

Production of synbiotic beverage using *Geotrichum candidum* and pineapple (*Ananas comosus*) juice

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Abstract

The aim of this study was to develop a fermented synbiotic beverage using potentially probiotic yeast *Geotrichum candidum* with prebiotics and pineapple juice. Five variants of pineapple juice were prepared using prebiotics, namely, *G. candidum* culture (Gc), Gc+Inulin, Gc+Fructooligosaccharides, Gc+Lactulose and a mixture of prebiotics with Gc culture Gc+Inulin + FOS+Lactulose. Pineapple juices supplemented with prebiotics were fermented with *G. candidum* for 24 h at 30°C. The synbiotic juices were assessed for key technological attributes, including pH, titrable acidity, shelf-life, viability of cells, and sensory acceptability. Functional properties of *Geotrichum* include survival under simulated gastrointestinal transit, antibacterial activity, antioxidative activity, cholesterol-lowering activity, γ -aminobutyric acid (GABA) production, and acridine orange binding. The aforementioned probiotic attributes of the formulated synbiotic juice were subsequently evaluated throughout a storage period of one month. The results showed that the synbiotic pineapple beverage could be stored at 4°C for up to 28 days with satisfactory sensory acceptability and shelf-life. *G. candidum* retained > 96, 94, and 93% viability following sequential exposure to lysozyme, lysozyme-SGF, and lysozyme-SGF-SIF, respectively. Furthermore, during 28 days of storage, no significant differences in viability were observed among fermented samples with or without prebiotics supplementation. Overall, this study revealed that a synbiotic beverage formulated from pineapple juice and prebiotics using *G. candidum* culture possesses significant functional and nutritional benefits supporting its potential application as a health-promoting functional beverages.

Introduction

Fermented or fortified foods have been effectively marketed as probiotic products and assessed for their capacity to provide beneficial microorganisms. Functional foods are becoming increasingly popular among consumers due to their potential therapeutic benefits. Topolska et al. (2021) identify three categories of functional foods: probiotics, prebiotics, and synbiotics, which combine probiotics and prebiotics. Functional foods are defined as those that provide essential nutrients while also containing components that may assist in disease treatment or lower the risk of disease onset (Varela-Pérez et al. 2022). Consuming certain fruits, vegetables, and animal products high in bioactive chemicals has been demonstrated in epidemiological studies to lower the incidence of cancer and a number of metabolic illnesses (Karasawa and Mohan 2018).

Dairy products have historically functioned as a vehicle for the transmission of probiotic bacteria to humans. Lactic Acid Bacteria, a collection of microorganisms that encompasses numerous probiotics, is a crucial participant in the fermentation of milk (Zibaei-Rad et al. 2023). Fermented milks, due to their substantial amounts of live bacteria, exert a probiotic effect that enhances intestinal microflora balance and overall health (Prado et al. 2008). The predominant probiotic products are promoted as fermented milk and yogurt; but, with the increasing vegetarian population in industrialized nations, there is a burgeoning market for vegetarian probiotic products (Nikolaou et al. 2023; Meenu et al. 2024). Globally, traditional non-dairy fermented beverages are manufactured. Due to their appealing flavor profiles and

perception as healthy, refreshing options, there is significant interest in developing functional beverages derived from fruit juice that incorporate probiotics (Mousanejadi et al. 2023; Pereira et al. 2011). Despite their unique use as probiotic carriers, juices provide a suitable habitat for bacteria without significantly impacting their sensory qualities, resulting in high microbial viability.

Prebiotics are usually prescribed prior to the consumption of probiotics, as they are nondigestible dietary elements that help the host by promoting the growth and functioning of the microbiota (Gibson and Roberfroid 1995). Prebiotic formulations have typically been studied to determine how cereal ingredients, including whole grains, barley husks, rye bran, oat milk fiber residues, and arabinoxylan fibers, affect metabolic activities (Berger et al. 2014; Connolly et al. 2012; Damen et al. 2011).

Geotrichum candidum is a listed microorganism with a documented history of safe usage in fermented foods, according to the International Dairy Federation and the European Food and Feed Cultures Association (Bourdichon et al. 2012).

Pineapple (*Ananas comosus* (L.) Merrill) is a tropical infructescence with a favorable acidity-to-sugar ratio, making it a popular fruit in both producing and importing countries. It is a rich source of polyphenolic chemicals, including gallic acid, chlorogenic acid, and ferulic acid, which have been shown to have antioxidative, antimutagenic, and anticarcinogenic properties, as well as protective effects against heart disease and cataracts (Baljeet et al. 2013). It also contains bromelain, an enzyme that aids digestion, as well as other nutrients that have anticancer, antidiabetic, and antihypertensive properties. It also contains good amounts of vitamins A, B, and C, as well as minerals, including calcium, magnesium, potassium, and iron (Ahmad et al. 2015).

Because of the aforementioned reasons, the objective of the present study was to formulate a synbiotic pineapple juice with a functional probiotic *G. candidum* strain and the widely used food-grade nontoxic and effective prebiotics inulin, fructooligosaccharides (FOS) and galactooligosaccharides such as lactulose (Gibson 1999). *G. candidum*, selected for the present study, possesses potential probiotic properties such as acid-bile-NaCl and phenol tolerance, antimicrobial activity against food-spoilage, and gastrointestinal tract pathogens, autoaggregation and coaggregation abilities. The strain also binds promutagen acridine orange (AO). The safety aspect of *G. candidum* was established by evaluating its antibiotic susceptibility, hemolysis, biogenic amine production, DNase activity, and mucin degradation. Synbiotic pineapple juices were prepared and evaluated for the survival of *G. candidum* in gastrointestinal transit, antimicrobial activity, antioxidative activity, cholesterol lowering activity, γ -aminobutyric acid (GABA) production, AO binding, sensory qualities, storage stability, and microbial viability. The abovementioned probiotic properties were subsequently evaluated of the synbiotic pineapple juice during a storage period of one month.

Materials and methods

Yeast strain and culture conditions

Geotrichum candidum, (GenBank accession number – KM282102), a *proprietary* strain of Enformtech, New Zealand, was grown in GPY (glucose, peptone, and yeast extract) agar plates and incubated at 30°C for 24 h. The activated culture was inoculated in 100 mL GPY medium, incubated at 30°C for 24 h for biomass production.

The following bacterial strains were used as indicators for the antagonism assay: *Escherichia coli*, *Enterobacter aerogenes*, *Salmonella typhi* MTCC 98, *Serratia marcescens* MTCC 97, *Shigella* sp., *Pseudomonas aeruginosa* MTCC 2587, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Yersinia enterocolitica* MTCC 4858, *Enterococcus faecalis*, *Micrococcus luteus* and *Bacillus* sp. were grown in Nutrient broth and *Listeria monocytogenes* MTCC 1143 and *Staphylococcus aureus* MTCC 1144 were grown in brain heart infusion broth for 18 h at 37°C.

Inulin, FOS, and other chemicals were purchased from Himedia (Mumbai, India). Lactulose was purchased from Sigma (USA).

Sample collection and formulation of synbiotic pineapple juice (*Ananas comosus*)

Pineapples were procured from a local market in Rajkot, Gujarat, India. Fruits were thoroughly washed under running tap water, followed by rinsing with distilled water. After peeling, the pineapple pieces were rinsed again with sterile distilled water. Pineapple juice extracted using a juice extractor then cooled and stored at 4°C for further analysis. The initial pH of the juice was adjusted to 6.7 with 4 M NaOH. 100 mL juice was transferred into 250 mL bottles, pasteurized for 2 min at 88°C (Moreira et al. 2017). The bottles were cooled to room temperature, inoculated with *G.candidum* (2%), fermented at 30°C for 24 h, and stored at 4°C for 28 days. Synbiotic juice was prepared by fermenting juice supplemented with prebiotics inulin, FOS and lactulose (1%). Five variants of pineapple juice namely, *G. candidum* culture (Gc), Gc+Inulin, Gc+FOS, Gc+Lactulose and a mixture of prebiotics with Gc culture Gc+Inulin+FOS+Lactulose. Formulated synbiotic juice was evaluated for the survival of *G. candidum* under simulated gastrointestinal transit, antimicrobial activity, antioxidative activity, cholesterol lowering activity, GABA production, and binding of acridine orange. The abovementioned probiotic properties were also evaluated during one-month storage period.

pH, titratable acidity and viable cell count of juice samples

2 mL juice samples were collected to determine the pH and titratable acidity. Pour plate technique was performed on GPY agar at an interval of 7 days to determine the viability of cells (log CFU/mL).

Prebiotic utilization test

G. candidum was evaluated for its ability to utilize the prebiotics, inulin, FOS, and lactulose as described by Kaplan and Hutkins (2000). 10 µL of 24 h culture was spot-inoculated on GPY agar supplemented with (1% w/v) of the respective prebiotics and pH indicator bromocresol purple (300 mg/L). The plates were incubated at 37°C for 48 h.

Functional probiotic properties

Survival in gastrointestinal transits

The viability of *G. candidum* cells was determined during gastrointestinal transit. Electrolyte solution, simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to (Pithva et al. 2014). 100 µL cell suspension ($A_{600}=1$) was incubated for 10, 120 and 180 min with 1 mL electrolyte, SGF, and SIF, respectively. Viability of the cells after each exposure, i.e., lysozyme, lysozyme-SGF, and lysozyme-SGF-SIF was determined by plating with appropriate dilutions on molten GPY agar. A cell suspension without gastrointestinal enzymes was utilized as a control, and cell viability was quantified as log CFU/mL.

Cholesterol removal assay

Cholesterol removal was estimated according to (Gilliland et al. 1985). A cell suspension (2%) of *G. candidum* was inoculated in GPY medium supplemented with 100 µg/mL cholesterol, incubated at 30°C for 24 h and centrifuged (10,000 ×g, 10 min, 4°C). 0.5 mL supernatant was mixed with 3 mL ethanol (95%), vortexed for 1 min, held at 65°C in a water bath for 10 min and allowed to cool to room temperature. 3 mL distilled water and 5 mL hexane were added, and vortexed for 1 min, and held stationary to allow the phases to separate. Hexane layer was collected in a sterile test tube, dried at 60°C in a water bath, residues were dissolved in 4 mL o-phthalaldehyde reagent, vortexed for 1 min and held for 10 min at room temperature. 2 mL conc. H₂SO₄ was added to the mixture, vortexed, and A_{550} measured. The cholesterol removal efficiency was calculated using cholesterol standard. Cholesterol removal (%) was estimated using the formula $(A) = [(B-C)/B] * 100$, where B represents the absorbance of the blank and C represents absorbance of the supernatant.

GABA production

GABA production by *G. candidum* culture was detected using thin layer chromatography on activated silica gel plates (Silica gel 60 F₂₅₄: Merck, Darmstadt, Germany) as described by (Lee et al. 2010). TLC was performed using solvent system: n-butanol: acetic acid: water (5:2:2, v/v/v), and detected by spraying 2% (w/v) ninhydrin and held for 10 min at 80°C.

1-diphenyl 2-picryl hydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity of the cells was determined according to the method of (Shimada et al. 1992), with minor modifications. 50 µL of the cell suspension ($A_{600}=1$) was added to 1 mL freshly prepared 0.2 mM DPPH solution (in methanol), and held in the dark for 30 min. PBS was used as a control. The DPPH scavenging activity (%) was estimated according to the formula: $(A_b - A_t / A_b) * 100$, where A_b = absorbance of the blank and A_t = absorbance of the test.

Binding of AO

AO binding assay was conducted with *G. candidum* cells treated with lysozyme, lysozyme-SGF, and lysozyme-SGF-SIF, according to the method of (Pithva et al. 2015). The binding assay mixture consisted of 800 µL phosphate buffer, cell suspension ($A_{600} = 1$, 10^9 cfu/mL) and 100 µL AO at a final concentration of 50 µg/mL. The assay mixture was incubated on a shaker (90 rpm, 37°C, 30 min), and centrifuged ($5,000 \times g$, 10 min, 4°C). Supernatant was collected and scanned (200–700 nm) using UV–Visible spectrophotometer (UV1601, Shimadzu, Japan).

Antibacterial activity

Inhibition of the growth of test pathogens by *G. candidum* cells treated with SGF, SGF-SIF was determined according to Schillinger and Lucke's (1989).

Sensory profile

The sensory panel consisted of 40 untrained individuals aged between 25–35 years. The product acceptability including appearance, texture, taste, color, and overall acceptance, was evaluated immediately after juice processing using 9-point hedonic scale (1 = disliked very much and 9 = liked very much). In a single session, each individual evaluated five juice samples. Each synbiotic juice was assigned a unique random code to ensure unbiased assessment.

Statistical analysis

The values given here are the mean of two independent experiments in triplicate $\pm$ standard deviation ($\pm$ SD). Microsoft Excel 2010 was used to analyze the data via one-way analysis of variance (ANOVA). P values of <0.05 were considered significant. The correlation coefficient between cell density and AO bound was calculated.

Results

Synbiotic juice preparation

Commercially available pineapple juices generally have pH ranging from 3.5 to 4.0; therefore, prior to fermentation, the pH was adjusted to approximately 6.7 using 4 M NaOH. The juice containing prebiotics was fermented with *G. candidum* at 30°C for 24 h and stored at 4°C. The fermented juices had pH in the range of 4.62–4.86 and a titratable acidity of 0.4 g/100 mL as shown in Table 1.

Prebiotic utilization test

G. candidum utilized all three prebiotics inulin, FOS and lactulose and produced acid, as evidenced by the formation of yellow zone against a purple background on GPY agar plates.

Functional probiotic properties

Survival of *G. candidum*

The viability of the *G. candidum* strain in five fermented juice formulations, measured initially and at 7 days of interval over 28 days of storage is shown in Fig. 1. All fermented juice samples initially contained approximately 7.7 log CFU/mL of viable cells. After 7 days of storage at (4°C), the viability of *G. candidum* decreased in all the fermented beverages, reaching approximately 7.38 log CFU/mL. No significant differences in cell concentration were observed after 14 days of storage at 4°C. Throughout this period, prebiotic supplementation did not enhance probiotic viability, as no significant differences were detected among the formulations. However, after 21 days of storage, the formulations containing Gc+FOS and Gc+Lactulose showed a significant reduction in viable cell counts (approximately 6.9 log CFU/mL). Thereafter, no significant differences in viability were observed among fermented samples with or without prebiotics supplementation.

Survival during gastrointestinal transits

G. candidum retained >96, 94, and 93% viability after sequential exposure to lysozyme, lysozyme-SGF, and lysozyme-SGF-SIF, respectively. No significant reduction in viability was observed for Gc (without prebiotic) and Gc with inulin following sequential exposure to lysozyme, lysozyme-SGF, lysozyme-SGF-SIF. In contrast, formulations containing FOS, lactulose, and IFL (inuline+FOS+lactulose) showed reductions in viability of 0.18, 0.53 and 0.18 log cycles, respectively (Fig. 2). Notably, *G. candidum* cells in juice supplemented with IFL exhibited high survival rates (>93%, 96%, and 98%) after sequential exposure to gastrointestinal transit.

Cholesterol removal assay

Cultures from synbiotic juices demonstrated cholesterol removal efficiency ranging from 36–61%. Notably, *G. candidum* formulations exhibited significantly higher cholesterol lowering activity than other prebiotic combinations after 24 h of incubation (Table 2).

GABA production

The formulation of *G. candidum* with pineapple juice supplemented with prebiotics exhibited prominent GABA spots on TLC plates, matching the R_f value of the standard GABA. TLC profiling of the culture supernatant of *G. candidum* grown in GPY medium containing 5% monosodium glutamate, along with the control (GPY +5% MSG – uninoculated) is shown in Fig. 3. The appearance of GABA spots in the culture supernatants indicates the conversion of glutamate to GABA, confirming the presence of glutamate decarboxylase (GAD) enzyme activity that is essential for GABA production.

DPPH scavenging activity

DPPH radical scavenging activity of the cells obtained from synbiotic juices (37-54%) was significantly lower than that of the cell-free culture filtrate CFC (80-90%) of *G. candidum* grown on GPY supplemented with prebiotics (Fig. 4).

Influence of AO binding after lysozyme-SGF-SIF treatment

G. candidum cells exhibited AO binding during gastrointestinal transit, although binding efficiency decreased after sequential exposure to lysozyme, lysozyme-SGF and lysozyme-SGF-SIF (Fig. 5). Maximum AO binding was observed after lysozyme treatment in the prebiotic-fortified juices. AO binding ranged from 39 to 45 µg/mL. No difference was observed between control and inulin-fortified synbiotic juices after gastrointestinal simulation, whereas cells from IFL-fortified synbiotic juices showed a gradual decline in AO-binding capacity.

Antibacterial activity

Cells harvested from synbiotic juices exhibited antibacterial activity against Gram-positive *Bacillus* spp., whereas no inhibitory activity was detected against Gram-negative gastrointestinal pathogens (Fig. 6). The degree of inhibition varied with tested pathogens and the type of prebiotics used in the juice formulation.

Sensory profile

Prior to storage, the sensory evaluation of synbiotic juices was performed using a hedonic scale, and the results are presented in Fig. 7. The characteristic scores for all formulations were above 6.0, corresponding to rating from "liked moderately" to "liked extremely." The mean sensory scores of the synbiotic pineapple juice formulations showed significant differences in color and appearance, flavor, body and texture, and overall acceptability ($P < 0.05$). All formulations exhibited comparable scores for color, appearance, and flavor, except for the Gc+Inulin pineapple juice. Among the formulations, Gc+Lactulose obtained the highest score (8.2) for color and appearance, as well as body and texture. Overall acceptability was highest for the synbiotic pineapple juices Gc+Lactulose and Gc+IFL, as evidenced by the sensory attributes.

Discussion

The development of an effective probiotic product requires the selected microorganism to demonstrate safety, functionality, and stability during processing, storage, and gastrointestinal transit. In the present study, *Geotrichum candidum* exhibited desirable probiotic potential, particularly when incorporated into synbiotic juice formulations supplemented with inulin, FOS, and lactulose. The inclusion of prebiotics significantly improved cell viability compared with the control (without prebiotics), confirming their protective and nutritive roles. The enhanced persistence of *G. candidum* may be attributed to its ability to metabolize these prebiotics as selective fermentable substrates, thereby promoting cellular stability and metabolic activity during storage and gastrointestinal simulation (Slavin 2013; McLaughlin et al. 2015; Gibson et al. 2017).

Survival under simulated oral and gastrointestinal conditions represents a critical determinant in probiotic selection. Probiotic cells are initially exposed to simulated lysozyme-containing saliva, followed by gastric acidity and digestive enzymes in the stomach and intestine. Gastric pH during fasting (1.0–1.5) increases postprandially with gastric transit lasting approximately 90–120 min (Gardiner et al. 2000;

Dunne et al. 2001). In this study, *G. candidum* maintained substantial viability after sequential exposure to simulated saliva, gastric fluid (120 min), and intestinal fluid (180 min). Among the tested synbiotic combinations, lactulose-containing formulations demonstrated significantly greater survival. Lactulose may enhance stress tolerance by serving as a readily fermentable substrate and by contributing to osmotic balance and membrane stabilization under acidic conditions. These findings align with previous reports demonstrating that synbiotic systems improve probiotic resistance to gastrointestinal stress and enhance functionality (Markowiak et al. 2017).

Hypercholesterolemia poses a major risk factor for cardiovascular diseases, and probiotic-mediated cholesterol reduction has attracted considerable attention. One proposed mechanism involves bile salt hydrolase (BSH) activity, leading to deconjugation of bile salts, disruption of micelle formation, and reduced intestinal cholesterol absorption (Araki et al. 1996; Fuller 1989). Probiotic yeasts such as *Saccharomyces boulardii*, *Pichia kudriavzevii*, and *Saccharomyces cerevisiae* have previously been investigated for their potential to assimilate cholesterol (Psomas et al. 2003). Chen et al. (2010) reported 7–21% cholesterol removal by *Galactomyces* spp. after 24 h incubation. In contrast, the synbiotic juice formulations developed in the present study achieved cholesterol removal of 36–61% (100 µg/mL) within 24 h, indicating enhanced cholesterol assimilation capacity. This improvement may result from synergistic interactions between *G. candidum* and the incorporated prebiotics, which could stimulate metabolic pathways associated with cholesterol assimilation and bile salt modification.

In addition to antioxidant potential, *G. candidum* demonstrated the ability to produce GABA, a non-proteinogenic amino acid that functions as a principal inhibitory neurotransmitter in the central nervous system. GABA has been associated with antihypertensive effects, improved sleep quality, enhanced cognitive performance, antidiabetic activity, and stress reduction (Diana et al. 2014; Wang et al. 2016). The potential of *G. candidum* to biosynthesize GABA further supports its application in functional food formulations targeting neurological and metabolic health.

Oxidative stress caused by reactive oxygen species contributes to the pathogenesis of chronic diseases including atherosclerosis, cancer, liver disorders, and inflammatory conditions (Wang et al. 2006). The observed DPPH radical scavenging activity indicates that *G. candidum* possesses notable antioxidant potential. The antioxidant activity may be associated with hydrogen-donating metabolites, cell wall components, or bioactive compounds produced during fermentation. Probiotic microbes with antioxidant properties may therefore contribute in reducing oxidative damage *in vivo*, enhancing the functional value of synbiotic beverages. This potential of the *G. candidum* can be explored to produce ethnomedicine with probiotic properties, and no cytotoxicity.

Dietary exposure to mutagens and carcinogens – including mycotoxins, heavy metals, acrylamide, heterocyclic amines, and polycyclic aromatic hydrocarbons – poses substantial health risks (Sugimura et al. 2004; Hur et al. 2007; Gokmen and Senyuva 2009). Probiotic microorganisms may exert antimutagenic effects by binding or metabolizing toxic compounds, thereby reducing their bioavailability and interaction with intestinal epithelial cells (Goldman and Shields 2003). The presence of *G. candidum*

in the gastrointestinal tract may therefore contribute to detoxification processes through adsorption or biotransformation mechanisms.

Microbial spoilage and food-borne pathogens remain major challenges in food safety. Certain probiotic yeasts have been recognized for their antimicrobial properties, offering potential as natural biopreservatives. Previous studies demonstrated that *G. candidum* inhibits *Listeria monocytogenes* and other Gram-positive and Gram-negative bacteria (Dieuleveux et al. 1998b). In agreement with these findings, the present study showed inhibitory activity against food spoilage organisms, including *Bacillus cereus*, *Bacillus subtilis*, and *Bacillus megaterium*. The antimicrobial action is likely attributable to the production of organic acids, hydrogen peroxide, volatile compounds, and specific metabolites such as phenyllactic acid, indoleacetic acid, and phenylethyl alcohol. Among these, phenyllactic acid has been reported as the most potent antibacterial agent (Dieuleveux et al. 1998a). Such antimicrobial activity enhances the dual functionality of *G. candidum* as both a probiotic and natural preservative agent in functional beverages.

Overall, the findings of this study demonstrate that synbiotic juice formulations containing *Geotrichum candidum*, particularly in combination with lactulose, exhibit enhanced survival under gastrointestinal stress, significant cholesterol assimilation capacity, antioxidant activity, GABA production, antimutagenic potential, and antimicrobial effects against food-spoilage organisms. Overall acceptability for pineapple juice was the highest, as evidenced by the attributes. These findings suggest that the selection of appropriate prebiotics can significantly enhance the sensory quality of synbiotic pineapple juices, which may be valuable for industrial-scale synbiotic pineapple juice development. These multifunctional properties strongly support the application of *G. candidum* as a promising probiotic yeast for the development of functional synbiotic beverages. However, further *in vivo* studies and clinical validation are warranted to confirm its health-promoting efficacy and safety, along with long-term benefits in human beings.

Conclusion

Synbiotic juices exhibit probiotic properties and utilize prebiotics such as inulin, FOS, and lactulose as carbon sources. When *G. candidum* is supplemented with different prebiotics, inulin, FOS, and lactulose alone and in combination, *G. candidum* shows considerable viability in synbiotic juices up to 28 days of storage and also after consecutive exposure to simulated gastrointestinal fluids. The probiotic strain *G. candidum* demonstrated a number of functional probiotic properties, including the ability to bind to acridine orange, GABA production, antioxidative activity, cholesterol-lowering activity, survival under simulated gastrointestinal transit and also possesses antibacterial activity against food spoilage pathogens. Therefore, synbiotic juices prepared with *G. candidum* and prebiotics can be useful in improving overall human health.

Declarations

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Data availability

We do not have any research data outside the submitted manuscript file.

Competing interest

The authors have no relevant financial or non-financial interests to disclose.

Ethical approval

Not applicable.

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Author contributions

Mayuree Naliyapara: Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Sheetal Pithva:** Writing – review & editing, Writing – original draft. **Von Johnson:** Writing – review & editing, Writing – original draft. **Bharatkumar Rajiv Manuel Vyas:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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Tables

Table 1 pH and titratable acidity of the synbiotic juice formulation containing prebiotics prepared by fermenting with *G. candidum* at 30°C for 24 h.

<i>Geotrichum candidum</i> + Prebiotic	pH	Titratable acidity (g/100 mL)
Gc	4.62	0.4
Gc + Inulin	4.67	0.4
Gc + FOS	4.60	0.4
Gc + Lactulose	4.86	0.4
Gc + IFL	4.71	0.4

Table 2 Cholesterol removal (%) by the synbiotic juice containing prebiotics prepared by fermenting with *G. candidum* at 30°C for 24 h

<i>Geotrichum candidum</i> + Prebiotic	Cholesterol removal (%)
Gc	61±0.0
Gc + Inulin	36±0.1
Gc + FOS	38±0.2
Gc + Lactulose	44±0.1
Gc + IFL	41±0.2

Figures

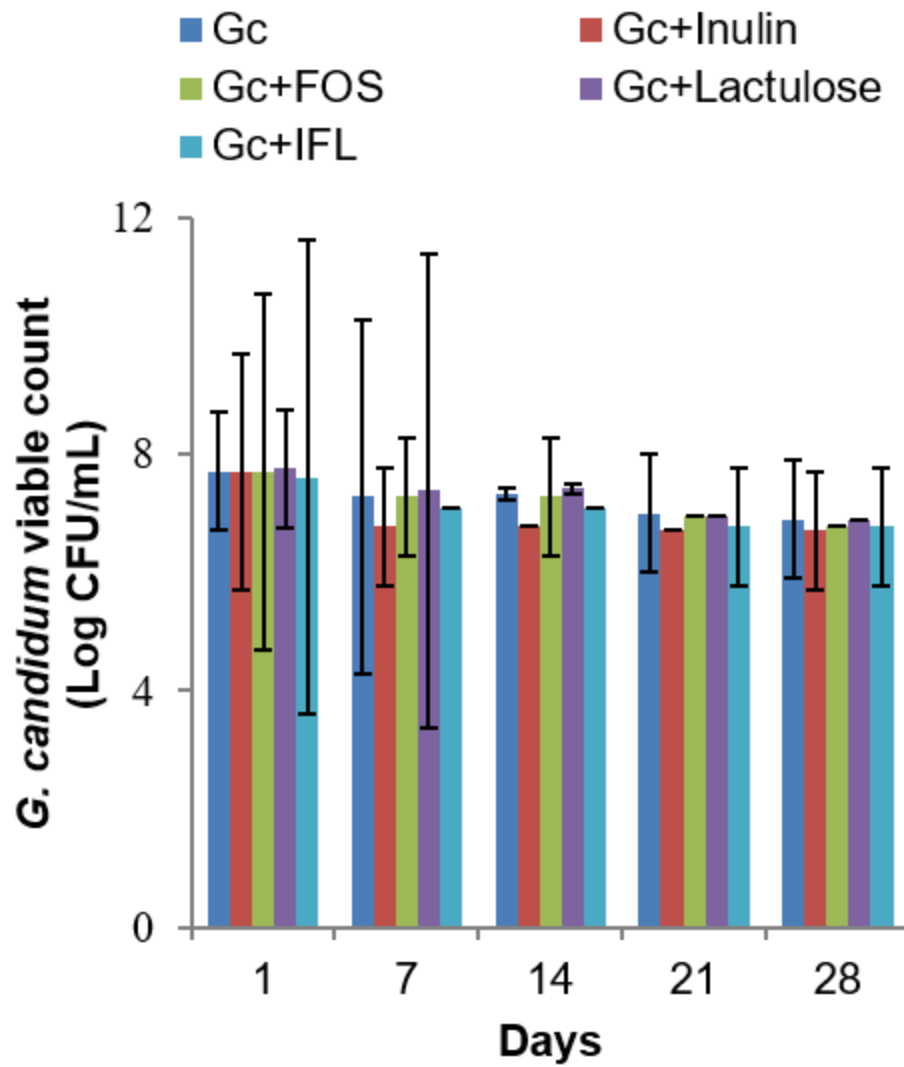


Fig 1

Figure 1

Viable count (log CFU/mL) of *G. candidum* in synbiotic juice stored at 8-10°C for 28 days. Error bars indicate standard deviation (n=3).

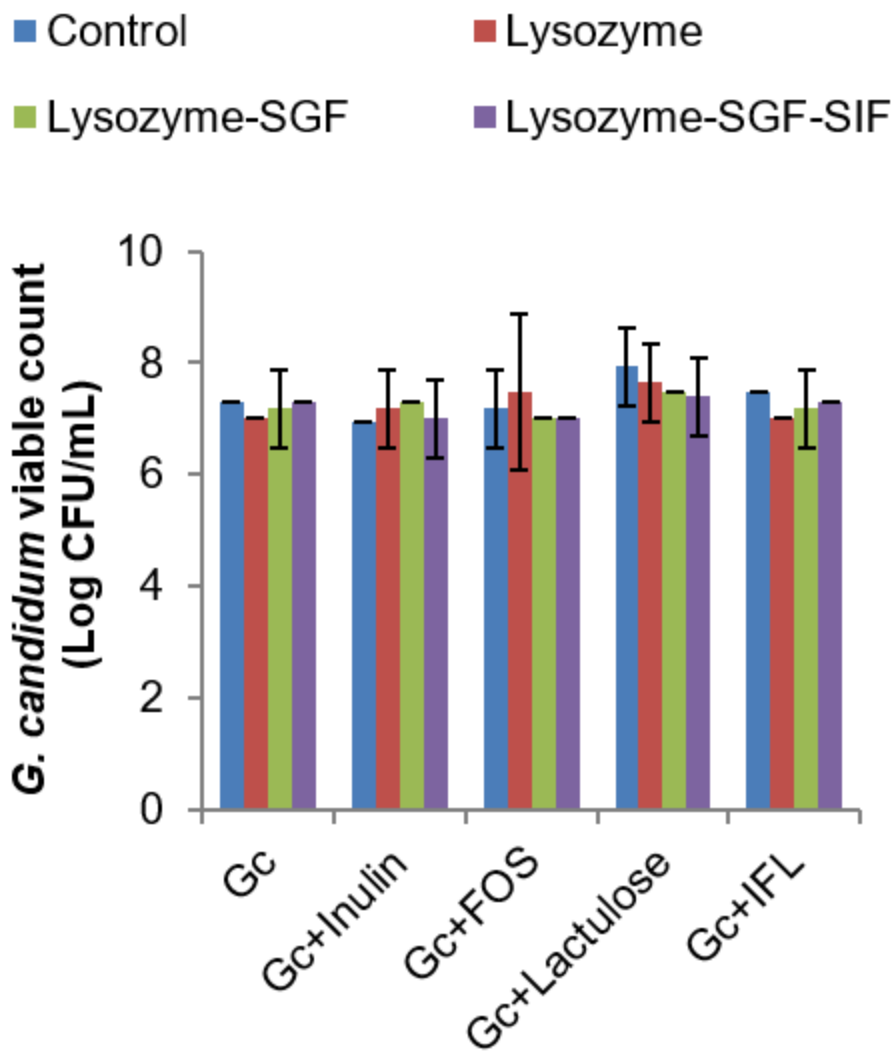


Fig 2

Figure 2

Viable count (log CFU/mL) of *G. candidum* cells stored at 8-10°C (control) upon sequential exposure to Lysozyme, Lysozyme-SGF, and Lysozyme-SGF-SIF. Error bars indicate standard deviation (n=3).

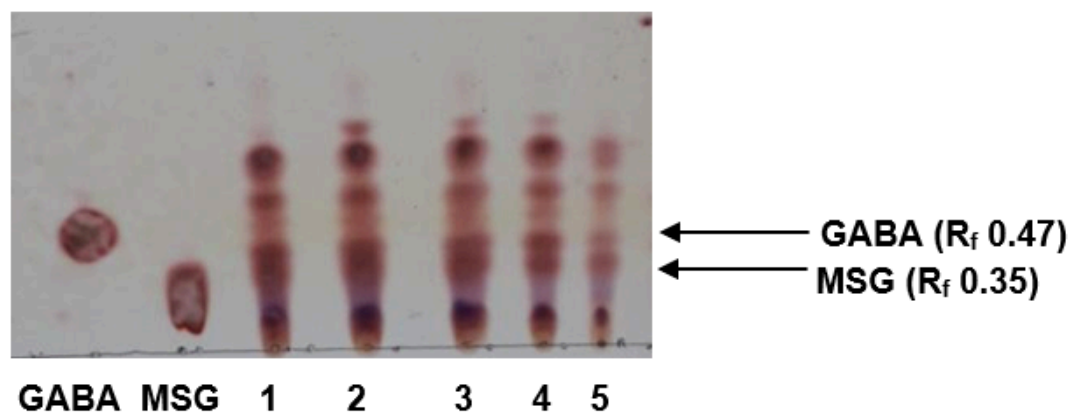


Fig 3

Figure 3

Thin-layer chromatography of GABA formation from synbiotic juices containing prebiotics fermented by *G. candidum* at 30°C for 24 h. Lane: GABA standard; MSG standard; GABA; juice 1 Gc, juice 2 Gc+Inulin, juice 3 Gc+FOS, juice 4 Gc+Lactulose, juice 5 Gc+IFL respectively. TLC was performed using the following solvent system: n-butanol: acetic acid: water (5:2:2), and the chromatogram was developed by spraying 2 % ninhydrin after incubation for 10 min at 80°C.

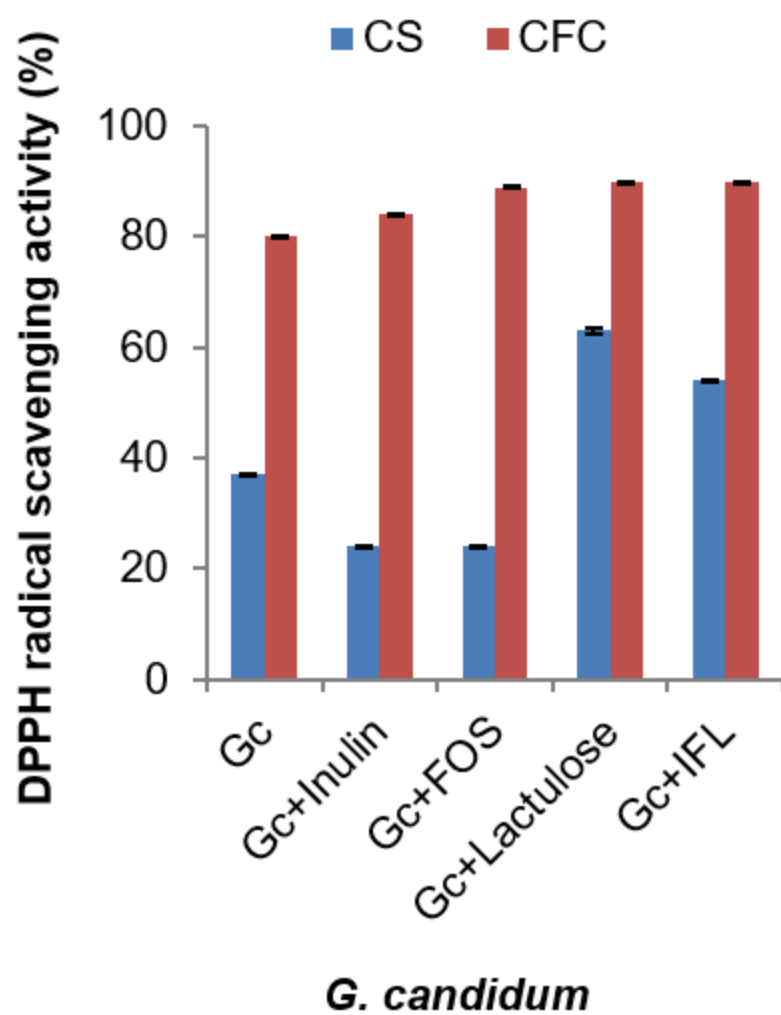


Fig 4

Figure 4

DPPH radical scavenging activity of the *G. candidum* cells synbiotic juice containing prebiotics fermented by *G. candidum* at 30°C for 24 h. Error bars indicate standard deviation (n=3).

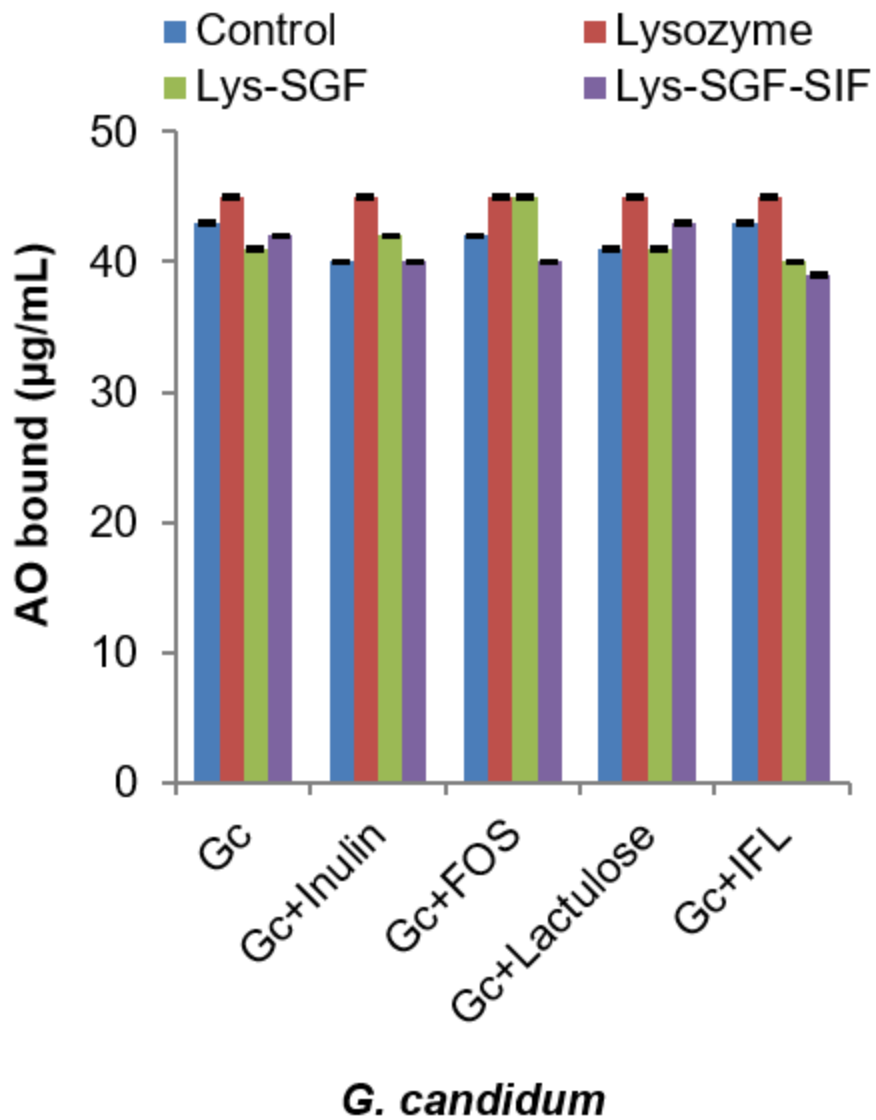


Fig 5

Figure 5

AO binding by the cell biomass of synbiotic juice containing prebiotics and *G. candidum* treated with Lysozyme, Lysozyme-SGF, and Lysozyme-SGF-SIF. Error bars indicate standard deviation (n=3).

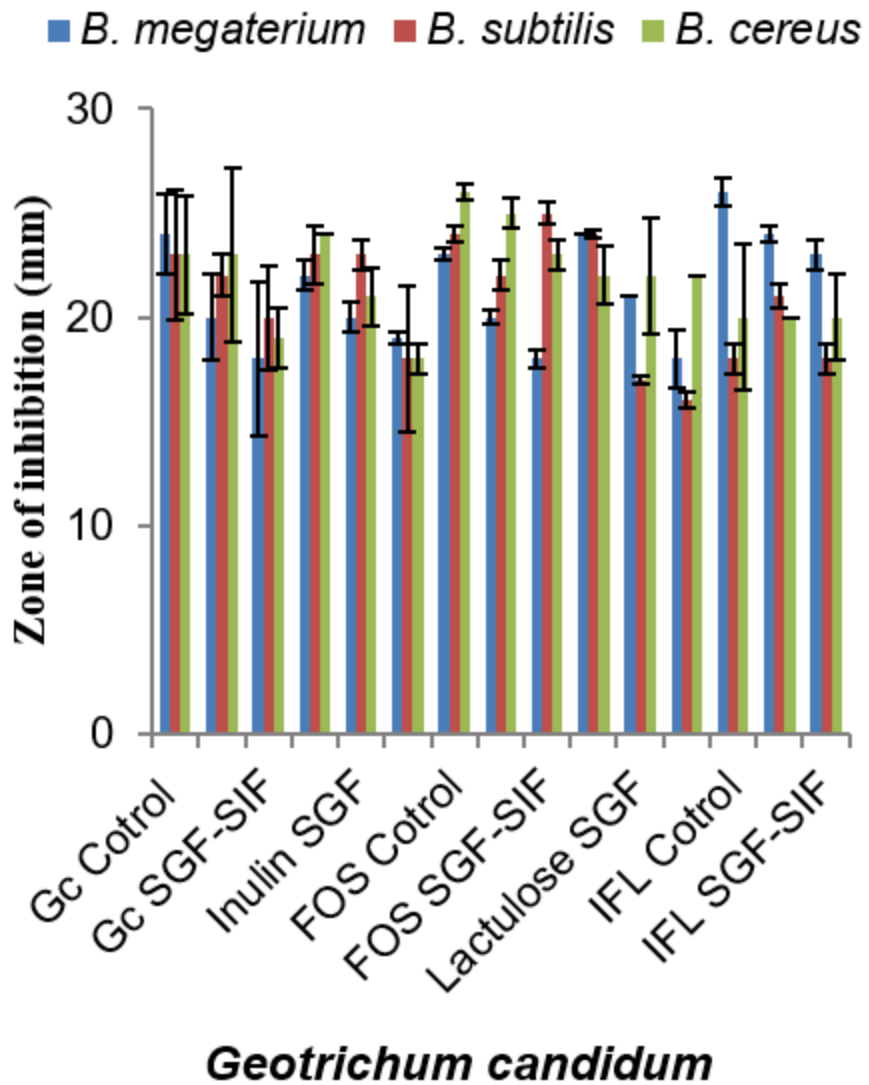


Fig 6

Figure 6

Antibacterial activity of *G. candidum* harvested from synbiotic juice containing prebiotics treated with SGF, and SGF-SIF determined by a spot overlay assay against Gram-negative and Gram-positive bacteria. Error bars indicate the standard deviation (n=3).

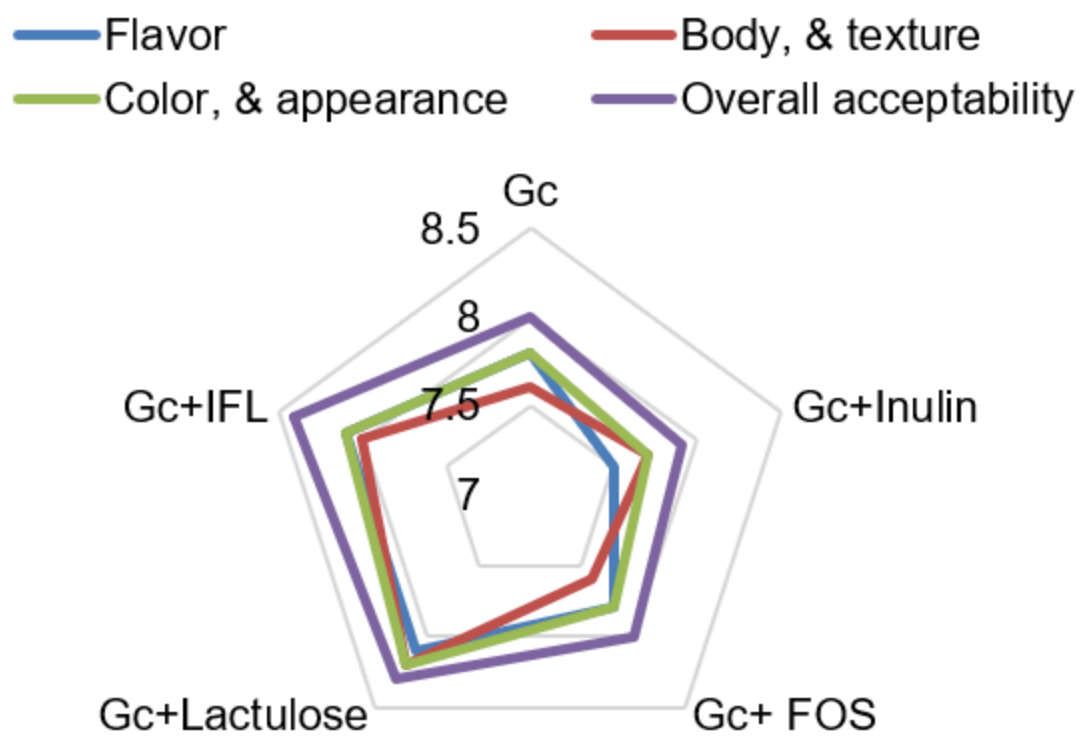


Fig 7

Figure 7

Radar plot of the sensorial analysis of the Gc, Gc+Inulin, Gc+FOS, Gc+Lactulose, Gc+IFL pineapple juice.