J. Dairy Sci. 97:3300–3313
http://dx.doi.org/10.3168/jds.2013-7728

Preventive effect of fermented Maillard reaction products from milk proteins in cardiovascular health

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ABSTRACT

The aim of this study was to determine the dual effect of Maillard reaction and fermentation on the preventive cardiovascular effects of milk proteins. Maillard reaction products (MRP) were prepared from the reaction between milk proteins, such as whey protein concentrates (WPC) and sodium caseinate (SC), and lactose. The hydrolysates of MRP were obtained from fermentation by lactic acid bacteria (LAB; i.e., *Lactobacillus gasseri* H10, *L. gasseri* H11, *Lactobacillus fermentum* H4, and *L. fermentum* H9, where human-isolated strains were designated H1 to H15), which had excellent proteolytic and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities (>20%). The antioxidant activity of MRP was greater than that of intact proteins in assays of the reaction with 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt and trivalent ferric ions; moreover, the effect of MRP was synergistically improved by fermentation. The Maillard reaction dramatically increased the level of antithrombotic activity and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) inhibitory effect of milk proteins, but did not change the level of activity for micellar cholesterol solubility. Furthermore, specific biological properties were enhanced by fermentation. *Lactobacillus gasseri* H11 demonstrated the greatest activity for thrombin and HMGR inhibition in Maillard-reacted WPC, by 42 and 33%, respectively, whereas hydrolysates of Maillard-reacted SC fermented by *L. fermentum* H9 demonstrated the highest reduction rate for micellar cholesterol solubility, at 52%. In addition, the small compounds that were likely released by fermentation of MRP were identified by size-exclusion chromatography. Therefore, MRP and hydrolysates of fermented MRP could be used to reduce cardiovascular risks.

Received November 17, 2013.
Accepted February 18, 2014.
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Key words: prevention of cardiovascular diseases, antioxidant activity, lactic acid bacteria, Maillard reaction, milk protein

INTRODUCTION

Cardiovascular diseases (**CVD**), which are currently an issue in both adults and children and are often caused by unhealthy diet, stress, and lack of exercise (i.e., lifestyle), have been the leading cause of death worldwide in recent years, with consistent increases in mortality year over year. Many of the major risk factors for coronary disease have been identified, and researchers are studying different modifiable factors that may influence CVD. Several studies have focused on the effects of various biological or chemical compounds on CVD, such as inhibition of angiotensin-converting enzyme (**ACE**), reduction of cholesterol solubility, fibrinolytic activity, antithrombotic activity, and antioxidant activity, among others (Houston, 2005; Chung et al., 2008; Miguel et al., 2009).

Under oxidative stress, oxidative modifications of low density lipoprotein occur, leading to atherosclerosis. Free radicals generated from reduction of oxygen may cause cellular damage and contribute to atherosclerosis, arthritis, diabetes, and carcinoma (Halliwell, 1994; Aviram, 2000). The major effect of antioxidants is to prevent the formation of oxidized low density lipoprotein and severe tissue injury. Thrombin is one of the major pathogenic factors in CVD. It is a multifunctional protease generated at sites of vascular injury that acts to trigger fibrin formation, platelet aggregation, and chemotaxis for monocytes, fibroblasts, and vascular smooth muscle cells (Coughlin, 1994). However, excessive generation of fibrin due to activation of the coagulation cascade by thrombin leads to thrombosis, triggering blockage of a vessel and causing various cardiovascular disorders (leading to CVD). Hypercholesterolemia (elevated serum cholesterol) also plays a major role as it leads over time to atherosclerosis and CVD. Serum cholesterol is produced both through

Table 1. Chemical composition (means ± SD) of whey protein concentrate (WPC) and sodium caseinate (SC) used in this study

Component	WPC	SC
Moisture (%)	4.6 ± 0.2	4.0 ± 0.3
Protein (% dry basis)	97.7 ± 1.0	97.8 ± 0.8
Fat (%)	0.3 ± 1.0	1.0 ± 0.3
Ash (%)	1.9 ± 0.3	3.7 ± 0.2
Lactose (%)	7.66 ± 0.1	0.14 ± 0.2

hepatic cholesterol synthesis, the rate of which is controlled by 3-hydroxy-3-methylglutaryl-CoA reductase (**HMGR**), and through intestinal cholesterol uptake. Many studies have been performed in which the control of serum cholesterol levels via HMGR inhibition or suppression of cholesterol uptake through reduction of cholesterol solubility have been evaluated (Daniel et al., 2003; Ngamukote et al., 2011; Duangjai et al., 2013).

Milk proteins have many important health benefits and hold considerable nutritional value. Additionally, they are precursors of diverse bioactive compounds that can be released by enzymatic hydrolysis during food processing (including intestinal digestion and the fermentation of milk). The bioactive peptides derived from milk proteins may have antioxidant, immunomodulating, antithrombotic, antihypertensive, and antibacterial properties (Meisel, 1997; Gobbetti et al., 2000; Smacchi and Gobbetti, 2000). In particular, Maillard reaction products (**MRP**), which are produced by reactions between carbonyl and amine groups, not only produce food characteristics such as aroma, color, flavor, and texture (Fayle and Gerrard, 2002) but also increase the antioxidant activity of milk proteins (McGookin and Augustin, 1991; Chevalier et al., 2001). Moreover, our group has reported that the biological characteristics and antioxidant activity of milk proteins were improved by the combination of the Maillard reaction and enzymatic hydrolysis (with commercial proteases; Oh et al., 2013).

Few studies exist on the potential preventive cardiovascular effects (reduction of cholesterol uptake, inhibition of thrombin and HMGR, fibrinolytic activity) of milk-derived compounds. Therefore, the qualities of MRP fermented by lactic acid bacteria (**LAB**) were studied. Furthermore, the aim of this study was to determine the effects of MRP and fermented MRP on antioxidant activity, antithrombotic activity, HMGR inhibition activity, and cholesterol reduction activity for reduction of cardiovascular risks.

MATERIALS AND METHODS

Chemicals

Whey protein concentrate-80 (**WPC**) and sodium caseinate (**SC**) were obtained from Davisco Foods International Inc. (Le Sueur, MN) and Kerrygold (Dublin, Ireland), respectively, and their compositions are presented in Table 1. Lactose monohydrate was purchased from Junsei Chemical Co. (Tokyo, Japan). The chemicals, including *o*-phthaldialdehyde (**OPA**), 1,1-diphenyl-2-picrylhydrazyl (**DPPH**), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (**ABTS**), potassium persulfate, 2,4,6-tripyridyl-s-triazine (**TPTZ**), L-ascorbic acid, iron (III) chloride hexahydrate, fibrinogen, thrombin, and cholesterol used in this study were purchased from Sigma Chemical Co. (St. Louis, MO). Disodium monophosphate, monosodium phosphate, and iron (II) sulfate heptahydrate were from Showa Chemicals (Osaka, Japan). All chemicals used were of analytical grade.

Preparation of MRP from Milk Proteins

Whey protein concentrate, SC, and lactose were dissolved in 0.1 *M* sodium phosphate buffer (pH 7.4) at a 1:5 (wt/wt) ratio of protein (10 mg) and sugar (50 mg). The reaction was allowed to proceed with shaking at 60 rpm in a water bath at 55°C for 1 d. The pH was not controlled during the reaction. Then, reaction mixtures were extensively dialyzed against 0.1 *M* sodium phosphate buffer (pH 7.4) 3 times within 24 h and were lyophilized.

Fermentation of MRP from WPC-Lactose and SC-Lactose

A total of 20 strains of lactic acid bacteria strains isolated from humans and plants (human-isolated strains were designated H1 to H15; plant-isolated strains were designated P1 to P5)in this study were obtained from the Food Microbiology Laboratory, Division of Food Bioscience and Technology, Korea University (Seoul, Korea). All strains were activated 3 times successively in de Man, Rogosa, and Sharpe (**MRS**) broth (Difco Laboratories, Detroit, MI) at 37°C for 18 h before use. All stock cultures were maintained at −80°C with sterile 50% (vol/vol) glycerol as a cryoprotectant. The strains were subcultured 3 times before use. Fermentation of MRP from WPC-lactose and SC-lactose was performed in MRP solution (Table 2). Bacterial cells (10^9 cfu/mL) were inoculated into MRP solution and incubated at 37°C. Samples were withdrawn after 12, 24, 36, and 48 h of fermentation and were adjusted to pH 7.5 with 1 *N* NaOH. Samples were then centrifuged at 2,000 × *g* for 30 min, and the supernatants were collected.

Proteolytic Activity

Proteolytic activity was measured according to the method described by Nielsen et al. (2001) using the