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Optimization of the prebiotic & probiotic concentration and incubation temperature for the preparation of synbiotic soy yoghurt using response surface methodology

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A B S T R A C T

This research work aimed to develop synbiotic soy yoghurt with improved product characteristics and consumer acceptability. Response surface methodology was employed to investigate the combined effects of FOS concentration (2–10 % w/v), ST-LA inoculum size (2–4% v/v), and fermentation temperature (37–42 °C) on fermentation time (h), hardness (g), whey separation (%) and overall acceptability on 9 point hedonic scale of synbiotic soy yoghurt. A second-order polynomial response surface equation and response surface graph revealed that the experimental variables significantly affected the studied responses. Determination coefficients (R^2) were higher than 87% which showed that the developed models were well fitted to the experimental data. Synbiotic soy yoghurt was prepared using optimized FOS concentration (8.1% w/v), culture combination ST-LA with inoculum size 3.6% v/v, 1:1 ratio and incubated at temperature 41 °C for 5.25 h. The optimized product was well set with very less whey separation (1.14%). The developed product showed good nutritional, textual and sensory characteristics.

1. Introductions

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit to the host (Araya et al., 2002). Prebiotics are non-digestible substances such as fructooligosaccharide, which provide beneficial physiological effect on the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria (FAO/WHO, 2001). A food product containing both probiotics and prebiotics is named as synbiotic (Homayouni, Ehsani, Azizi, Yarmand, & Razavi, 2007) resulting in an increase in the probiotic counts and the reduction of pathogen microorganisms in the gut.

Various food products have been developed as carriers for probiotics, mainly of dairy origins because consumers are commonly allied with fermented dairy products. Among dairy fermented products, yoghurt is considered as the most popular vehicle for the delivery of probiotics for the consumer. A total of 78% of current probiotic sales in the world today are delivered through yoghurt (Cargill, 2009). Yoghurt is produced by adding two starter cultures, *Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus* to milk. Several health benefits have been reported for traditional yoghurt and this healthy image is enhanced by supplementation with probiotics. Few drawbacks, however, associated with the fermented milk products, mainly the increasing prevalence of the lactose intolerance and the level of cholesterol make it essential to explore some other non-dairy sources as suitable substrate for the probiotics (Granato, Branco, Nazzaro, Cruz, & Faria, 2010).

Soy is an excellent raw material for the development of probiotic non-dairy functional foods to overcome the limitations associated with dairy products. Preparation of a yoghurt-like product from non-dairy raw material such as soy with probiotics and prebiotics is a novel development in the field of fermented functional foods (Mishra & Mishra, 2013). The benefits of soy have drawn much attention in the past and numerous soy products have been evaluated as possible probiotic vehicles. Donkor, Henriksson, Vasiljevic, and Shah (2005) reported that the protein in fermented soy milk could encourage the growth of many probiotic strains such as *Lactobacillus acidophilus*,

Abbreviations: FOS, Fructooligosaccharide; TPA, Texture profile analysis; TA, Texture profile.

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Lactobacillus casei and *Streptococcus thermophilus*. Soy-based fermented foods may provide additional benefits for the consumer due to their various functional properties such as hypolipidemic, anticholesterolemic and antiatherogenic and reduced allergenicity (Lopez-Lazaro & Akiyama, 2002).

Development of a fermented soy product containing probiotics requires strain selection for the ability to grow in the substrate, as well as the ability to compete or even establish a synergy between strains (Champagne, Green-Johson, Raymond, Barrete, & Buckley, 2009). Several technological aspects such as good sensory properties, viability during processing and stability in the product during storage have to be considered in probiotics selection. Soy milk used as a substrate for the preparation of yoghurt has low acidification rate and slow growth of probiotic bacteria which take longer time to complete the fermentation. Longer fermentation time produces undesirable changes in the product which is not acceptable to the consumer (Donkor, Henriksson, Vasiljevic, & Shah, 2007). The growth and acidification of lactic acid bacteria are very limited due to the low concentration of soluble carbohydrates in soy milk.

In addition, the texture and taste of soy yoghurt are essential attributes for product acceptability by the consumer. Due to the unpleasant beany flavour, insufficient acidity, rigid and brittle gel structure soy yoghurt has not been generally accepted among consumer. Firmness and water holding capacity are the two major parameters that of uttermost concern for the manufacturer of set type yoghurts. These characteristics, related to the gel structure, contribute to a smooth mouth feel of the product. To improve the growth of probiotic bacteria and production of organic acid, soymilk needs to be supplemented with prebiotics. Incorporation of prebiotics also improves the sensory profile, physico-chemical and rheological characteristics of probiotics fermented soy products (Donkor et al., 2005). Among prebiotics, fructooligosacchride (FOS) is non-digestible fructan frequently used as a functional food ingredient that offers a unique combination of interesting nutritional properties and important technological benefits. Dosage levels of FOS in the range of 2–50% (w/w) are recommended for various food formulations (Franck, 2002). Incubation temperature directly influences the bacterial growth and, hence, the aroma and texture of the product. Inoculum level can alter the rate of acidification; moreover, it is essential to guarantee a desired level of final bacteria during the shelf life of the product (Kristo, Biliaderis, & Tzanetakis, 2003). It is important to supply information on process conditions, including incubation temperature and the amount of inoculums to enhance the quality of the yoghurt.

Torriani, Gardini, Guerzoni, and Dellaglio (1996) found the response surface methodology as an efficient tool in the evaluation of the simultaneous effect of some important technological and microbiological parameters on the acidification rate and the growth of starter bacteria during the fermentation process of yoghurt. Response surface methodology (RSM) has successfully been used to determine and optimize the rheological properties and gelation kinetics of yoghurt (Kristo et al., 2003). Gouveia, Fiadeiro, and Queiroz (2008) reported that RSM is an advantageous optimization technique in which the process can be studied simultaneously enabling identification and quantification of significant interactions between the variables and predict the optimum conditions for the process through predictive models.

Although some published studies have dealt with yoghurt production from soymilk, not much data is available, providing a complete assessment of the product, including supplementation with prebiotic and their effects on acidification, viability of probiotic, rheological & textural properties and sensory attributes of the product. The present work was undertaken to develop a process technology for the synbiotic soy yoghurt using response surface methodology. Response surface methodology was also used to model and evaluate the combined effects of FOS, fermentation temperature, and probiotic inoculum level on fermentation time, textural properties, sensory characteristics and whey separation.

2. Materials and methods

2.1. Microbial cultures

Probiotic cultures, *L. acidophilus* NCDC11 (LA), *Streptococcus salivarius* subsp. *thermophilus* NCDC118 (ST) procured in freeze dried form from the National Collection of Dairy Culture, National Dairy Research Institute, Karnal, India were used in this study. The cultures of *Lactobacilli acidophilus* were activated by inoculating in MRS and *St. thermophillus* in M17 broth followed by incubation for 24 h at 37 °C. The activated cultures were used after three successive transfers for the preparation of stock cultures for soy yoghurt making. Stock cultures were prepared by mixing 24 h grown activated cultures with sterilized 80% glycerol (v/v) in a 1:1 ratio in eppendorf tubes and stored at −40 °C for further analysis. For the preparation of the inocula, 100 mL of sterile MRS and M17 broths were inoculated with 1 mL of a thawed stock culture and incubated at 37 °C until a pH of 4.5 was reached (Champagne et al., 2009). The incubation time varied between 12 to 14 h as a function of the strain. The cultures were centrifuged (4500 × g, 10 min, 4 °C), pallet washed in peptone water and re-suspended with peptone water to obtained the final concentration of inoculum 10.74 to 10.98 log cfu/ml. This concentration was selected on the basis of preliminary experiments which reduced fermentation time and maintained the desirable viable culture concentration during storage (Mishra & Mishra, 2013).

2.2. Production of soy milk & soy yoghurt

Soy milk was prepared as per method described by Mishra & Mishra, 2013. Soy milk samples thus prepared contained, total solid 10–12%, total soluble solids 8-10 °Brix, protein 4.89–5.15 % and fat 1.83–2.23 % having pH 6.10–6.22. Prebiotics, FOS (Swetoos, EnSigns Health Care Pvt. Ltd., India) was mixed in soy milk before steaming.

Different fermentation temperature (°C), FOS concentration (% w/v) and ST-LA inouclum size (% v/v) were the variables tested for the preparation of synbiotic soy yoghurt, according to the experimental design outlined in Table 1. Twenty batches containing 3 sets of yoghurt each was prepared in 100 mL plastic cups, containing 50 mL of FOS supplemented soy milk by inoculation of ST-LA (1:1) under sterilized condition. Samples were incubated at the experimental temperature (37–42 °C, Table 1) until pH reached 4.5 (selected as the end point of fermentation). After incubation, samples were kept at 4 °C for further analysis.

2.3. Monitoring of pH value of yoghurt during fermentation

The pH value of the milk during fermentation was measured in certain intervals (every 10 min) using a pH meter (Model: 361; Make: Systronics) with a glass electrode standardized at the experiment temperatures over the range from 7.0 to 4.0.

2.4. Texture profile analysis (TPA)

Texture profile analysis of fermented soymilk was measured using a Texture Analyzer (Model: TA.XT-2, Make: Stable micro