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The utilization of oat polar lipids produced by supercritical fluid technologies in the encapsulation of probiotics

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ABSTRACT

Oats contain ingredients that have potential health-promoting properties. Oat lipids contain several essential fatty acids. The polar lipids in oats include glycolipids and phospholipids, which are typically extracted from oat flakes with polar organic solvents. Supercritical carbon dioxide (SC-CO_2) has been proposed as an alternative to organic solvents in the food sector for environmental and safety reasons. SC-CO_2 was used in this study without and with ethanol as a co-solvent to isolate the polar lipid fractions from oat flakes with or without heat treatments. The polar lipids were collected as a solution in ethanol and precipitated using SC-CO_2 as an antisolvent. The fatty acid compositions of different lipid fractions were determined. The precipitated oat polar lipids were tested as an encapsulative and protective agent of probiotics in a human digestive tract simulation. The protective effects of the oat polar lipids were evaluated by measuring the gas production, microbial activity, acetic and lactic acid production, and pH changes in different test mediums. The results demonstrate that the oat polar lipids are able to protect *Bifidobacterium breve* in a phosphate buffer, thereby providing a useful stabilization method to improve the shelf life of probiotic products.

1. Introduction

Oats are an important source of nutrients; they contain the protein, digestible carbohydrates and dietary fiber fractions required for a balanced human diet. For a long time, oats were mainly used as feed for animals, but recent findings have pointed out new possibilities for the health-promoting properties of oats and oat products (Brennan & Cleary, 2005).

The oil content in oats is high, typically 2–12% (Zhou, Robards, Glennie Holmes, & Helliwell, 1999), and the lipid content appears to vary widely depending on the varieties of oat or the cultivar (Lockhart & Hurt, 1986). However, an increase in the total lipid content seems to be accompanied by an increase in all individual fatty acids (Zhou, Glennie Holmes, Robards, & Helliwell, 1998). Youngs, Puskulcu, and Smith (1977) reported that the majority of oat lipids are bound in the endosperm. White, Fisk, and Gray (2006) found most of the lipid bodies in the aleurone and germ fractions of milled oats, and Doehlert, Moreau, Welti, Roth, and Mcmullen (2010) reported that oat kernels appear to contain much higher polar lipid concentrations than other plant tissues. Oat lipids contain several essential fatty acids (Youngs, 1986) and a very high level of antioxidants (Peterson, 2001), but the high lipid content makes oat lipids susceptible to rancidity problems during processing (Lehtinen, Kiiliäinen, Lehtomäki, & Laakso, 2003).

The separation of oat lipids is usually based on their different polarity, and the extraction efficiency of cereal lipids is strongly dependent on solvent-related parameters, such as the solvent, extraction time and method used (Alkio, Aaltonen, Kervinen, Forssell, & Poutanen, 1991; Youngs, 1986). Oats contain a large proportion of glycolipids, for example digalactosyl diacylglycerol (DGDG) (Andersson, Demirbuker, & Blomberg, 1997; Aro, Järvenpää, Könkö, Huopalahti, & Hietaniemi, 2007; Fors & Eriksson, 1990; Forssell, Kervinen, Alkio, & Poutanen, 1992). Moreover, due to its surface-active properties, researchers have shown that oat oil rich in polar lipids forms a fine particle emulsion, which has been used in self-emulsifying systems in drug delivery (Gren and Kaufman, 2002; Odeberg, Kaufmann, Kroon, & Höglund, 2003). During aqueous dispersing, oat polar lipids spontaneously form liposomal structures (microcapsules), and therefore they are able to encapsulate various sensitive substances within capsular structures.

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In our previous paper (Aro et al., 2007), we described a method for isolating and precipitating the pure polar lipid fraction from oat flakes using a supercritical antisolvent technique (*SAS removed*). In this study, we tested the influence of heat treatment on oat flakes in order to enhance the extractability and stability of neutral lipids, and thus increase the yield of polar lipids during an additional precipitation process. The chemical characteristics of the oat-based, polar lipid precipitate and delipidated oat flakes were determined. The precipitated oat polar lipids were further divided into different lipid classes.

Within the food industry, the encapsulation of probiotics is used to protect the cells against an unfavorable environment. Moreover, the coating material must be food grade and able to form a barrier to protect the encapsulated substance (Burgain, Gaiani, Linder, & Scher, 2011). Rokka and Rantamäki (2010) recently reviewed the most commonly used technologies and the future challenges for the microencapsulation of probiotics. The aim of this study was to test different conditions for isolating the oat polar lipids with supercritical carbon dioxide (SC-CO_2). Further, the probiotic bacterial suspensions of *Bifidobacterium breve* and polar oat lipids produced as a result of SC-CO_2 testing were prepared with and without maltodextrose. We tested the possible protective effect of the oat polar lipids for bacteria living in the human colon using refrigerator storage; after this, we simulated the human digestive tract using a model of the digestive tract of a pig.

2. Materials and methods

2.1. Oat material and heat treatment

Oat seeds were mechanically flattened into flakes in order to enhance the extraction process, as described in Aro et al. (2007). The thickness of the flakes was approximately 0.5 mm and their diameter varied from 3 to 7 mm. The flakes were kept at a temperature of +4 °C until they were used.

Two different hot steam treatments were tested. Part of the flakes were treated for 40 min in 90–95 °C steam (flakes 40/95), and the other part for 20 min in 75–80 °C steam (flakes 20/80). In both treatments, the water content of the flakes before processing was 17%. After processing, the flakes were dehydrated to 10% humidity with air flow. The processes were performed using industrial-scale equipment (Polar Mills Ltd, Vaasa, Finland) to best simulate the typical kilning process used in the cereal industry (Gates et al., 2008).

2.2. Production of oat-based polar lipids and chemical analysis methods

The extraction and antisolvent processes with supercritical fluids and the analysis of the basic chemical composition of the flakes and the fractions were performed in the same manner as described previously by Aro et al. (2007).

To identify the polar lipid groups in the oat polar lipid extract, the extract was divided further using liquid chromatography, as described by Ahvenainen (2010); this was done based on a modified analytical separation method first proposed by Kurvinen, Kuksis, Sinclair, Abedin, and Kallio (2000). In brief, using a silica gel column (LiChrospher® Si 60 (250 mm × 4 mm or 250 × 10 mm, dp 5 μm) Merck KGaA, Darmstadt, Germany), the polar lipids that had dissolved in chloroform-methanol (2:1) were first separated into three fractions using an isocratic elution solution, chloroform-methanol-isopropanol-water (65:30:1:5, by volume). Peaks were recorded using UV detection at 214 nm. The first fraction was further separated into four sub-fractions using another elution solution, chloroform-methanol-isopropanol (76:13.5:0.5, by volume). The chromatographic peaks in the fractions were identified using both solvent systems with the following reference compounds: digalactosyl diglyceride (DGDG) (Sigma Aldrich, St Louis, MO, USA), monogalactosyl diglyceride (MGDG) (Sigma Aldrich, St Louis, MO, USA), steryl glucoside (SG) (Larodan, Malmö, Sweden), and a commercial "POL Mix 71" mixture of phospholipids containing phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl inositol (PI), phosphatidylserine (PS) and phosphatidyl glycerol (PG) (Larodan, Malmö, Sweden). The quantitative analysis of polar lipids was performed using and HPLC-ELSD system, and the fatty acid composition was determined using GC-FID. Both methods have been described in detail earlier (Kivini, Järvenpää, Aro, Huopalahti, & Ryhänen, 2004).

The sugars (fructose, glucose, maltose, raffinose, lactose and saccharose) from the flakes were analyzed before and after the delipidation using an HPLC equipped with a refractive index detector. Briefly, the sugars were extracted using water and cleaned up using a C18 solid-phase extraction cartridge (Waters, Milford, MA), as described by the Nordic Committee on Food Analysis (No 148/1993). The analytical column used was a Luna NH_2 (150 mm × 3 mm, dp 5 μm, Phenomenex, Torrance, CA), and acetonitrile-water (75:25) was used as a mobile phase.

2.3. Suspension preparation

A suspension of the precipitated oat polar lipids and water was prepared using a ratio of 3–100 (w/v). The suspension was mixed with a magnetic stirrer for 30 min at 45 °C. Freeze-dried *B. breve* pellets were added to the suspension at the level of 1.7×10^8 CFU, and the mixing process was continued until all of the pellets had been totally mixed into the suspension. Another similar suspension was prepared, but commercial Malto6 maltodextrose (Func Food Finland Ltd, Tampere, Finland) was added to the suspension using a ratio of 3–100 (w/v). Both suspensions were freeze-dried and stored in −18 °C until used.

2.4. Test mediums and products

During the storage simulation, the test products were kept refrigerated in test mediums for either one day or one week. Altogether four products were tested: the pure test media (negative control), *B. breve* alone, was encapsulated with oat polar lipids or with a combination of oat polar lipid encapsulation and maltodextrose as a prebiotic.

The level of amended *Bifidobacteria* was approximately 1.3×10^8 bacterial cells per simulation. The storage part of the simulations was run separately for either one day or one week, resulting in a total of 16 treatments; each treatment consisted of four parallel simulation vessels.

2.5. Human digestive tract simulation

All the samples were shaken for 2 h with a pepsin-HCl solution at a pH below 2.5, and for 3 h with a pancreatin solution at a pH of 6.5. To simulate the growth conditions prevailing in the human colon, a supernatant of swine colon digesta was used to provide substrates for *B. breve* and so that the vast majority of the microbes from colon digesta could be left out. At the end of the colon model, gas production was measured using a glass syringe and a pH with a specific electrode. The total microbial numbers were enumerated by a flow cytometer using staining with a DNA-specific Cyto 24 dye and settings adjusted for counting the bacteria. The concentrations of acetic acid and lactic acid were analyzed by gas chromatography using a packed column (80/120