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Development of fermented oat flour beverage as a potential probiotic vehicle

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ABSTRACT

Dairy products have conventionally been used as carriers of probiotics. However, lactose intolerance, cholesterol, allergenic milk proteins, and the trend towards vegetarianism motivated the search for non-dairy products as potential probiotic carriers. Cereals may represent an excellent choice due to their high nutritional value and consumption all around the world. In the present study, an oat based fermented product 'probiotic fermented oat flour' (PFOF) was developed using a probiotic strain *Lactobacillus plantarum* M-13 and honey. The bacterial isolate *L. plantarum* M-13 has previously been characterized for several probiotic functional attributes. For PFOF development, process variables, i.e., concentrations of oat flour (8.0% w/v) and honey (3.0% w/v), and incubation time (48 h) were optimized based on a Box-Behnken design. Optimization enhanced the viable cell count of *L. plantarum* M-13 in PFOF from 14.4 log cfu/ml (unoptimized conditions) to 16.9 log cfu/ml, i.e. by 17.4%. With respect to linear terms, the variable incubation time had the most substantial positive influence on viable cell count of *L. plantarum* M-13, while with respect to interactive terms, the variables incubation time and honey had the maximum effect. Good viability of *L. plantarum* M-13 was observed in PFOF over a period of three wk of storage at room temperature and with refrigeration. Furthermore, sugar content decreased and lactic acid content increased during storage. The PFOF remained free of *Enterobacteriaceae* contamination, although fungal and yeast contaminants were found.

1. Introduction

The interest in developing probiotics and/or prebiotics as dietary supplements is due to their potential to enhance the gut microbial composition and activities, and overall health (Rigo-Adrover et al., 2016). Fermented dairy products have so far been the predominant commercial carriers of probiotics, however, health concerns like lactose intolerance, allergies to milk proteins, cholesterol and saturated fatty acid content has prompted research on the development of non-dairy carriers of probiotics (Kumar, Vijayendra, & Reddy, 2015). Among various non-dairy options as potential probiotic vehicles, cereals offer many advantages, e.g., they are grown and consumed all over the world, and represent rich sources of energy, vitamins, and minerals. Besides, the presence of specific prebiotics, i.e., nondigestible carbohydrates (soluble fibers, oligosaccharides and resistant starches) in the cereals may stimulate the growth of probiotics and other beneficial gut bacteria (lactobacilli, bifidobacteria etc.), and help to maintain healthy gut conditions (Kumar et al., 2015). Furthermore, lactic acid bacteria (LAB) fermentation of cereals may enhance the bioavailability of several minerals, digestibility, and the organoleptic properties of foods (Enujiugha & Badejo, 2015). Probiotic fermented cereal grains like maize, sorghum, wheat, oat, millet, barley and rye are commonly used for preparing beverages, gruels and porridges (Kaur, Jha, Sabikhi, & Singh, 2014).

Cereal based probiotic products have been developed previously like a probiotic wheat bread (Soukoulis et al., 2014), a probiotic oat flake beverage (Luana et al., 2014) and a probiotic ragi malt (VidyaLaxme, Rovetto, Grau, & Agrawal, 2014). Cereal based multiple probiotic beverages were produced by fermentation of oat, barley and malt substrates (Salmeron, Thomas, & Pandiella, 2014). Among various cereals as probiotic vehicles (Bernat, Chafer, Martinez, Garcia, & Chiralt, 2015; Luana et al., 2014; Salmeron et al., 2014) oat may be an apt choice due to its abundant functional properties. Oat (*Avena sativa*) is one of the important annual crops grown in temperate regions of the world. Oat is rich in health benefitting components like β-glucan, dietary lipids, proteins, starch, and antioxidant phenolic compounds, and is known to have anticancerous and hypocholesterolaemic properties (Rasane, Jha, Kumar, & Sharma, 2015). In addition, availability of soluble fibers, both oligosaccharides and polysaccharide in oat have a prebiotic effect (Kaur et al., 2014). Despite this, fewer reports

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are available on usage of oat as a potential probiotic carrier (Gupta, Cox, & Ghannam, 2010; Bernat et al., 2015).

Honey, a natural sweetener, contains several bioactive compounds such as vitamins, phenolics, flavonoids and fatty acids. Honey has potential biological activities such as antioxidant, immunomodulatory, antiproliferative and neurological properties (Muhammad et al., 2016). Additionally, honey has a prebiotic potential due to the presence of fructooligosaccharides. Thus, honey may be a suitable component of functional foods (Das et al., 2015).

Statistical based optimization provides insight into process dynamics, and illustrates the complex interactions between the process variables, contrary to the conventional one-variable-at-a-time approach (Singh & Bajaj, 2016). Response surface methodology (RSM) based optimization has been used for development of new products and processes, and/or for the improvement of existing ones (Gupta, Sharma, Singh, Gupta, & Bajaj, 2015). The Box-Behnken design, a type of RSM is suitable for industrial research as it is an economical design, and requires only three levels for each factor (Tekindal, Bayrak, Ozkaya, & Genc, 2012). Various probiotic foods have been developed using statistical optimization (Selvamuthukumaran & Khanum, 2015). A probiotic cereal-based baby food was developed by fermentation with *Lactobacillus casei* and *L. plantarum*, using RSM (Rasane et al., 2015). Similarly, a process for production of probiotic oat milk was optimized using *Lactobacillus reuteri* and *Streptococcus thermophilus* as starter cultures (Bernat et al., 2015).

Lactobacillus plantarum M-13 was previously isolated from *kalarei*, and characterized for functional attributes (Gupta, 2015; Gupta & Bajaj, 2017). *Kalarei* is a locally made fermented food product that is produced by fermentation of cow or buffalo milk with mixed cultures of lactic acid bacteria. It is a dense cheese that is usually consumed with bread after sauting and salting. *Kalarei* may be a suitable source for targeting novel probiotics. The isolate *L. plantarum* M-13 showed excellent survival under simulated gastrointestinal tract (GIT) conditions, and had several desired functional properties like adhesion ability, autoaggregation and coaggregation potential, extracellular enzyme producing ability, antibacterial activity and antibiotic susceptibility (Gupta, 2015; Gupta & Bajaj, 2017).

Considering the need for non-dairy based probiotic products, the current study was done to develop probiotic fermented oat flour (PFOF) by using *L. plantarum* M-13, and honey. The fermented product PFOF was studied for the viability of *L. plantarum* M-13, and other biochemical properties.

2. Materials and methods

2.1. Chemicals and raw materials, probiotic organism

All the chemicals used were of analytical grade and were obtained from Sigma-Aldrich Chemicals Ltd. (St. Louis, MO, USA), HiMedia Laboratories Ltd. (Mumbai, India), Ranbaxy Fine Chemicals Ltd. (Mumbai, India), Qualigens Fine Chemicals Ltd. (Mumbai, India), and Merck and Co. Inc. (White House Station, NJ, USA). Whole oat grains were obtained from a local grain market (*Kanak Mandi*, Jammu, India). In India oat is cultivated for fodder and grain purposes. The oat grains were washed, and dried at 40 °C in a hot air oven (Optics Technology, New Delhi, India) for 12 h, and then ground in a blender (Bajaj Electricals Ltd., Mumbai, India). The ground oat flour was sieved to get the particle size of less than 2 mm. The sieved oat flour was stored in an airtight container. Honey was purchased from a local horticulture farm in Jammu, where honey bees had access to mustard flowers. The probiotic organism used in the current study was *L. plantarum* M-13, an isolate from *kalarei*. For carrying out various experiments *L. plantarum* M-13 was grown in MRS broth for 18 h at 37 °C with shaking (180 rpm) to reach log phase (Andrabi, 2014; Andrabi, Bhat, Gupta, & Bajaj, 2016).

2.2. Fermentation for production of 'probiotic fermented oat flour' PFOF

A suspension of oat flour was prepared in distilled water (8%, w/v), and sterilized. Honey was pasteurized at 90 °C for 20 min, and added aseptically (3%, w/v) into the oat flour suspension. An overnight grown culture of *L. plantarum* M-13 was inoculated (1%, v/v) into the oat flour suspension to get an initial cell count of 10^9/ml. The contents were fermented in 250 ml Erlenmeyer flasks at 37 °C with shaking (180 rpm). Samples were drawn at different time intervals, and examined for viability of the *L. plantarum* M-13 (standard plate count technique), titratable acidity (as lactic acid content), sugar content and pH.

For viability analysis of the *L. plantarum* M-13, 1.0 ml sample of PFOF was subjected to vigorous vortex, and then serially diluted with 9.0 ml of sterile saline (0.1% NaCl, w/v). Then 0.1 ml of appropriately diluted PFOF sample was spread plated on MRS agar. The plates were incubated at 37 °C for 72 h. The colonies appeared on the plates were counted as colony forming units (cfu), and results were expressed as log cfu/ml. For measurement of other parameters of PFOF, i.e., titratable acidity (as lactic acid content), sugar content and pH, PFOF sample was centrifuged at 10,000 × *g* for 10 min (Eppendorf Centrifuge 5804 R, Hamburg, Germany), and supernatant was used for analysis. For the titratable acidity assay, 10.0 ml of centrifuged PFOF supernatant was titrated against 0.1 N NaOH using phenolphthalein as an indicator (pH 8.3). Titration was initiated by adding 2–3 drops of phenolphthalein to the sample, followed by drop wise addition of 0.1 N NaOH with continuous swirling. The appearance of a stable light pink color indicated the point of neutrality, i.e., the end point of the titration. The amount of NaOH used (titre) was recorded, and titratable acidity was expressed by using the following equation: 1.0 ml of 0.1 N NaOH = 0.0090 g of lactic acid.

The total sugar content in PFOF was quantified by using the phenol sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). In a hot acidic medium glucose is dehydrated to hydroxymethyl furfural and forms a colored product with phenol that has an absorption maximum (λ_{max}) at 490 nm (UV–VIS Spectrophotometer UV-1800, Shimadzu Corp., Kyoto, Japan). One ml of the appropriately diluted PFOF supernatant was mixed with 1.0 ml of phenol (5%, v/v), and then 5 ml of sulphuric acid (96%, v/v) was added. The contents were incubated at room temperature for 20 min, and then at 30 °C in a water bath for 30 min. The absorbance was measured at 490 nm. The amount of sugar in the sample was determined by using glucose as the standard. The pH of the PFOF sample was determined using a pH meter (Hanna Instruments, Woonsocket, RI, USA).

2.3. RSM based optimization of variables for development of PFOF

Three process variables, i.e., amounts of oat flour and honey, and incubation time (time period of fermentation) were optimized using RSM (Design-Expert 6.0 software, Stat Ease, Inc., Minneapolis, MN, USA), for production of PFOF. The low and high values of the variables were used at three coded levels (−1, 0, +1) (Tables 1 and 2). The significance level (p-value) of each variable was determined using the Student's *t*-test with $p < 0.05$ indicating a statistically significant difference.

A total of 17 experiments were carried out based on the design

Table 1
Range of independent variables for Box-Behnken design based on RSM for production of 'probiotic-fortified oat flour' PFOF.

Independent variable	Unit	Symbol	Coded level		
			-1	0	+1
Oat flour	%, w/v	A	3	5	8
Honey	%, w/v	B	2	3	4
Incubation time	h	C	6	27	48