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Effects of exopeptidase treatment on antihypertensive activity and taste attributes of enzymatic whey protein hydrolysates

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ABSTRACT

The objectives of this study were to investigate the effects of exopeptidase treatment on ACE-inhibitory activity, antihypertensive activity and taste of whey protein hydrolysates (WPHs). WPH with high ACE-inhibitory activity (IC_{50} = 0.15 mg/mL) was treated with carboxypeptidase (Accelerzyme® CPG), aminopeptidase (Peptidase R), or an aminopeptidase and proteinase mixture (ProteAX). The exopeptidase-treated hydrolysates exhibited ACE-inhibitory activity (IC_{50} = 0.24–0.34 mg/mL) and decreased systolic blood pressure (–12 to –31 mm Hg) in spontaneously hypertensive rats for 24 h after a single administration of 100 mg/kg body weight. The highest ACE-inhibitory activity was associated with the 200–1000 Da fractions in all exopeptidase-treated hydrolysates. Exopeptidase treatment significantly lowered bitterness, increased umami and salty tastes, and increased overall acceptability of the starting WPH, changes that may be due to release of certain terminal amino acids. Therefore, exopeptidase treatment may be a viable debittering method for bitter-tasting, antihypertensive protein hydrolysates, before incorporation into functional foods.

1. Introduction

Hypertension, the main risk factor for cardiovascular disease, accounts for 45 and 51% of heart disease- and stroke-related deaths worldwide, respectively, and causes more than nine million deaths annually (World Health Organization, 2013). In Canada, synthetic angiotensin I-converting enzyme (ACE) inhibitors are the class of drugs most commonly prescribed for cardiovascular diseases (Public Health Agency of Canada, 2009). These drugs inhibit ACE, an enzyme that promotes sodium retention and vasoconstriction, to effectively control hypertension (Ritter, 2011). Although effective, synthetic ACE inhibitors are associated with undesirable side effects such as chronic dry cough or angioedema, the latter of which can lead to serious respiratory distress requiring ventilatory support or,

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in rare cases, death (Weber & Messerli, 2008). Therefore, there is growing interest in natural ACE inhibitors in the form of peptides from food protein hydrolysates, which have not been associated with the aforementioned side effects, for use in functional foods aimed to help in the management of hypertension.

However, bitterness has been experimentally correlated with ACE-inhibitory activity in protein hydrolysates (Cheung & Li-Chan, 2010) and predicted by computer modeling of peptides, particularly those of shorter chain lengths (Pripp & Ardö, 2007; Tan et al., 2013). Furthermore, the most bitter fraction of a whey protein hydrolysate also had the highest ACE-inhibitory activity (Cheison, Wang, & Xu, 2007). This relationship between ACE inhibition and bitterness may exist because both attributes have been associated with low molecular weight peptides containing hydrophobic amino acid residues. Hydrophobic amino acid residues, especially at the C-terminal, have been shown to be highly influential of ACE-inhibitory activity in peptides (Sagardia, Roa-Ureta, & Bald, 2013; Wang et al., 2011; Wu, Aluko, & Nakai, 2006a, 2006b). Fractions with molecular weights <1000 Da have also been shown to have higher ACE-inhibitory activity than higher molecular weight fractions (O'Loughlin, Murray, FitzGerald, Brodkorb, & Kelly, 2014; Pihlanto-Leppälä, Koskinen, Piilola, Tupasela, & Korhonen, 2000). Similarly, hydrophobic amino acid residues, particularly at the C-terminal, and molecular weights <1000 Da have been correlated with peptide bitterness using computer modeling (Kim & Li-Chan, 2006).

Since hydrolysates often taste bitter, an attribute that can decrease acceptance and even lead to the rejection of foods (Temussi, 2011), the use of hydrolysates as functional food ingredients can risk decreased consumer acceptance of the target food product. Consumers are reluctant to compromise poor taste for health benefits in functional foods (Verbeke, 2006), with factors such as flavor and texture influencing the continued consumption of new food products regardless of their health benefits (Barrios, Bayarri, Carbonell, Izquierdo, & Costell, 2008). Various methods have been explored for debittering protein hydrolysates to increase their palatability, such as the removal of hydrophobic peptides, masking of bitter taste, and encapsulation of hydrolysates (Hernández-Ledesma, Contreras, & Recio, 2011). Treatment with exopeptidases, which are enzymes that release terminal amino acid residues from peptides, is another promising debittering method that reduces the bitterness of hydrolysates without decreasing yields or requiring the addition of taste-active compounds (Saha & Hayashi, 2001). Aminopeptidases and carboxypeptidases are two types of exopeptidases that cleave amino acid residues from the N- and C-terminal of peptides, respectively. Exopeptidase treatment can decrease hydrolysate bitterness by releasing hydrophobic amino acids from the N-terminal (Nishiwaki, Yoshimizu, Furuta, & Hayashi, 2002), C-terminal (Fu, Li, & Yang, 2011), or both terminals (Ge & Zhang, 1996). Although effective for debittering, however, hydrolysis with exopeptidases may result in cleaving off amino acid residues pertinent to ACE-inhibitory activity. To date, there is limited knowledge on the effects of exopeptidase treatment on ACE-inhibitory activity, or any other bioactivity, of protein hydrolysates. The effect of exopeptidase treatment on the full taste profile of protein hydrolysates has also been rarely addressed.

The objective of this study was to assess whether exopeptidase treatment is an appropriate debittering method for hydrolysates with ACE-inhibitory activity. Hydrolysates of whey protein have been widely studied for ACE-inhibitory activity (Abubakar, Saito, Kitazawa, Kawai, & Itoh, 1998; O'Loughlin et al., 2014; Pihlanto-Leppälä et al., 2000; Wang, Mao, Cheng, Xiong, & Ren, 2010; Wang et al., 2012) and have been reported to have bitter taste (Cheison et al., 2007; Leksrisompong, Gerard, Lopetcharat, & Drake, 2012; Leksrisompong, Miracle, & Drake, 2010). Therefore, whey protein was selected as the substrate for producing hydrolysates in the current study. The effects of exopeptidase treatment on *in vitro* ACE-inhibitory activity, molecular size distribution, *in vivo* antihypertensive activity in spontaneously hypertensive rats, as well as taste, overall acceptability and amino acid profile of the resulting whey protein hydrolysates were investigated.

2. Materials and methods

2.1. Materials

NZMP™ whey protein isolate (WPI) 895 donated by Fonterra Co-operative Group (Rosemont, IL) was used as the protein source for hydrolysates production. Protease M "Amano" SD, Protease P "Amano" 6SD, Thermoase PC10F, Protin SD-AY10, Protin SD-NY10, Peptidase R, and ProteAX were donated by Amano Enzyme U.S.A, Ltd. (Elgin, IL). Validase® Papain Liquid, Maxazyme® NNP DS, and Accelerzyme® CPG were donated by DSM Food Specialties B.V. (Delft, The Netherlands). WPH 4003 was donated by PGP International (Eagan, MN), Hilmar™ 8350 and Hilmar™ 8390 were donated by Hilmar Ingredients (Hilmar, CA), and NZMP™ WPH 917 (Fonterra Co-operative Group) was donated by Caldic Canada Inc. (Delta, BC).

Trichloroacetic acid (TCA), food grade hydrochloric acid (HCl), sodium hydroxide (NaOH) and caffeine meeting Food Chemical Codex requirements, and citric acid anhydrous meeting United States Pharmacopeia requirements were from Fisher Scientific (Fairlawn, NJ). Hippuryl-His-Leu-OH (HHL) was from New Bachem (Torrance, CA), while 2,4,6-trinitrobenzenesulfonic acid (TNBS) was from Thermo Scientific (Rockford, IL). Rabbit lung angiotensin I-converting enzyme, spectrophotometric-grade ethyl acetate, blue dextran, vitamin B_{12}, L-carnosine, and Leu were from Sigma-Aldrich (St. Louis, MO). An antifreeze protein was donated by A/F Protein Canada Inc. (St. Johns, NF). Trp and Ala were from Bio Basic Inc. (Markham, ON). All other laboratory chemicals were of reagent grade. Monosodium glutamate was from Ajinomoto North America, Inc. (Teaneck, NJ). Iodized table salt (Windsor®; Anjou, QC) and sugar (Rogers Sugar Inc.; Vancouver, BC) were purchased locally.

2.2. Hydrolysate production

Hydrolysates were produced in the University of British Columbia (UBC) Food Science program pilot plant (Vancouver, BC) following safe food handling practices. WPI was dissolved in distilled-deionized water (ddH_2O) at 3 g WPI/100 mL, resulting in a solution with pH 6.86 ± 0.09, and preheated with constant stirring in a temperature-controlled water bath (Blue M Electric Company, Blue Island, IL). Incubation