Food Chemistry 257 (2018) 163–171

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Structural characteristics of low bitter and high umami protein hydrolysates prepared from bovine muscle and porcine plasma

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ARTICLE INFO

Keywords:
Protein hydrolysates
Bovine muscle
Porcine plasma
Enzymatic debittering
Enzyme specificity

ABSTRACT

The aim of this study was to use different enzyme mixtures to investigate the influence of peptide characteristics and taste of protein hydrolysates from bovine muscle and porcine plasma. Minced beef and porcine plasma were hydrolysed using 10 food-grade enzymes, including Protease A, Protease P, ProteAX, Flavourzyme, Alcalase, Papain, Bromelain, Protamex, Neutrase and Sumizyme BNP-L. The relationship between degree of hydrolysis (DH), molecular weight (MW) distribution, enzyme specificity, and sensory characteristics of hydrolysates were investigated. The results demonstrated that Protease A, a mixture of endo- and exo-peptidases, was the optimal protease to generate hydrolysates with low bitterness. Endopeptidases (Papain and Bromelain) elicited bitter taste of protein hydrolysates. A positive correlation was suggested between umami taste and MW distribution (< 0.5 kDa), while bitterness was positively correlated with MW distribution (0.5–1 kDa). Overall, hydrolysis with enzyme preparations containing endo- and exo-peptidases was effective to reduce bitterness of hydrolysates.

1. Introduction

The global demand for food with high protein content has dramatically increased in recent years (Mullen et al., 2017). The meat industry annually generates a significant amount of protein-rich raw materials (Toldrá, Mora, & Reig, 2016). These products are abundant in high-quality proteins with excellent nutritional value (Toldrá et al., 2016). For instance plasma, the part of the blood after removal of red blood cells, contains 6–8% protein, which is mainly composed of albumin, globulins and fibrinogen (Bah, Bekhit, Carne, & McConnell, 2013). However, they are mainly used for production of low-value products, such as fertilizers, animal feed, pet food, etc (Alao, Falowo, Chulayo, & Muchenje, 2017). Therefore, better utilisation of these raw materials is not only crucial from the perspective of sustainability, but also provides new protein ingredients and products as well as the potential revenue for food industry (Mullen et al., 2017). Enzymatic hydrolysis is an efficient process to extract proteins from meat by-products and obtain high value-added ingredients, e.g. protein hydrolysates (Lafarga & Hayes, 2016). Nevertheless, a major challenge that may affect the sensory property of protein hydrolysate-based products is the bitter and unpalatable taste (FitzGerald & O'Cuinn, 2006). Thus, it is imperative to limit bitter taste of protein hydrolysates for further application as protein ingredients.

Bitter peptides have been widely investigated to improve taste properties and the physiochemical characteristics of hydrolysates related to bitter taste have been partially elucidated (Cho, Unklesbay, Hsieh, & Clarke, 2004; Kim & Li-Chan, 2006; Maehashi & Huang, 2009). The hydrophobicity of peptides is one of the most crucial factors for bitterness (Maehashi & Huang, 2009). It is hypothesized that peptides with a high average hydrophobicity tend to have an intensely bitter taste according to the Q-rule (Ney, 1971). However, peptide sequence, bulkiness and spatial structure of peptide have been shown to exert an impact on bitter taste perception (Kim, Yukio, Kim, & Lee, 2008). It has been documented that bitter peptides are composed of less than eight amino acids (Maehashi & Huang, 2009). Bitterness increases as the peptide length is elongated, as the extended peptide chain length may increase interactions with bitter receptors (Kim & Li-Chan, 2006; Liu et al., 2013). Bitterness is also influenced by degree of hydrolysis (DH). DH is defined as the percentage of peptide bonds cleaved during enzymatic hydrolysis, which is a predictor of reduced average molecular weight (MW) of peptides (Adler-Nissen, 1979). The intensity of bitterness and DH is positively correlated when DH values are very low (Newman et al., 2014). As proteolysis proceeds, however, more hydrophobic amino acids are exposed, leading to increased bitterness (Spellman, Ocuinn, & Fitzgerald, 2009). With extensive hydrolysis (at high DH), bitter peptides can be further degraded into smaller peptides

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https://doi.org/10.1016/j.foodchem.2018.02.159
Received 11 December 2017; Received in revised form 27 February 2018; Accepted 28 February 2018

or free amino acids, resulting in reduced bitterness. The proteases used to generate hydrolysates can also influence bitterness due to different peptide profiles caused by their different specificities (Heiniö, Nordlund, Poutanen, & Buchert, 2012).

In recent years, a variety of methods have been employed to reduce bitterness of hydrolysates, such as removal of hydrophobic peptides, masking of bitter taste, enzymatic debittering and encapsulation of hydrolysates (Saha & Hayashi, 2001). Enzymatic hydrolysis of bitter peptides by exopeptidases, including amino- and carboxy-peptidases has been reported to serve as a promising approach for debittering of protein hydrolysates without decreasing yields (Saha & Hayashi, 2001). Exopeptidases can selectively cleave peptide bonds at the N- or C-termini of bitter peptides, releasing free hydrophobic amino acids and further reducing bitter taste (Raksakulthai & Haard, 2003). Sequential hydrolysis by endo- and exo-peptidase has been shown to reduce the bitterness of wheat gluten hydrolysates (Liu, Zhu, Peng, Guo, & Zhou, 2016). Cheung, Aluko, Cliff, and Li-Chan (2015) claimed that exopeptidase treatment of whey protein hydrolysates decreased bitterness and increased the umami taste. Umami is a savoury taste, corresponding to the flavour of glutamates, especially monosodium glutamate (Beauchamp, 2009). Umami peptides have been isolated and identified in a variety of food products, which can elicit an intense umami taste (Zhang, Venkitasamy, Pan, Liu, & Zhao, 2017). Generally, they are typically acidic peptides with MW less than 500 Da (Lioe, Wada, Aoki, & Yasuda, 2007; Zhang et al., 2017). Recently, suppression of bitterness by umami peptides via the human bitter taste receptor has been reported (Kim, Son, Kim, Misaka, & Rhyu, 2015), which may serve as an alternative strategy to reduce bitter taste and increase consumer preference towards protein hydrolysates.

Even though some low bitter protein hydrolysates have been prepared from dairy or plant proteins with the aid of exopeptidase treatment (FitzGerald & O'Cuinn, 2006), there is scanty information on the generation of low bitter hydrolysates derived from meat muscle and by-products. Moreover, the relationship between taste properties of hydrolysates and their structural characteristics remains unclear. Therefore, the objective of this study was to develop a promising approach to prepare low bitter and high umami hydrolysates via simultaneous hydrolysis of bovine muscle and porcine plasma using endo- and exopeptidases and to unravel their structural characteristics accounting for the low bitter and high umami taste of hydrolysates.

2. Materials and methods

2.1. Materials

Porcine plasma (protein content, 6.2%) was collected from Danish Crown Ingredients (Copenhagen, Denmark). The minced beef (protein content, 21.4%) was obtained from a local slaughterhouse in Copenhagen. The samples were stored at −20 °C until further analysis. The protein composition of raw materials was determined by Kjeldahl analysis as per the AOAC method (Horwitz, 2000). Alcalase® 2.4 L FG (2.4 AU/g), Flavourzyme® (1000 LAPU/g), a mixture of endo- and exopeptidases, Protamex® (1.5 AU/g) and Neutrase® 0.8 L (0.8 AU/g) were obtained from Novozymes (Bagsvaerd, Denmark). ProteAX (1400 U/g), Protease P 6SD (600,000 U/g), Protease A 2SD (100,000 U/g) were supplied by Amano Enzyme Inc. (Nagoya, Japan) and they exhibited both endo- and exopeptidase activities. Papain P1 (100 TU/mg) and Bromelain (100 TU/mg) were donated by Enzybel-BSC (Waterloo, Belgium). Sumizyme BNP-L (45,000 U/g) was obtained from Shin-Nippon chemical Ltd. (Aichi, Japan). SGNIGFPGPK and GLV (> 98% purity) were purchased from WatsonBio, LLC. (Houston, USA). Sodium dodecyl sulfate (SDS), *o*-phthaldialdehyde (OPA), *N*-Acetyl-l-cysteine (NAC), l-leucine, l-tryptophan, myoglobin from equine skeletal muscle, insulin from bovine pancreas and other analytical reagents were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Preparation of protein hydrolysates

A 100 g of minced beef was diluted 1:2 with water, while plasma was not diluted due to the low solid content. Afterwards, the temperatures and pH of samples were adjusted to the optimal values for the selected protease in a water bath. When equilibrium was reached, the proteases were added to the solutions to a final concentration of 0.5% (w/w) with continuous stirring. The optimal temperatures and pH values suggested by the manufacturers were as follows: Alcalase (pH 7.5, 50 °C), Flavourzyme (pH 7.0, 50 °C), Protamex (pH 7.5, 55 °C), Neutrase (pH 7.0, 50 °C), ProteAX (pH 7.0, 50 °C), Protease P 6SD (pH 7.0, 40 °C), Protease A 2SD (pH 7.0, 50 °C), Papain P1 (pH 7.0, 55 °C), Bromelain (pH 7.0, 40 °C) and Sumizyme BNP-L (pH 7.0, 50 °C). Protein hydrolysates of 20 mL were withdrawn after 1, 2, 3, 4, and 5 h, respectively. At each hydrolysis time point, the hydrolysis was terminated by heating at 95 °C (minced beef) and 85 °C (plasma) for 15 min. Thereafter, the samples were cooled to room temperature and the supernatant was recovered by centrifugation at 8000*g* for 15 min using a bench centrifuge, Sigma Laborzentrifugen 3 k15 (Osterode am Harz, Germany).

2.3. Determination of the yield of hydrolysate

The dry matter of hydrolysates was measured using Memmert Universal Oven UF110 (Schwabach, Germany) by determining the moisture loss of samples at 105 °C until constant weight. The yield was expressed as the percentage of dry matter content of supernatant in relation to the dry matter of hydrolysates.

2.4. Determination of DH

The OPA method was used to determine DH of hydrolysates (Spellman, McEvoy, O'cuinn, & FitzGerald, 2003), which is based on specific reaction between OPA and amino groups in the presence of a thiol group. Briefly, the OPA reagent was prepared by combining 10 mL of 50 mM OPA in methanol, 10 mL of 50 mM NAC in Milli-Q water, 5 mL of 20% (w/v) SDS and 75 mL of borate buffer (0.1 M, pH 9.5). The OPA reagent was kept from the light and stirred for 60 min prior to use. The OPA assay was initiated by mixing an aliquot of 10 μL of sample or standard and 1.2 mL of OPA reagent. After incubation for 10 min at room temperature, the absorbance was measured at 340 nm using a Thermo Scientific UV–Vis spectrophotometer, model Helios Omega (Loughborough, UK). Leucine was used as the standard for calculation of the equivalent amount of free amino groups for DH determination. The total amount of amino groups was determined in protein samples by incubation with 6 mol/L HCl at 110 °C for 24 h. The DH was calculated using the following equation:

$$\mathrm{DH} = \frac{(NH_2)_{Tx} - (NH_2)_{T0}}{(NH_2)_{Total} - (NH_2)_{T0}} \times 100\%$$

where $(NH_2)_{Tx}$ is the free amino group content of hydrolysates (μmol/mL), $(NH_2)_{To}$ is the free amino group content of unhydrolysed sample (μmol/mL) and $(NH_2)_{Total}$ is the total amount of free amino groups in protein samples (μmol/mL).

2.5. Estimation of peptide MW distribution

MW distribution of hydrolysates (supernatants) was analysed by size exclusion chromatography under isocratic conditions using a Phenomenex BioSep™ SEC-S2000 column (300 mm × 4.6 mm) (Torrance, CA, USA) on an ultra-high performance liquid chromatography (UHPLC) system (Thermo Scientific Dionex Ultimate 3000, Denmark). Data were processed and acquired via Chromeleon 7.0 Chromatography Data System software. Ten microliters of each hydrolysate (1 mg/mL) were injected, eluted isocratically using 30% acetonitrile containing 0.1% trifluoroacetic acid at a flow rate of 0.5 mL/min and monitored at 214 nm. The MW calibration curve was