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Study of soy-fortified green tea curd formulated using potential hypocholesterolemic and hypotensive probiotics isolated from locally made curd

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ABSTRACT

Recently there has been an increased demand for functional foods to reduce the risk of cardiovascular diseases mainly related to hypercholesterolemia, because of undesirable side effects of traditional drugs (statins). Hence, in the quest for natural and safer alternatives, this work is aimed to bring together the health-promoting properties of probiotics, soymilk, bovine milk and green tea into one product, i.e., soy-fortified green tea curd (GTC). This study includes isolation and characterization of microbes for probiotic attributes, from locally made curd which could reduce cholesterol and produce angiotensin-converting enzyme inhibitors in vitro. The best isolate was used for the production of soy-fortified GTC, and the effect of refrigerated storage on bacterial viability, tea polyphenol contents, and organoleptic properties was investigated. CI1 (*Enterococcus faecium*) depicted best probiotic potential amongst the 15 isolates. Soy-fortified GTC depicted higher probiotic viability for a longer duration during refrigerated storage and greater ACEI activity than unfortified GTC.

1. Introduction

Recently the food industry has shown a growing interest in the so-called functional foods due to increase in consumers' desire for a healthier lifestyle and so for foods from the natural origin with enhanced nutritional and therapeutic values. Functional foods are more preferred over medications to reduce the risk of hypercholesterolemia and hypertension, which are the two major contributing factors in development of cardiovascular diseases (CVD) in order to avert various side effects from the traditional drugs. Hypercholesterolemia and hypertension are projected to be the chief cause of fatality worldwide by 2020. However, frequent and prolonged administration of the traditional drugs like statins induces undesirable secondary effects like myopathy and cognitive impairment. Hence, to develop more natural alternatives, several researchers have studied the in vitro and in vivo cholesterol reducing potencies of beneficial lactic acid bacteria (LAB) termed as probiotics. Thus, there is growing interest in the use of nutraceutical and pharmaceutical products formulated using probiotics and prebiotics alone or in combination (synbiotic) having cholesterol-lowering and blood pressure reducing properties. Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host (Hill et al., 2014). Probiotic bacteria are used in the food industry due to various beneficial properties including reduction in bowel irritation, immune-modulation, anti-hypertension and cholesterol reduction (Miremadi, Sherkat & Stojanovska, 2016). Pereira, McCartney and Gibson (2003) proved that the oral administration of probiotics and prebiotics significantly reduce the serum cholesterol levels in human subjects. Hypocholesterolemic effects of probiotics can be accredited to bile salt hydrolase activity, bacterial cell wall attachment, and physiological role of the short-chain fatty acids (fermentation end products) (Miremadi et al., 2016).

A fermented dairy product like yogurt is an ideal vehicle for consumption of the probiotics (Batista et al., 2015). In order to enhance the taste, valuable components and growth of yogurt bacteria, green tea extract and soymilk are known to be used as additives. But there is no report of their use together in a single functional food. Hence, the present study was designed to combine the health-promoting effects of probiotics, soymilk (prebiotics, angiotensin-converting enzyme

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inhibitory i.e., ACEI peptides, and low lactose level), bovine milk (ACEI peptides), and green tea (polyphenols) into one nutrition-packed functional food product i.e., soy-fortified green tea curd (GTC). We hypothesize that soy fortification of green tea curd improves its ACEI property and the viability of potential probiotic starters during storage under refrigeration.

Probiotic organisms possess several proteolytic enzymes which entail bovine and soy milk protein hydrolysis to form small peptides and free amino acids essential for their growth. Peptides generated as a result of fermentation by bacteria are never completely utilized by them (Leclerc, Gauthier, Bachelard, Santure & Roy, 2002) and huge amounts may accumulate in the medium. Numerous bioactive peptides are released by proteolysis of the milk proteins, having ACEI, immune-modulatory, hypocholesterolemic, antioxidative and antimicrobial effects (Miremadi et al., 2016). The angiotensin-converting enzyme (ACE; dipeptidyl carboxypeptidase, EC 3.4.15.1) plays a key role in the regulation of blood pressure. It leads to increase in blood pressure by catalyzing the production of angiotensin-II (a potent vasoconstrictor) and inactivating bradykinin (a vasodilator). Thereby, the inhibition of ACE leads to lowering blood pressure. The functionality of such bioactive peptides requires intestinal absorption and their transport to target organs. ACEI peptides are passed into the blood with human digestion of milk or yogurt since these peptides are resistant to the digestive-tract endopeptidases (Kitts & Weiler, 2003). Soymilk is utilized for production of beverages, acting as a source of prebiotics (raffinose and stachyose), and phytoestrogens (such as isoflavones). Soymilk fortified curd has several nutritional advantages over whole milk curd like the reduced level of cholesterol and saturated fat, as well as low levels of lactose (Panghal et al., 2018) for possible consumption by hypercholesterolemic and lactose intolerant people. Bioactive peptides generated from soymilk upon fermentation have numerous beneficial effects such as hypolipidemia, hypocholesterolemia, hypotension (by ACE inhibition), enhancement in arterial and endothelial functions, development of resistance to insulin, and weight loss (Friedman & Brandon, 2001). ACEI are now used worldwide as antihypertensive agents, leading to a remarkable reduction in blood pressure. Fermentation of bovine and soy milk by probiotic bacteria leads to the production of a variety of products such as short chain fatty acids, simple carbohydrates, peptides, amino acids and some vitamins (Champagne, da Cruz, & Daga, 2018). Moreover, fermentation of soymilk helps in modifying or improving its flavor and texture as well as enhancing its beneficial health properties (Donkor, Henriksson, Vasiljevic & Shah, 2005).

Since ancient times, green tea has been considered to have antioxidant and anti-cancer properties, they also promote oral health and other physiological functions such as anti-hypertensive effect, controlling body weight, anti-inflammatory activities, etc. Increasing interest in its health benefits has led to the inclusion of green tea in various functional foods (Granato, Santos et al., 2018). All beneficial effects of tea have been attributed to the activity of the polyphenolic compounds (like catechin and gallic acid) having strong antioxidant properties, i.e., they protect our body from damage due to free radical-induced oxidative stress. Seven tea catechins have been identified so far (−)-catechin (C), (+)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin 3-gallate (EGCG), (−)-gallocatechin-3-gallate (GCG) and (−)-catechin-3-gallate (CG) (Friedman, Levin, Lee & Kozukue, 2009). They all belong to the group of flavan-3-ols, part of the chemical family of flavonoids. Epidemiological studies show that tea polyphenols mainly catechins decrease mortality from cardiovascular diseases and slow down the aging processes. Tea catechins also have the novel characteristic of trapping reactive carbonyl species (RCS). The binding site for RCS trapping is the A-ring of the catechins, whereas the preferred site for antioxidation is the B-ring. Green tea demonstrates a significant increase in plasma antioxidant capacity in humans which leads to reduced oxidative damage in macromolecules such as DNA and lipids (Xu, Yeung, Chang, Huang & Chen, 2004). Tea leaves are the only food product containing EGCG, an extremely active compound with eight–OH groups determining its extraordinary antioxidant activity. The presence of tea does not significantly ($P < 0.05$) influence the characteristic micro-organisms in yogurt. It has been reported that yogurt bacteria do not affect tea catechins when incubated together for 48 h. Green tea also does not show any effect on the total lactic acid level of the final product. Jaziri, Slama, Mhadhbi, Urdaci and Hamdi (2009) recommended the incorporation of tea into yogurt for imparting beneficial effects on human health.

This study aimed to characterize the probiotic potential of the locally made curd isolates according to ICMR-DBT (Indian Council of Medical Research-Department of Biotechnology) guidelines-2011 (Ganguly et al., 2011) and then use the potential probiotics for production of functional food, soy-fortified GTC. They were first screened for various probiotic attributes (acid and bile tolerance, cell surface hydrophobicity, tolerance to growth inhibitors like NaCl and phenol, tolerance to simulated gastric and small intestinal conditions, production of toxic biogenic amines, blood hemolysis and antimicrobial potentials, and antibiotic susceptibility). Then, their hypocholesterolemic and ACEI peptide-producing potential was evaluated in vitro. The potential probiotic isolate was then utilized for production of soy-fortified GTC. Soymilk was incorporated into the bovine milk base as a prebiotic source in a quest to increase the post-storage viability of the probiotic starter cultures in the GTC produced. The effect of refrigerated storage of the soy-fortified GTC on its tea polyphenol content, sensory acceptability, probiotic viability and antihypertensive potential were evaluated in comparison to soy-unfortified GTC.

2. Materials

Locally made curd samples (made using bovine milk) were collected from different parts of Odisha, India. *Lactobacillus helveticus* MTCC 5463 obtained from Anand Agriculture University, Gujarat, India was used as the reference LAB. Earlier known as *Lactobacillus acidophilus* V3, it exhibited potential probiotic properties along with the ability to reduce cholesterol. Its hypocholesterolemic effect was reported in human subjects by Ashar and Prajapati (2000).

3. Methods

3.1. Isolation of micro-organisms and screening for probiotic attributes

Micro-organisms were isolated from the locally made curd samples using MRS (de Man, Rogosa and Sharpe) media from Difco (BD, USA) after incubation for 48 h at 37 °C. Organisms were selected randomly based on their visually distinct colony morphology (size, shape and color) from the MRS agar plates, and the individual pure cultures were maintained.

The extent of acid tolerance of all the isolated bacteria was determined at pH ranging from 2 to 7.4. The bile tolerance for the screened acid tolerant bacteria was determined at 0.1, 0.2, 0.3 and 0.5% oxgall. The cell surface hydrophobicity of the strains was studied to determine their ability to bind and colonize the gut epithelium. The tolerance of the isolates to growth inhibitors like NaCl and phenol was determined so that they can be included in the synbiotic food formulations. The ability to produce toxic biogenic amines by the isolates was determined using decarboxylase media. The blood hemolysis potential was examined. The antimicrobial potential of the isolated strains was investigated using agar well diffusion assay. Antibiotic susceptibility patterns were determined by disk diffusion method using the Kirby-Bauer technique. The diameters of the zone of inhibition were compared with standards for Antimicrobial Disk Susceptibility Tests, CLSI (Formerly NCCLS) Vol. 32 No.3, Jan 2012, and interpreted as sensitive (s), intermediate (i) and resistant (r). The free cells of the two isolates were evaluated for their tolerance in the simulated stomach and

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