 0.05$
Z + M	Porcine pancreatic lipase temperature: 35 °C; pH = 7.0; time: 3 h; enzyme/substrate ratio 1.5% (w/w)	Papain temperature: 60 °C; pH = 6.0; time: 3 h; enzyme/substrate ratio: 1.0% (w/w)	$23.17^{e} \pm 0.11$
Y + M	Lipase temperature: 35 °C; pH = 7.5; time: 3 h; enzyme/substrate ratio 1.5% (w/w)	Papain temperature: 60 °C; pH = 6.0; time: 3 h; enzyme/substrate ratio: 1.0% (w/w)	$19.17^{c} \pm 0.12$
F	—	Protamex temperature: 40 °C; pH = 6.5; Time: 3 h; Enzyme/substrate ratio: 1.0% (w/w)	$12.71^{a} \pm 0.01$
Z + F	Porcine pancreatic lipase temperature: 35 °C; pH = 7.0; time: 3 h; enzyme/substrate ratio 1.5% (w/w)	Protamex temperature: 40 °C; pH = 6.5; Time: 3 h; Enzyme/substrate ratio: 1.0% (w/w)	$21.28^{d} \pm 0.16$

Values bearing different lowercase letters (a, b, c, d and e) were significantly different ($p \leqslant 0.05$).