Production of Functional High-protein Beverage Fermented with Lactic Acid Bacteria Isolated from Korean Traditional Fermented Food

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Abstract
The aim of this study was to manufacture functional high protein fermented beverage, using whey protein concentrate (WPC) and Lactobacillus plantarum DK211 isolated from kimchi, and to evaluate the physicochemical, functional, and sensory properties of the resulting product. The fermented whey beverage (FWB) was formulated with whey protein concentrate 80 (WPC 80), skim milk powder, and sucrose; and fermented with Lactobacillus plantarum DK211 as single, or mixed with Lactococcus lactis R704, a commercial starter culture. The pH, titratable acidity, and viable cell counts during fermentation and storage were evaluated. It was found that the mixed culture showed faster acid development than the single culture. The resulting FWB had high protein (9%) and low fat content (0.2%). Increased viscosity, and antioxidant and antimicrobial activity were observed after fermentation. A viable cell count of $10^9$ CFU/mL in FWB was achieved within 10 h fermentation, and it remained throughout storage at 15°C for 28 d. Sensory analysis was also conducted, and compared to that of a commercial protein drink. The sensory scores of FWB were similar to those of the commercial protein drink in most attributes, except sourness. The sourness was highly related with the high lactic acid content produced during fermentation. The results showed that WPC and vegetable origin lactic acid bacteria isolated from kimchi might be used for the development of a high protein fermented beverage, with improved functionality and organoleptic properties.

Key words: whey protein, fermented beverage, Lactobacillus plantarum, antioxidant activity, sensory analysis

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Introduction
Probiotic bacteria has been shown to have effects in enhancement of the immune system, and in prevention of gut, vaginal, and urogenital infections, diarrhea, and gastritis, by inhibiting enteric and foodborne microbial pathogens (Walsh et al., 2010). Consumers’ interest in functional foods containing probiotic bacteria and prebiotics has created a huge market, and their market share is still expanding (Rathore et al., 2012; Walsh et al., 2010). The consumer request for food with nutritional benefits is also quickly improved, which has generated the manufacture of various value-added dairy products (Shiby et al., 2013). Recently, the sales of ready-to-drink (RTD) protein drinks have been increased through mainstream supermarket distribution.

The value of proteins as an essential source of amino acids is well documented, but recently it has been recognized that dietary proteins exert many other functionalities in vivo, by means of biologically active peptides. Such peptides are inactive within the sequence of the parent protein, and can be released by digestive enzymes during gastrointestinal transit, or by fermentation or ripening during food processing (Korhonen, 2009). In particular, milk proteins are regarded as a source of energy and of essential amino acids, which are needed for growth and maintenance of physiological functions (Unal and Akaln, 2012).

Whey protein, a by-product recognized as a valuable food ingredient with important nutritional and functional properties, is gaining acceptance as a functional food ingredient. Commercial whey proteins are considered a GRAS (Generally Recognized As Safe) substance for food product applications (Sinha et al., 2007). A wide variety of whey ingredients are available for use in the manufacture of yogurt and fermented beverages, including sweet whey powder (SWP), whey protein concentrate (WPC), whey protein isolate (WPI) and specialized WPCs (Hugunin, 2008). Whey proteins possess high biological value, and are superior to other proteins, such as those of egg, soy and caseins of milk, mainly due to their high content of branched essential amino acids (Pescuma et al., 2010;
Whey-based lactic beverages represent an emerging segment of non-conventional dairy products that require sensory, physical, and chemical characterization for quality control and product development (Almeida et al., 2009). However, consumer acceptance of these health drinks depends on the development of nutritional beverages that maintain their desirable appearance, texture, and flavor characteristics during storage and consumption (Shiby et al., 2013). Numerous formulations of liquid products based on whey proteins have been developed to improve their characteristics (Almeida et al., 2009; Athanasiadis et al., 2004; Djurić et al., 2004; Pescuma et al., 2010: Shiby et al., 2013). However, the protein contents of products in their studies are less than 5%. Whey proteins that are not modified to have more heat stability will not be stable as the sole ingredient at levels above 3% protein, that is, they will gel or precipitate under high heat treatment (Rittmanic, 2008). A typical yogurt and Greek yogurt provides on average 3 and 6.7 g protein/100 g serving, respectively. The protein contents in commercial protein drinks or shakes are mostly between 6 and 10%.

The aim of this study was to formulate a novel functional fermented whey beverage containing high protein content, by using whey protein, and lactic acid bacteria isolated from Korean traditional fermented foods. However, a potential issue with culture is the use of vegetable-origin probiotic in a milk-based product containing high protein content; consequently, this probiotic would be potentially unsuited to growth in milk-based product. Moreover, the incubation time is highly related with the production capacity in plant. The reduction in fermentation time could increase the production capacity of plant, and significantly reduce production costs (Hugunin, 2008). A commercial strain, Lactobacillus plantarum (LP-5, Culture System Inc, USA), Staphylococcus thermophiles (ST-Body 1, Christian Hansen, Denmark), and Lactococcus lactis (L. lactis R704, Christian Hansen, Denmark) were used in this study depending on the purpose of use.

Whey protein concentrate (WPC 80) and skim milk were purchased from Sung Poon Co. (Korea) and Seoul milk (Korea), respectively. Table 1 shows the compositions of these dairy ingredients. Sucrose was obtained from CJ Co. (Korea). All other chemicals and reagents used were of analytical grade, and were purchased from Sigma-Aldrich (USA).

**Fermentation condition**

Samples were withdrawn every 2 h during 24 h incubation, for the measurements of pH, titratable acidity, and viable cell counts. Bacterial growth was observed with enumerated viable colony at the MRS agar plates incubated at 37°C for 48 h.

**Manufacture of fermented whey beverage**

Fermented whey beverage was prepared, using 11% (w/v) WPC 80, 2% (w/v) skim milk powder, and 10.3% sugar. Each strain was subcultured triplicate in MRS broth at 37°C. All dry ingredients were dissolved in sterile water, and homogenized with a homomixer (IKA, Japan) at 10,000 rpm. This mixture was then pasteurized at 70°C for 30 min, cooled to about 40°C, inoculated culture at a rate of 20 mL/L (10^8 CFU/mL), and fermented at 37°C for 10 h. The resulting FWB was distributed in sterile glass bottles, and stored at 4, 10, and 15°C for 28 d. Viable cell count, pH, and titratable acidity were measured after 0, 7, 14, 21, and 28 d of storage. The FWBs containing commercial strains (LH 166 or ST-Body 1) were also prepared as above, except for the fermentation time. The fermentation time of FWB with Lactobacillus helveticus LH 166 and that with ST-body were 13 h and 14.5 h, respectively.

| Table 1. The composition of whey protein concentrate (WPC) and skim milk powder (SMP) |
|-----------------------------------------|----------|----------|
| Concentration (g/100 g powder)         | WPC      | SMP      |
| Protein                                | 80       | 35       |
| Fat                                    | 0.1      | 52       |
| Lactose                                | 6.5      | 1.0      |

Hence, this study was conducted (1) to optimize the conditions to develop a fermented whey beverage with acceptable organoleptic properties, (2) to determine functional properties, such as antioxidant activity and antimicrobial activity, and (3) to assess the shelf life of the product.

**Materials and Methods**

**Strains and materials**

The strain, Lactobacillus plantarum DKL 121 was isolated from kimchi samples, and maintained in glycerol stocks at -20°C. Four commercial strains: Lactobacillus helveticus (LH 166, Culture System Inc, USA), Lactobacillus plantarum (LP-5, Culture System Inc, USA), Streptococcus thermophilies (ST-Body 1, Christian Hansen, Denmark), and Lactococcus lactis (L. lactis R704, Christian Hansen, Denmark) were used in this study depending on the purpose of use.

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