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Optimization of process conditions for developing yoghurt like probiotic product from peanut

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ABSTRACT

Functional food market is dominated by dairy based probiotic products mainly yoghurt. There is need to develop dairy alternatives due to allergenic milk proteins, lactose and high cholesterol content. In this paper, efforts have been made to develop yoghurt like probiotic product from peanut milk utilizing single probiotic culture and without any supplements. The conditions were optimized utilizing response surface methodology by studying the individual and interactive effects of three process variables *i.e.* inoculum concentration, incubation temperature and time. Inoculum concentration of 1.9%, incubation temperature of 38 °C and 12 h incubation time was found optimum for probiotic peanut yoghurt preparation.

1. Introduction

Probiotics are normally marketed as nutraceuticals in forms of capsules and powders or added to yogurt, which is most popular vehicle for incorporation of probiotic microorganisms. According to WHO 2006, Probiotics are defined as live microorganisms, which, when consumed in appropriate amounts (10^6 cfu/ml), result in a health benefit to the host. Probiotics intake improves the intestinal microbial balance of the host and lowers the risk of gastrointestinal diseases by stimulating the growth of beneficial microorganisms and reducing the amount of pathogens (Chiang & Pan, 2012; Cross, 2002; Fuller, 1989). Food is considered the more convenient way of delivering probiotics in daily diets as compared to capsules or powders. Dairy food products mainly yoghurts are ideal food matrix for delivering probiotics, owing to their high consumer acceptability and better viability of these organisms. But a number of factors like cholesterol content, allergy to milk proteins and lactose intolerance necessitate exploring other non-dairy alternatives. Several reports on development of probiotic foods from different matrices like cereals, oilseeds, fruits and vegetables etc. are available (Angelov, Gotcheva, Kuncheva, & Hrstozova, 2006; Bansal, Man0.gal, Sharma, & Gupta, 2014; Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003). Attempts have also been made for developing yoghurt like probiotic products from non-dairy sources like soy milk utilizing single (Bansal, Mangal, Sharma, Yadav, & Gupta, 2015) or mixed cultures (Farnworth et al. 2007; Ghorbania, Pourahmada, Fallahpourb, & Assadib, 2012; Stijepic, Glusac, Milosevic, & Mikulec, 2013). Peanut milk (water extract of peanut) like soymilk, is a low-cost substitute for dairy milk for the developing countries. Peanut is a good source of protein, minerals essential fatty acids such as linoleic and oleic acids and antioxidant such as *p*-coumaric acid that may contribute to potential health benefits by their consumption (Duncan, Gorbet, & Talcott, 2006; Talcot, Passeretti, Duncan, & Gorbet, 2005). Peanut milk is extensively used as a dairy alternative in India and other developing countries and by people/children who are lactose intolerant or allergic to milk proteins (Kouane, Zhang, & Chen, 2005). The current interest in peanut milk/milk products is

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motivated by the fact that dairy and dairy products are always priced too high for the low income earners. Another factor, no less important, is the growing awareness of the nutritional benefits of vegetable proteins in low cholesterol diets by health conscious people (Kouane et al., 2005). Like soy products fermented with lactic acid bacteria, lactic acid fermented peanut milk/curd (Giyarto, Djaafar, Rahayu, & Utami, 2012; Isanga and Zhang 2009; Lee & Beuchat, 1991; Sunny-Roberts, Otulona, & Iwakun, 2004; Yadav, Singh, Bhowmik, & Patil, 2010) may act as a suitable carrier for probiotic to the host. The major challenge for development of non-dairy probiotic foods is the slow growth of probiotic bacteria on these substrates and less probiotic count. A product must contain at least 10^6 cfu/ml viable probiotic bacteria to classify as probiotics. Non-dairy probiotics are generally produced using mixed cultures or additives/gelling agent, which further create problem in establishing exact probiotic count thus limiting its commercial production. Probiotic Soy yoghurts possess characteristic beany flavor. Therefore, present study was undertaken with the aim of developing a non-dairy probiotic product so as to cater to the needs of lactose intolerant and vegan consumers and a process for development of monoculture based probiotic peanut yoghurt was standardized utilizing response surface methodology.

2. Materials & methods

2.1. Preparation of peanut milk

Preparation of peanut milk was done by using milk extractor. Peanuts were soaked in 0.5% $NaHCO_3$ (1:3 kernels to 0.5% $NaHCO_3$) for 16–18 h as per method of Saio (1986). The soaked peanuts were then dehusked, washed with water and ground with hot water (1:6 kernels to water) in the grinder for 8 min. Pressure blanching of peanuts was done in autoclave at 121 °C at 15psi for 3–5 min. The grinded product is allowed to reach the deodorizer through the pressure exerted by gas in grinder. This deodorization aims at removing the peanut aroma from the milk. The slurry formed was sieved by muslin cloth to obtain the peanut milk.

2.2. Culture preparation

Seven probiotic bacterial strains namely *Lactobacillus brevis* MTCC no. 1750, *Lactobacillus casei* MTCC no. 1423, *Lactobacillus fermentum* MTCC no. 903, *Lactobacillus fermentum* MTCC no. 1745, *Lactobacillus plantarum* MTCC no. 6160, *Lactobacillus plantarum* MTCC no. 1407, and *Streptococcus faecalis* T110 (renamed as *Enterococcus faecalis*) were procured from IMTECH, Chandigarh and local market. The procured strains were received in the form of lyophilized cultures. The cultures were revived on MRS media (Himedia make). Pure cultures were maintained on MRS agar/broth at 37 °C till further use. Two strains namely, *Lactobacillus fermentum BBE4*, *Lactobacillus fermentum BBE5* isolated and characterized in our laboratory were also used for the experiments. Probiotic inoculum was prepared by inoculating active culture of probiotic bacteria in sterilized MRS broth. It was incubated at 37 °C till OD 1 is achieved.

2.3. Preparation of peanut yoghurt

A preliminary trial was conducted utilizing the above mentioned probiotic cultures in order to screen the cultures that have the capability to ferment peanut milk in to set type yoghurt. For this active cultures (OD 1) grown overnight in MRS broth for about 16 h at 37 °C were taken. The peanut milk was warmed and inoculated with 1% of culture and incubated at 37 °C for 10–16 h. Then on the basis of overall sensory rating results the best culture showing promising response was selected (Data not shown). Response surface methodology was applied to further optimize the fermentation conditions for probiotic peanut yoghurt development. Quadratic polynomial model was fitted to each response except firmness as *per* the equation given below:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_{i^2} + \sum_{i=1}^{3} \quad \sum_{j=i+1}^{3} \beta_i X_i X_j \tag{1}$$

where Y is the response, β_o constant, β_i the linear coefficient, β_{ii} the quadratic coefficient and β_{ij} the interaction coefficient. X_i and X_j are independent variables.

2.3.1. Experimental design

Twenty treatments were performed according to face central composite design with 3 factors and 3 levels of each variable. The factors or independent variables of the design were inoculum concentration, incubation temperature and incubation time. Coded and actual levels of experimental design are given in Table 1.

2.3.2. Sensory quality

Sensory characteristics of yoghurt samples in terms of colour/appearance, texture, flavor, odour and overall acceptability, on 9 point hedonic scale, were evaluated by a group of semi-trained panelists. Water was provided for mouth rinsing between evaluations of different samples to avoid the carryover effect of the aftertaste.

2.3.3. Physicochemical analysis

In order to determine different responses to variables; inoculum concentration, incubation time and incubation temperature various physiochemical parameters of peanut yoghurt like acidity, syneresis, viscosity, firmness or texture, and probiotic count were analyzed. Titratable acidity was estimated using the method of AOAC (2000), by titration of sample with 0.1 N NaOH solution containing 1% phenolphthalein as an indicator. To determine synersis (ml/100 ml or %), yoghurt samples were kept on filter paper over glass beaker for 16 h at 4 °C to separate the water from yoghurt. The water was collected in measuring cylinder and % synersis was calculated as per the formula given below:

$$\text{Synersis} = \frac{\text{Volume of water collected after drainage}}{\text{Volume of yoghurt sample before drainage}} \times 100$$

Viscosity was analyzed using rapid viscoanalyzer (Techmaster, Newport scientific, Australia). Samples underwent controlled cooling from 43 °C to 15 °C as per the test configuration given below (Bennett, Harte, & Smithers, 2001):

Textural properties *i.e.* firmness and strength of the yoghurt samples were determined using Texture Analyzer (TA-HDi, Stable Micro System, UK) operated in the compression mode with settings viz. load cell force 5.0 kg, pretest speed 2 mm/s, test speed 2 mm/s, post-test speed 2 mm/s, distance travel 10 mm with a 60°conical probe.

2.3.4. Microbiological analysis

For probiotic product development, the viability of probiotic bacteria is of prime importance. Total viable number of *Streptococcus faecalis* T110 on MRS agar was determined by serial dilution and standard plate count method.

2.4. Analysis of data

Response surface methodology (RSM) was adopted in