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Yogurt fortified with GABA-producing strain and *Ganoderma lucidum* industrial waste

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Abstract: This study aimed to produce yogurt with *Ganoderma lucidum* residues and selected probiotic bacteria. To select the most potent GABA-producing strain (GABA – γ-aminobutyric acid), nine probiotic bacteria were subjected to the glutamate decarboxylase (GAD) activity assay. *Limosilactobacillus reuteri* DSM 17938 was selected and used in preparing fresh yogurts, with and without supplementation of *Ganoderma* residues obtained after water extraction [GW (*Ganoderma* waste) 2% (w/v)]. A decrease in pH during fermentation and the occurrence of syneresis were investigated. Lactic acid bacteria (LAB) viability and anti-*Escherichia* activity were estimated. Further, the cytotoxic effect of yogurt extracts on the human colon cancer cell line (HCT116) was surveyed. Besides functionality, the sensory attributes were evaluated. The pH values did not significantly change with the GW addition while increasing the LAB counts [up to 9.76 ± 0.11 log colony-forming units (CFU) mL^{-1}] and the cytotoxic effect on HCT116 cells. Yogurt produced with selected bacteria and GW had the most valuable anti-coli effect against *E. coli* 0157: H7 and *E. coli* ATCC 35218 (American Type Culture Collection, Rockville, Maryland) (4.81 ± 0.62 and 5.64 ± 0.29 CFU mL^{-1}, respectively). Although the added GW increased the yogurt functionality, it had a slightly negative effect on the taste and texture of the partially modified recipe. Yogurts fortified with GABA-producing strain and GW could potentially reduce relapse rates of depressive disorders.

Keywords: fermented beverages; psychobiotics; sustainability; depression; anti-*Escherichia* activity

Recent studies have estimated the value of the global yogurt market at almost a hundred billion dollars, with predictions to increase by more than 50% in the next ten years (Alied Market Research 2022). Thus, the search for low-cost and functional innovative yogurt ingredients is stimulated. In the food industry, the medicinal fungus *Ganoderma lucidum* is highlighted as a remarkable functional ingredient. As it is well known, the economically important fungus *G. lucidum* is an inexhaustible source of biologically active compounds, such as triterpenoids, polysaccharides, triterpenes, sterols, lectins, steroids, fatty acids, and proteins/peptides, with proven health benefits (Veljović et al. 2019). These compounds, among many others, have cytotoxic, antimicrobial, neuroprotective, antioxidant, anti-inflammatory, antitumor and immu-

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nomodulatory effects (Oke et al. 2022). Because of its huge potential, hundreds of *Ganoderma*-based products are part of the modern world market (e.g. food and beverages). Since the *G. lucidum* fruit body has a woody texture, large quantities of *Ganoderma* residues are generated and available as a by-product and low-cost raw material. Following the idea of sustainability in food production, *Ganoderma* residues could be added as functional ingredients in popular fermented beverages (e.g. yogurt). To the best of our knowledge, none of the commercial foods and beverages contains *Ganoderma* residues remaining after industrial extract production. The chemical profile of *G. lucidum* extracts that dictates bioactivity strongly depends on the characteristics of the used solvent. Hot water is a favourable solvent for polysaccharide and polyphenol extraction (Tang et al. 2016), while in the remaining solid residues triterpenoids, mainly ganoderic acids, are present in a higher percentage.

Besides being a source of bioactive compounds, fungal residues also have a valuable role as a carrier for probiotic bacteria in yogurt production. Probiotic bacteria are known to exert several beneficial effects, including reducing pro-inflammatory cytokines, protecting the digestive tract from inflammation, and combating pathogenic microbes (Mu et al. 2018). Some probiotic bacteria can synthesize the enzyme glutamate decarboxylase (GAD), which catalyzes the decarboxylation reaction of glutamate to γ-aminobutyric acid (GABA) and are therefore considered GABA-producing strains (Tette et al. 2022). Data from the literature highlight the link between chronic inflammation of the digestive tract and depression (Jørgensen et al. 2014). Moreover, depressive disorders can also arise due to the disruption of GABAergic signalling (Tette et al. 2022). Thus, probiotics are nowadays seen not only as the first-choice therapy in the treatment of digestive tract ailments, but also as a supplementary therapy in the treatment of depressive disorders. Due to the current global economic recession secondary to the COVID-19 pandemic, the rate of depressive disorders is elevated (Guerra and Eboreime 2021). Despite the progress made in the development of commercial antidepressants, side effects and inconsistent scientific reports dealing with the addiction caused by their long-term use have directed the flow of current research towards seeking new approaches in the treatment of depressive disorders (Poluektova et al. 2021). Dietary therapy represents an additional valuable method to improve the success of conventional antidepressant therapy (Borys 2021). In particular, dietary therapy that includes fermented dairy beverages, goods with potential neurobiotic properties that deliver live bacteria, bacterial metabolites and prebiotics has increasingly been seen as a potential adjunctive treatment for depression (Rocks et al. 2020).

The aim of this study was to design and examine a yogurt fortified with GABA-producing strain and *Ganoderma* residue obtained after water extraction (GW). In order to achieve this, probiotic strains were tested for GABA-producing potential. The most potent strain was selected and used in the preparation of fresh yogurts. The functionality and sensory properties of fresh yogurts supplemented with 2% (w/v) GW were further studied. A decrease in pH during fermentation and the occurrence of syneresis were investigated. To highlight functionality, yogurts were analysed from the microbiological point of view. Lactic acid bacteria (LAB) viability and anti-*Escherichia* activity were estimated. Further, the cytotoxic effect of yogurt extracts on the HCT116 was surveyed. Finally, a sensory evaluation was conducted.

MATERIAL AND METHODS

Material. Pasteurized cow milk (Zapis Tare Dairy Plant, Serbia) was purchased from the local market (Belgrade, Serbia). Lyophilized starter culture YC-X11 was obtained from Chr. Hansen (Hørsholm, Denmark). Bacterial strains *Escherichia coli* 0157: H7 and *E. coli* ATCC 35218 were obtained from the Department of Microbiology, Faculty of Biology, University of Belgrade, Serbia. All probiotic strains, *Limosilactobacillus reuteri* DSM 17938, *Lacticaseibacillus rhamnosus* GG (ATCC 53103), *Lactobacillus acidophilus* NCFM HOWARU, *Lactobacillus lactis* subsp. *lactis* H-559, *Lactobacillus delbrueckii* subsp. *bulgaricus* LB12, *Bifidobacterium animalis* subsp. *lactis* BB 12, *Bacillus coagulans*, *Lacticaseibacillus rhamnosus* L-64 and *Lactiplantibacillus plantarum* 299v were part of the microbial collection at the Institute of General and Physical Chemistry, Belgrade, Serbia. The human colon cancer cell line HCT116 (ATCC CCL-247) was obtained from the Oncology Institute of Vojvodina, Serbia. Selected strains of bacteria originated from the ATCC collection. Dried *G. lucidum* solid residues, remaining after industrial water extract production, were obtained from Ganoherb Technology (Fujian) Corporation (Shangjie, China). Dried waste materials were ground into a homogeneous mixture by a laboratory mill, whereas granulation of obtained particles was in the range of 0.5–1.5 mm. De Man-Rogosa-

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Sharpe (MRS) broth, MRS agar, Mueller Hinton Broth (MHB) and eosin methylene blue (EMB) agar were obtained from HiMedia (Mumbai, India). Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin mixtures, phosphate-buffered saline (PBS), trypsin from the porcine pancreas, glutamic acid, bromocresol green, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich (Steinheim, Germany).

Detection of GABA-producing strains. To select the most potent GABA-producing strain, a colorimetric GAD assay was performed as described by Santos-Espinosa et al. (2020) with a slight modification. For this purpose, 5 mL of nine LAB overnight cultures were centrifuged (4 000 rpm, 10 min). Prior to the screening, all strains were cultivated in MRS broth for 48 h at 37 °C under microaerophilic conditions. After centrifugation, cells were washed with phosphate-buffered saline (1× PBS) twice and homogenized with 0.5 mL GAD solution (1 g glutamic acid, 0.3 mL Triton X-100, 90 g NaCl, and 0.05 g bromocresol green in 1 L of distilled water, adjusted to pH 4), and incubated at 37 °C for 4 h under microaerophilic conditions. After this time, the colorimetric reaction (change from green to blue) was visually observed, and the colour of the tested strains was compared.

Yogurt manufacture. Yogurts were prepared at a laboratory scale using pasteurised cow milk (2.8% milk fat). The milk, heat-treated at 85 °C for 10 min, was cooled down to 43 °C, and monosodium glutamate (MSG 0.1% w/v) and a lyophilized starter culture YC-X11 [0.02%, 50 U (DVS units – direct vat set cultures units)] containing *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* were added. The inoculated milk was filled into glass containers (100 g). The probiotic yogurt samples were additionally inoculated with *L. reuteri* (log 8 CFU mL^{-1}). Both control and probiotic yogurt were subjected to fermentation with and without 2% (w/v) GW. Fermentation was set at 43 °C until pH 4.6 was reached (~4 h). After fermentation, the yogurt samples were stirred and stabilized by cooling (4 °C for 24 h). Freshly prepared yogurts were used in further testing.

Fermentation kinetics and susceptibility to syneresis. Fermentation kinetics was evaluated by measuring the pH change during yogurt fermentation at an interval of 30 min – 1 h until the samples reached a pH value of 4.6 and after stabilisation (cold storage at 4 °C, 24 h). The pH value was determined at approximately 10 °C using a pH meter with a gel-filled electrode (WTW™ SenTix™ 41 pH, Massachusetts, USA). Susceptibility to syneresis of yogurt samples was estimated as previously described (Jovanović et al. 2020).

LAB viability. The viability of the LAB was evaluated upon fermentation using the pour plate technique and serial dilutions in phosphate-buffered saline (1× PBS). LAB were enumerated using MRS agar under microaerophilic incubation at 37 °C for 48 h. The results were expressed as the log of the mean number of the colony-forming units (log CFU mL^{-1}).

***In situ* anti-*Escherichia* activity.** Anti-*Escherichia* activity was investigated as previously described by Jovanović et al. (2021). *Escherichia* strains: *E. coli* 0157: H7 and *E. coli* ATCC 35218 were incubated in MHB at 37 °C overnight. Prior to fermentation, milk samples were inoculated with LAB bacterial starter culture 0.2 g (YC-X11) and 8 log CFU mL^{-1} of *E. coli* strains. Next, MSG (0.1% w/v) was added and milk samples were subjected to fermentation with and without *Limosilactobacillus reuteri* (8 log CFU mL^{-1}) and 2% (w/v) GW. Fermentation was set at 43 °C until pH 4.6 was reached (~4 h). The obtained yogurts were stabilized for 24 h. After the stabilization, yogurt serial decimal dilutions were prepared, plated on EMB agar, and incubated for 24 h at 37 °C under aerobic conditions. The results were expressed as log CFU mL^{-1}.

Cytotoxicity. For cytotoxicity testing, yogurt extracts were prepared as described by Rafiq et al. (2018) with a slight modification. In brief, yogurt supernatants (i.e. extracted whey) were obtained by centrifugation at 20.00 g for 30 min at 4 °C and subjected to lyophilization. Next, lyophilized samples were resuspended in distilled water. Extracts (10 mg mL^{-1}) were filtered using a 0.22 μm syringe filter. The cytotoxic effect was estimated by employing an MTT assay. Prior to cytotoxicity evaluation, the HCT116 cell line was maintained as a monolayer in DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% foetal bovine serum, 1% penicillin/streptomycin mixtures, and 2 mM of L-glutamine. After reaching 90% confluence, the cells were harvested and seeded into 96-well plates at a density of 2×10^4 cells/well and incubated overnight at 5% CO_2, 37 °C. Then, HCT116 cells were exposed to diluted extracts in the following concentrations: 0.75, 0.5 and 0.25 mg mL^{-1} and incubated for 24 h. After incubation, the medium containing the test substances was displaced with MTT (final concentration 0.5 mg mL^{-1}) and incubated for additional 3 h. At the end of the incubation with MTT, the medium was removed, and the formazan crystals were dissolved in DMSO (dimethyl sulfoxide). The viability of the cells was determined by measuring the absorb-

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ance at 570 nm using a microplate reader (Multiskan FC; Thermo Scientific, Shanghai, China). Three independent experiments in sextuplicate were performed. External wells were excluded.

Sensory analysis. Sensory evaluation of yogurt samples supplemented with GW was conducted as described in Jovanović et al. (2020). The overall sensory quality was determined using a scoring method 0–5. Yogurt samples were evaluated in terms of the most prominent sensory attributes, i.e. appearance, texture, odour, and taste. For this purpose, a panel of seven members who completed the training for the selected assessor according to the requirements of the standard ISO 8586-1 was employed. Panellists were instructed to cleanse their palates between samples using water and toasted bread. The scores assigned by the panel were weighted with corresponding coefficients of importance (CI) selected according to the influence of each attribute on overall sensory quality. Maximum overall quality was obtained by adding up the scores of each tested sensory attribute per sample, which was previously multiplied by the CI.

Statistical analyses. All measurements were done in triplicate and data were expressed as mean value ± standard deviation (SD). The experimental data were subjected to the analysis of variance (one-way ANOVA). The significant differences between the mean values of data obtained from the fermentation kinetics, syneresis rates, bacterial counts and *in situ* anti-*Escherichia* activity were analysed by Tukey's multiple comparison test, while data from MTT assay were analysed by Dunnett's test. All statistical analyses were performed using the software GraphPad Prism 6.0 (GraphPad Software Inc., San Diego, USA) and Excel 2016 (Microsoft).

RESULTS AND DISCUSSION

Selection of GABA-producing strain. In the mammalian central nervous system, GABA is the most abundant inhibitory neurotransmitter. Abnormalities in GABA levels and GABA receptor dysfunction are associated with the pathogenesis of several neurological and psychiatric disorders, including depression. Certain health-conferring bacteria can synthesize GABA and when administered, they can elevate GABA levels and alleviate depression-like behaviour. Decarboxylation of glutamate (GAD ABC genes) appears as the most common pathway of GABA production that relies on the machinery of probiotic bacteria (Poluektova et al. 2021; Zhou et al. 2022). Herein, to determine the most potent GABA-producing strain, nine probiotic bacteria were subjected to the GAD colorimetric assay. This colorimetric test detects the level of activity of the GAD enzyme, which is directly related to the potential of GABA production since this enzyme is responsible for the conversion of glutamate to GABA (Santos-Espinosa et al. 2020). The test uses a bromocresol green indicator that is very sensitive to proton consumption during the decarboxylation reaction and relies on a colour change, qualitatively indicating which strains have the ability to produce GABA. Based on visual assessment and the presence of yellow-green and green-turquoise coloration (Woraharn et al. 2014) the tested LAB strain exerts low and moderate GAD activity. Results obtained showed that among the tested strains *L. reuteri* DSM 17938 was the most potent GABA-producing strain (Figure 1). It is expected that *L. reuteri* was proved to be the most potent GABA-producing strain. Recent findings presented by Zhou et al. (2022) describe the neurobiotic capacity of *L. reuteri*. *L. reuteri* co-administered with *Ligilactobacillus murinus (*formerly *Lactobacillus murinus)* can diminish depression-like behaviour in Dcf1 KO (dendritic cell-derived factor 1 knockout) mice, increase GABA levels and restore GABA-related receptor expression (Zhou et al. 2022). The selected strain, *L. reuteri* DSM 17938, was used for yogurt preparation in further work.

Fermentation kinetics and syneresis of yogurt samples. Based on the obtained results, GW and

Figure 1. Assessment of glutamate decarboxylase (GAD) activity of lactic acid bacteria (LAB) strains

A rapid colorimetric assay using bromocresol green as a pH indicator was used for the assessment of GAD activity.

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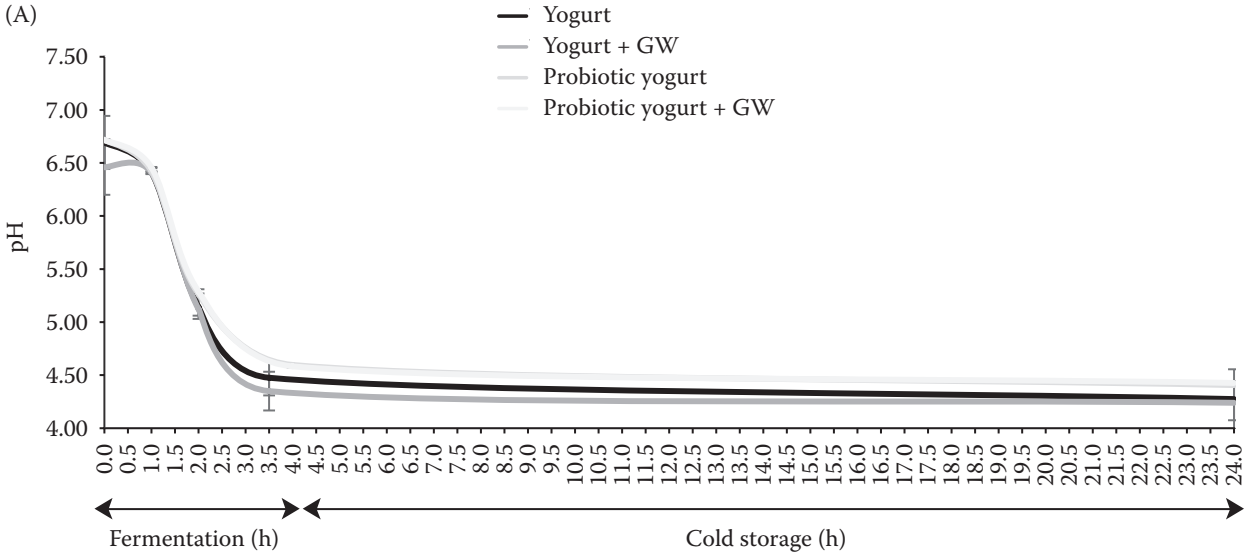

Figure 2. Decrease of pH value (A) and amount of whey (%) (B) separated from yogurt samples without and with *Limosilactobacillus reuteri* and 2% (w/v)

GW – *Ganoderma lucidum* residues obtained after water extraction

L. reuteri did not disrupt the fermentation process of yogurts. The fermentation kinetics of the tested samples is shown in Figure 2A. As can be observed, no statistically significant change in pH values during the fermentation and stabilization process between the yogurt samples was detected. The time required to reach a pH value of about 4.6 was up to 4 h of incubation. Our results are in line with the findings obtained by Vanegas-Azuero and Gutiérrez (2018), who demonstrated that the enrichment of yogurt samples with β-glucans from *G. lucidum* did not significantly affect the fermentation kinetics and that the required time for yogurt fermentation was up to 5 h. Next, syneresis rates were not significantly different between the tested samples (Figure 2B).

LAB viability. The LAB counts were monitored in fresh yogurt samples. High LAB viability was detected in all samples (Table 1), while the addition of GW increases the LAB number. Data obtained are in line with Wu et al. (2020) findings, which indicate that *G. lucidum* spores affect the intestinal microbiota by increasing the number of several bacterial genera (*Lactobacillus*, *Turicibacter*, and *Romboutsia*), as well as the viability of *L. reuteri* strains. The increased viability of LAB (up to 9.76 ± 0.11 log CFU mL^{-1}) present in fresh yogurt samples may be beneficial against microbial contamination and contribute to the health benefits that characterize the prepared products.

***In situ* anti-*Escherichia* activity.** *Escherichia coli* O157: H7 has been widely recognized as an important human pathogen (Akdemir 2007). It can be found in yogurt, cheese, and other fermented dairy products because it has the ability to tolerate extremely acidic

Table 1. Lactic acid bacteria (LAB) viability (n = 3, mean ± SD; log CFU mL^{-1})

Samples*	LAB viability
Yogurt	8.92 ± 0.06[a]
Yogurt + GW	9.18 ± 0.83
Probiotic yogurt	9.16 ± 0.31
Probiotic yogurt + GW	9.76 ± 0.11[a]

*Yogurt samples were fermented with YC-X11 starter culture; probiotic yogurt samples were fermented with YC-X11 + *Limosilactobacillus reuteri* DSM 17938; GW – *Ganoderma lucidum* residues obtained after water extraction; [a]values with the same letter are significantly different in comparison with each other according to Tukey's multiple comparison test (P < 0.05); CFU – colony forming units; SD – standard deviation

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Table 2. Anti-*Escherichia* effect (n = 3, mean ± SD; log CFU mL^{-1})

Strains	GC	Yogurt	Yogurt + GW	Probiotic yogurt	Probiotic yogurt + GW
E. coli 0157: H7*	7.37 ± 0.30^{a}	7.73 ± 0.74^{b}	6.96 ± 0.18^{c}	7.38 ± 0.37^{d}	4.81 ± 0.62^{abcd}
E. coli ATCC 35218	7.37 ± 0.47^{a}	7.17 ± 0.53^{b}	7.05 ± 0.21^{c}	6.34 ± 0.41	5.64 ± 0.29^{abc}

GC – growth control; **Escherichia coli* strain growth in MHB; MHB – Mueller Hinton Broth; GW – *Ganoderma lucidum* residues obtained after water extraction; ^{abcd} values with the same letter within the same row are significantly different in comparison with each other according to Tukey's multiple comparison test (P < 0.05); CFU – colony forming units; SD – standard deviation

conditions due to the presence of an acid-induced oxidative system, an acid-induced arginine-dependent system and a glutamate-dependent system (Coşansu 2018). Moreover, commercial yogurt products were implicated in *E. coli* O157: H7 outbreaks (Akdemir 2007). Here, the viability of *Escherichia* strains: *E. coli* 0157: H7 and *E. coli* ATCC 35218 was investigated in yogurts fermented with and without GW. The most prominent anti-*Escherichia* properties were detected for GW-supplemented probiotic yogurt (Table 2). A reduction in *E. coli* numbers may be associated with a large initial LAB count required to produce adverse effects on the pathogens. LAB starter culture as well as probiotic bacteria *L. reuteri* could exert an antagonistic effect against *E. coli*. The LAB metabolic activity responsible for liberating potent antibacterial peptides from milk proteins could be a limiting factor for the growth of *E. coli* (Jovanović et al. 2021). Additionally, several studies demonstrated that *Ganoderma* extracts as well as its active compounds exhibit significant anti-*E. coli* activity. Mishra et al. (2018) demonstrated that peptide fractions isolated from the mycelium and body of *G. lucidum* have anti-*Escherichia* activity. Furthermore, Al-Ansari et al. (2020) showed that silver nanoparticles synthesized by ethanol extract of *G. lucidum* have antioxidant, antitumor, and antimicrobial effects and that *E. coli* is highly sensitive to these nanoparticles.

Cytotoxic properties. The health benefits of yogurt consumption are well established for all age groups of consumers, including infants and older adults (Adolfsson et al. 2004). Abundant evidence indicates that regular consumption of yogurt can contribute to a favourable gut microbiome that has powerful anti-pathogenic properties. In addition to the beneficial effect of fermented drinks on the pronounced suppression of pathogens, various studies confirm that yogurts also exert other health-promoting effects. Still, studies investigating the role of fermented beverages in colon carcinogenesis have shown inconsistent results (Adolfsson et al. 2004; Michels et al. 2020). Thus, using the human colon carcinoma cell culture HCT116, an evaluation of the cytotoxic potential of the yogurt extracts was carried out. For all tested samples, a statistically significant reduction in cell viability was observed for at least one tested concentration (Figure 3). However, cells were most susceptible to an extract

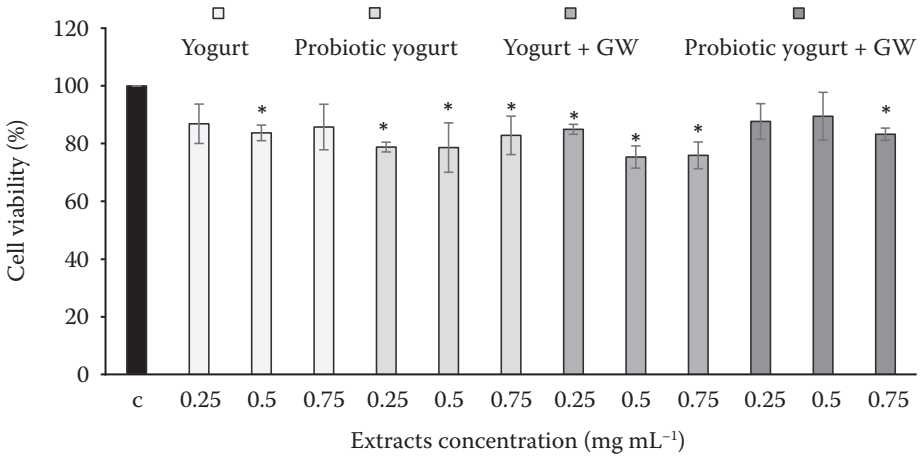

Figure 3. Viability of HCT116 cells treated with extracts of yogurts with and without *Ganoderma lucidum* residues obtained after water extraction (GW)

*significant difference in means between samples and cell growth control (C) according to Dunnett's test (P < 0.05)

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Table 3. Sensory evaluation conducted by a panel of seven members (n = 2, mean ± SD)

Sample	Sensory attributes*				
	appearance	odour	taste	texture	% max. overall quality
Yogurt	12.54 ± 0.41	18.71 ± 0.27	31.25 ± 0.49	23.14 ± 0.56	85.64
Probiotic yogurt	14.68 ± 0.14	19.43 ± 0.26	33.50 ± 0.30	29.36 ± 0.13	96.96
Yogurt + GW	10.82 ± 0.47	16.86 ± 0.70	15.75 ± 1.23	16.93 ± 1.31	60.36
Probiotic yogurt + GW	11.14 ± 0.49	17.86 ± 0.47	13.50 ± 1.39	16.07 ± 1.25	58.57

*coefficient of importance (CI) was assigned to each sensory attribute: appearance (CI = 3), smell (CI = 4), taste (CI = 7), and texture (CI = 6); GW – *Ganoderma lucidum* residues obtained after water extraction; SD – standard deviation

obtained from yogurt supplemented with GW (up to 25% inhibition of the cell viability). Similarly to obtained results, in our previous research, it was pointed out that yogurt-extracted whey inhibited HCT116 cell viability to some extent (Jovanović et al. 2021). More pronounced inhibition of colon cancer cell viability could be accomplished by increasing the dose of GW and adding additional probiotic strains or parabiotics at the beginning of yogurt preparation. Kim et al. (2022) confirm that the presence of parabiotics, based on heat-treated *Lactobacillus* strains, induces apoptosis of human colorectal cancer cells (RKO). Also, the active compounds of *G. lucidum* such as triterpenes: ganodermantriol, lucidenic and ganoderic acids, show significant pharmacological properties against cancer (Ahmad 2020).

Sensory evaluation. It is certain that the addition of new, functional ingredients, such as GW, affects the organoleptic properties of fermented beverages. Therefore, the evaluation of the sensory properties of the newly developed yogurts is essential for the optimization of the recipe and gaining insight into the acceptability of the product. Similarly to the results presented in the study by Li et al. (2011), the panel rated yogurt samples with GW as sensory acceptable but with lower ratings (60.36% and 58.57%; Table 3). Taking into account that *Ganoderma* has a bitter taste, it is probably an explanation for lower marks for the taste of samples with added fungal residues. The obtained preliminary sensory data can be used in the next phase of the formulation of fermented beverages containing GW and GABA-producing strains.

CONCLUSION

To our knowledge, none of the commercial beverages contains *Ganoderma* residues. Based on GABA-producing activity, among nine LAB strains, the probiotic bacterium *L. reuteri* DSM 17938 was selected as the most potent strain and further utilised for yogurt preparation. It is important to highlight that GW and *L. reuteri* did not disrupt the fermentation process of yogurts. During the yogurt production process, the pH values did not significantly change, while the addition of GW increases the LAB counts. GW added to yogurt increased anti-*E. coli* effect and cytotoxic effect of newly developed products. Anti-*E. coli* effect was demonstrated on two strains *E. coli* 0157: H7 and *E. coli* ATCC 35218, while the cytotoxic effect on HCT116 cells. The evaluation of the sensory properties of the newly developed yogurts showed that the added GW and GABA-producing strains had a slightly negative effect on the taste and texture of the analysed product. Considering the unquestionable health benefits of consuming newly developed yogurt, the sensory attributes can be improved by adding natural sweeteners (e.g. honey, stevia). The innovative yogurt-based products will have a great potential to be part of the profitably modern world market.

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