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Ultra-high temperature plus dynamic high pressure processing: An effective combination for potential probiotic fermented milk processing which attenuate exercise-induced immune suppression in Wistar rats

*Pablo Christiano B. Lollo *, Priscila Neder Morato, Carolina Soares de Moura, Miguel Meirelles de Oliveira, Adriano Gomes Cruz, José de Assis F. Faria, Jaime Amaya-Farfan, Marcelo Cristianini*

Department of Food and Nutrition, Faculty of Food Engineering, University of Campinas, Campinas, São Paulo, Brazil

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ABSTRACT

Intense physical activity results in a substantial volume of stress and hence a significant probability of immunosuppression in athletes. Consumption of a probiotic can attenuate immune suppression induced by exhausting exercise in rats. A potential probiotic fermented milk was manufactured from milk treated by dynamic high pressure (P.F. Milk DHP) containing *Strep. Salivarius thermophilus* ssp. TA 040 and *Lactobacillus acidophilus* LA14 was fed for 2 weeks to Wistar rats, which then were brought to exhaustion on the treadmill. Two hours after exhaustion, the rats were killed and material was collected for the determination of serum parameter of health and immune system. The immune suppression exercise-induced was observed by alteration in lymphocytes, monocytes, leukocytes and neutrophil counts in all exhausted groups, but it was attenuated by consumption of P.F. Milk DHP. Thus, P.F. Milk DHP was effective in enhancing the immune system and could improve the health status as in Wistar rats.

1. Introduction

Fermented milk products, the main type of foods matrices that are supplemented by probiotic bacteria, have been the subject of numerous studies covering both technological aspects (Granato, Branco, Cruz, Faria, & Shah, 2010) and health benefits (Tripathi & Giri, 2014; Wang et al., 2012).

Indeed, it is well established that the regular consumption of probiotic food can benefit the health by improving the immune system, increasing resistance against infections in the upper respiratory tract of athletes (de Vrese et al., 2006),

* *Corresponding author*. Department of Food and Nutrition, Faculty of Food Engineering, University of Campinas – UNICAMP, 80, Monteiro Lobato Street, Campinas, São Paulo 13083-862, Brazil. Tel.: +55 19 98418305; fax: +55 19 35214060.
E-mail address: pablo.christiano@gmail.com (P.C.B. Lollo).

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common infectious illnesses and gastrointestinal infections (West et al., 2011). Stressful situations, such as those derived from intense physical exercise, can increase the incidence of gastrointestinal disease episodes, particularly of diarrhea during heavy training (Mackinnon, 2000), and are likely to cause an increased susceptibility to infections of the upper respiratory tract (Mackinnon, 2000); this is due in part to a decline in immune system functions as a result of exhaustion following successive sessions of intense and prolonged exercise (Pedersen & Hoffman-Goetz, 2000). Probiotics may also help in preventing illness during heavy training and competition, which is a priority for athletes, coaches and exercise scientists, who are all interested in minimizing gastrointestinal disorders, particularly diarrhea, during travel to international competitions, as it can adversely affect adaptation periods and physical performance (Pyne & Gleeson, 1998).

There are both direct and indirect benefits of dietary supplementation by probiotics for athletes, in that they reduce gastrointestinal disorders and improve the immune system, decreasing infections. Therefore, probiotics could be used to maximize athletic performance indirectly by preventing the immune suppression that can arise from prolonged sessions of intense physical exercise and therefore reducing the athlete's susceptibility to disease (Nichols, 2007) and the incidence of acute infections, diarrhea, and their associated symptoms (Guarino, Lo Vecchio, & Canani, 2009).

Dynamic high pressure (DHP), also known as high pressure homogenization, is a promising emerging technology that uses a similar principle to conventional mixing but achieves pressures up to 400 MPa, in which the high turbulence, shear and cavitation forces are the major effects causing changes in the fluid (Floury, Bellettre, Legrand, & Desrumaux, 2004). This technology has been widely studied in the alteration of some milk constituents, including: reducing the size of fat globules (Serra, Trujillo, Quevedo, Guamis, & Ferragut, 2007) and changing the functionality of proteins (Serra, Trujillo, Guamis, & Ferragut, 2009); and recently providing evidence of aspects of the functionality of probiotic cultures such as improved acid tolerance, bile tolerance, and protease activity (Muramalla & Aryana, 2011; Tabanelli et al., 2012). Dairies using probiotic milk processed by DHP reported a higher viability of *Lactobacillus paracasei* in fermented milk after 30 days of storage (Patrignani et al., 2007) and of *Lactobacillus acidophilus* in cheese (Crescenza) after 12 days of storage (Burns et al., 2008).

In addition, fat exerts great importance on the characteristics of fermented milk, when processed by DHP (Oliveira, Augusto, Cruz, & Cristianini, 2014). Previous studies conducted in our laboratory demonstrated that it is possible to increase the consistency of fermented milk with reduced fat concentration (2%). The milk is processed thermally (95 °C/5 min) before the DHP process, this procedure favors synergistic effect between precipitation of β-lactoglobulin (heat treatment) and changes in fat globule. Thus, the manufacturing of fermented milk increased the parameters of viscoelasticity (G') by 15% for the sample processed at 180/5 MPa compared to the control sample (5/15 MPa) (124.67Pa·sn'). Furthermore, confocal microscopy images showed that DHP changed the characteristics of the matrix, increasing the interactions between protein and fat, due to the drastic reduction in the size of fat globules (Oliveira et al., 2014). In this sense, the authors of this study focused on developing fermented milk with higher consistency using a low fat concentration of 2%. To achieve that, it was necessary to perform a process of heat treatment combined with DHP. Moreover, the effects of dynamic high pressure were isolated, being the only variable the high pressure processes. Thus, the results found in this study were caused only by the different pressures applied to milk.

In this sense, the aim of this study was to evaluate the efficiency of potential probiotic fermented milk manufactured from milk treated simultaneously using ultra-high temperature and dynamic high pressure in maintaining the immune system of rats exercised to exhaustion after receiving probiotic supplementation for 14 days.

2. Materials and methods

2.1. Animals

Male (21-day-old, specific pathogen-free) Wistar rats, bred at the Multidisciplinary Centre for Biological Research (University of Campinas, SP, Brazil), were housed (~22 °C, 55% relative humidity, alternating 12-h light/12-h dark cycles) in individual growth cages, with free access to commercial chow (Labina, Purina, Brazil) and water at all times, until they reached a body weight (BW) of 160.8 ± 7.1 g. The research methodology was approved by the Ethics Committee on Animal Experimentation (CEEA-UNICAMP, protocol 2345-1). The animals were randomly assigned to one of 8 groups, depending on whether they were fed a diet of probiotic fermented milk, conventional fermented milk, or fermented milk produced from milk treated by dynamic high pressure at 100 and 200 MPa, and whether rats were exercised or remained sedentary. 15 days daily, was given by gavage 4 mL each of beverages tested, the control group received 4 mL of water administered by gavage also (Fig. 1). This study was conducted in the University of Campinas, Campinas/SP – Brazil and adhered to the animal care standards of the American College of Sports Medicine.

2.2. Fermented milk processing

The probiotic fermented milk processing is completely described in (Oliveira et al., 2014). UHT milk (2% fat, 3% protein and 10.4% total solids w/v%; Shefa, São Paulo, Brazil) was submitted to heat treatment (90 °C for 5 min), cooled to 60 °C, and subjected to high-pressure processing using a dynamic Panda Plus (GEA-Niro-Soavi, Parma, Itália). The first two stages at 15 MPa and the second at 5 MPa (homogenization treatment conventionally applied in the dairy industry) were used as the control treatment, and the other treatments were subjected to high dynamic pressure in a single stage at 100 and 200 MPa. The milk was then immediately cooled to 43 °C, and cultures of *Strep. Salivarius thermophilus* ssp. TA 040 and *L. acidophilus* NFCM® (Danisco, São Paulo, Brazil) were added to obtain concentrations of approximately 8 and 7 log cfu/mL, respectively (Taverniti, Scabiosi, Arioli, Mora, & Guglielmetti, 2014). The inoculated milk was fractionated in glass jars (150 mL) and subsequently submitted to fermentation at 43 °C; the pH was