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Antioxidant activity of milk protein hydrolysate in alloxan-induced diabetic rats

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ABSTRACT

The effect of milk protein concentrate (MPC) and milk protein concentrate hydrolysate (MPCH) as antioxidant agents were investigated in rats. Six groups of normal and type-2 diabetic rats were used: (1) healthy normal rats were used as control, (2) healthy normal rats were treated with alloxan as the diabetic group, (3) healthy normal rats treated orally with MPC, (4) diabetic rats treated orally with MPC, (5) healthy normal rats treated orally with MPCH, and (6) diabetic rats treated orally with MPCH. The results of this study concluded that treatments with MPC or MPCH reduced the thiobarbituric acid reactive substance level in normal and diabetic rats. Meanwhile, MPC or MPCH improved the activities of antioxidant enzymes (catalase, superoxide dismutase, reduced glutathione, glutathione-S-transferase, and glutathione peroxidase) in normal and diabetic rats. From the present data, it was concluded that both MPC and MPCH contain potent antioxidants and could improve the health of animals/rats with diabetes mellitus.
Key words: bioactive peptide, type 2 diabetes, free radical, antioxidant enzyme

INTRODUCTION

Bovine milk is an excellent source of proteins of high biological value. It is characterized by a complete AA profile and exceptionally high digestibility. Apart from their obvious nutritive value, milk proteins and products of their degradation (peptides) exert a wide range of biological functions (Szwajkowska et al., 2011). Milk proteins are considered the most important sources of bioactive peptides; these remain inactive within the sequence of the parent protein until they are released by either gastrointestinal digestion or food processing. Bioactive peptides have been defined as specific protein fragments that have a positive effect on body functions or conditions and may ultimately influence health (Kitts and Weiler, 2003). Their beneficial health effects are classified as antimicrobial, immunomodulatory, blood-pressure lowering, antithrombotic, antioxidant (**AO**), and opioid-like, in addition to being cholesterol lowering and enhancers of mineral absorption/bioavailability (Plaisancié et al., 2013).

The in vivo release of bioactive peptides from milk proteins in the gastrointestinal tract results from the action of digestive enzymes such as pepsin and pancreatic enzymes. The efficiency of physiological activity of biopeptides depends on their ability to maintain an integral state during transport to the various functional systems of the body (Vermeirssen et al., 2004; Picariello et al., 2010). Also, studies indicated that bioactive peptides encrypted in major milk proteins are latent within the sequence of the parent protein molecule. They can be liberated by (1) gastrointestinal digestion of milk, (2) fermentation of milk with proteolytic starter cultures, and (3) hydrolysis by proteolytic enzymes. In relation to their mode of action, bioactive peptides may reach target sites at the luminal side of the intestinal tract, or after absorption, in peripheral organs (Kamau et al., 2010).

Most of studies showed that many of the known bioactive peptides have been produced using gastrointestinal enzymes, usually pepsin and trypsin. Angiotensin-converting enzyme inhibitory peptides and calcium-binding phosphopeptides (**CPP**), for example, are most commonly produced by trypsin (Meisel and FitzGerald, 2003; FitzGerald et al., 2004; Gobbetti et al., 2004; Korhonen and Pihlanto, 2006). Peptides generated from the digestion of various proteins are reported to have antioxidative activities. Studies with peptides containing histidine have demonstrated that these peptides can act as metal-ion chelators, active-oxygen quencher, and hydroxyl radical scavenger. The ability of protein hydrolysates to inhibit deleterious

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changes caused by lipid oxidation appears to be related to the nature and composition of the different peptide fractions produced, depending on the protease specificity (Pihlanto, 2006).

Antioxidative peptides from foods are considered to be safe and healthy compounds with low molecular weight, 5 to 16 AA residues, low cost, high activity, and easy absorption. They have some advantages in comparison to enzymatic antioxidants; that is, with simpler structure they have more stability in different situations and no hazardous immunoreactions (Sarmadi and Ismail, 2010). The peptides generated from the digestion of milk proteins are reported to have AO activity. These peptides are composed of 5 to 11 AA including hydrophobic AA, proline, histidine, tyrosine, or tryptophan (Kamau et al., 2010).

Caseins have been shown to provide antioxidant activity against thiobarbituric acid-reactive substance (**TBARS**) content in both Fe/ascorbate induced peroxidation of arachidonic-derived liposomes and model linoleic acid systems (Pihlanto, 2006). Casein phosphopeptides have been reported to have AO activity. Casein phosphopeptides are derived from enzymatic hydrolysis of casein and are rich in phosphoserine residues. The proposed mechanism of AO activity of CPP is linked to the presence of phosphate groups originating from the phosphoserine residues in close proximity to the peptide chain. This creates a polar and anionic domain that can sequester cationic metal ions. Casein phosphopeptides contain the functional domain SerP–SerP–SerP–Glu–Glu. Casein phosphopeptides have been shown to possess 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)-radical scavenging, hydroxyl, peroxyl, and metal chelating activity (Kitts, 2005; Kim et al., 2007).

Phelan et al. (2009) found that casein hydrolysates affected both cellular catalase (**CAT**) activity and glutathione (**GSH**) content in Jurkat cells. In addition, they found that casein hydrolysates contained a certain degree of electron donating capacity as determined by the ferric reducing antioxidant power (**FRAP**) assay (17–32 mmol L^{-1}). Pritchard et al. (2010) found that the bioactive peptides in commercial Cheddar cheese showed the highest inhibition of 2,2-diphenyl-1-picrylhydrazyl (**DPPH**). The results indicate that the higher the concentration of peptide, the higher the inhibition of DPPH. Hydrolysates of whey protein isolate have been shown to possess AO activity. Five-hour digestion with alcalase produced a hydrolysate with strong reducing power (FRAP). When fractionated on the basis of molecular mass, the low molecular weight fraction (0.1–2.8 kDa) was most potent (Peng et al., 2009).

The AO activity of the whey protein concentrate (**WPC**) was attributed to the Cys content and the ability to elevate cellular GSH (Gad et al., 2011). Therefore, the purpose of the present study was to assess the AO activity of milk protein hydrolysate in normal and diabetic rats.

MATERIALS AND METHODS

Cow milk protein concentrate (**MPC**) was obtained from Fonterra Ltd. (Auckland, New Zealand); its analysis as shown by the producer was 69.89% protein, 17% lactose, 1.4% milk fat, 7.2% minerals, and 4.6% moisture. Other chemicals were obtained as follows: trypsin 2,000 U/g (EX pancrease) was obtained from Loba Chemie, Mumbai, India. Alloxan was obtained from Alpha Co., Mumbai, India, and was dissolved in saline solution (0.9% sodium chloride, pH 7). The dose of alloxan (120 mg/kg of BW per d) was chosen to induce diabetes in rats according to previous studies of Mansour et al. (2002) and Sheweita et al. (2002). Acetonitrile, HPLC grade, was from Scharlau, Barcelona, Spain. Trifluoroacetic acid, HPLC grade, was from SDS, Peypin, France. The HPLC peptide standard mixture (product No. H 2016) was obtained from Sigma-Aldrich (St. Louis, MO).

Preparation of Milk Protein Hydrolysates

Preparation of milk protein hydrolysate was carried out according to the method of Otte et al. (2007). Protein solutions (2% wt/wt on a protein basis) were made by dispersion of approximately 2.85 g of milk protein concentrate (according to the protein content) in in 97.15 mL of distilled water to a final weight 100 g, and stirring for 1 h at room temperature. Solutions were hydrated overnight at 5°C, after that, the pH was adjusted to 6.8 or 7.5 by 1% (wt/vol) NaOH solution. Trypsin solution (3.2 U mL^{-1}) was made in distilled water immediately before use. The hydrolysis process was started by addition of trypsin at a concentration of enzyme (units) to substrate (protein) of 80:100 (2×), 160:100 (4×), and 320:100 (8×), and then 1 mL was withdrawn as control sample at zero time. The reaction mixture was incubated at 40°C; interval samples (1 mL) were withdrawn after 3, 6, 9, 20, and 24 h of hydrolysis. The enzyme was inactivated by heating at 90°C for 15 min, and then cooled for 20 min in ice bath and centrifuged (Sigma centrifuge 113, VWR International, Darmstadt, Germany) for 10 min at 10,000 × *g*, 4°C; the supernatant was used for further analyses by reverse-phase (**RP**) HPLC.

Analysis of Peptides by RP-HPLC

Separation of the peptide extracts was carried out using the HPLC system (Agilent Technologies 1260,