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Enhancing Functional Probiotic Yoghurt: Exploring the Synergy of Ginseng Extract and *Lacticaseibacillus rhamnosus* NRRL B-445 on Antibacterial, Antioxidant Activities, and Sensory Attributes through *In-Vitro* Evaluation

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Abstract

This research aimed to manufacture a functional ginseng-fortified probiotic yoghurt (GFPY) to play a dual role in antioxidant and antibacterial activities, to provide Egyptian consumers and the local probiotics market with a healthy, safe, and functional dairy product, as a diet-oriented prevention strategy against the onset of bacterial- and oxidative stress-related diseases. Total phenolics (TPC), antioxidant and antibacterial activities were determined in ginseng water extract (GWE), ginseng methanol extract (GME), and in GFPY formulations (0.5, 0.1 or 2% GME) which were examined for sensory properties, *L. rhamnosus* viability and survival of *B. cereus* and *E. coli*. GME gave higher TPC (11.38 mg GAE g^{-1}) and antioxidant activity (15.195% DPPH-RSA) than those by GWE (7.34 mg GAE g^{-1} and 7.035%, respectively), besides stronger inhibition zone against pathogens (P<0.05). For inhibitory and bactericidal activities, *B. cereus* and *E. coli* O157:H7 were the most sensitive towards GME at 2.0 and 1.5%, respectively. GFPY with 1.0 and 2.0% GME had more 2- and 3-fold antioxidants higher than those having 0.5%, respectively. When GME was increased to 0.5-1%, *L. rhamnosus* in GFPY increased and was still higher than 8 Log CFU mL^{-1} on storage, however, 2% was not positively effective. *B. cereus* and *E. coli* lost their survival in GFPY (2% GME) on the 2nd and the 5th day of storage, respectively. Unlike 2%, GFPY with 0.5% GME was very accepted. Combination of *L. rhamnosus* with ginseng could maximize the role of probiotics, ginseng, and yoghurt as a preferable vehicle, enhancing nutritional and health benefits.

Keywords: Ginseng; *Lacticaseibacillus rhamnosus*; Probiotic yoghurt; Antibacterial activity; Pathogens; Antioxidant effect.

1. Introduction

Chronic diseases are one of the major healthcare problems that affect a growing number of people all over the world [1]. The onset of common ones, such as diabetes and related vascular complications are proven to be promoted by oxidative stress which occurs owing to overproduction of reactive oxygen species, as byproducts of cellular metabolism, with high toxicity [2]. Functional foods fortified with the antioxidative effects of medicinal plants, and their derived bioactive compounds were suggested to develop a diet-oriented prevention strategy to decrease the expansion of chronic diseases. Among plants, Ginseng (Panax ginseng C.A. Mayer) a well-known traditional medicinal plant has been reported to demonstrate biological activities as an antioxidant [3]. Because of its large molecular size and high polarity, humans do not effectively digest ginseng and show limited utilization of its phytochemicals in their small intestine [4]. Since intestinal flora are involved in metabolic pathways, they can produce a variety of enzymes to digest the medicinal herbs into absorbable active small molecules, with the ability to motivate physiological changes in the body [5]. Accordingly, probiotic bacteria can enhance the maintenance of intestinal microflora and produce antioxidant substances via the fermentation of ginseng [6, 7]. Also, ginseng as a prebiotic may be beneficial to stimulate growth and activity of probiotics in both the intestine and the diet [8].

Among probiotics, *Lacticaseibacillus rhamnosus* was selected in the current study, to be incorporated into ginseng- fortified probiotic yoghurt (GFPY), as a functional dairy product for many reasons: (1), *L. rhamnosus* achieved many probiotic characteristics as they are bile resistant, endure passing through and colonize the gastrointestinal tract [9]. Some strains found to produce bacteriocins, with effectively antimicrobial activity against foodborne pathogens [10]. (2), they have been reported to produce β- glucosidase enzyme with biotransformation ability of ginseng to its actively minor molecules [11], with antioxidative effects [12]. (3), besides the production of lactic acid during milk fermentation, *L. rhamnosus* was of ability to release antioxidants from milk proteins [6, 13]. Furthermore, yoghurt as one of the most popular dairy products widely consumed for its nutritional value, can be a source of antioxidants derived from peptides and amino acids during fermentation by lactic acid bacteria [14]. However, dairy products are reported to have low antioxidant effects because of their low content of polyphenolic compounds [15]. Many researchers have enhanced the antioxidant activity of yoghurt by the addition of pomegranate, grape, sweet cherries [16] and ginseng or red ginseng extract (GE, RGE) [17, 18]. Thus, the conjunction of ginseng extract and probiotic

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L. rhamnosus was a target of this research to boost the antioxidant functionality of yoghurt. Also, yoghurt is being examined as a successful carrier for delivering both probiotic *L. rhamnosus* and ginseng extract.

On the other hand, WHO declared that infectious diseases are one of the leading causes of worldwide morbidity and mortality [19]. Infectious diseases caused by bacteria and their antibiotic resistance remain the most serious problem and represent an immense global predicament [20]. Furthermore, contamination of dairy products by food-borne pathogens is being major problem. Otherwise, consumers demand safe and highly nutritional quality of chemical additives food with extended shelf life. For this, natural medicinal herbs have been reported to affect several food-borne pathogens through the antibacterial activity of their extract or isolated compounds [20, 21].

Moreover, most research has focused on studying the methods of ginseng extraction, its composition, and its role as a pharmaceutical antioxidant. However, there is a deficiency in studying its antibacterial activity against foodborne pathogens, as well as its applicable use. This research attempted to fill this gap, especially in Egypt, where there is little awareness of the importance of ginseng as a food additive, thereby ginseng-fortified dairy products are absent or still scarce. Thus, this work is designed to boost the functionality of probiotic yoghurt with ginseng to serve not only for its antioxidant activity but also for its prospective role as an antibacterial agent, enhancing the safety of yoghurt, with acceptable organoleptic properties.

Therefore, the present study aimed to determine the total phenolics (TP), antioxidant activity, antibacterial activity against foodborne pathogens, and evaluation of inhibitory and bactericidal effects against sensitive pathogens. The GFPY were assessed for sensory properties and investigated for the stability of starter cultures and *L. rhamnosus*, as well as their pH changes on 15-day cold storage. Furthermore, the survival of *B. cereus* and *E. coli* in GFPY with different concentrations of GME was examined.

2. Materials and methods

2.1. Materials, strains, and reagents

Dried Korean red Ginseng root samples (Panax ginseng C. A. Meyer) were purchased from a local market in Cairo, Egypt. Fresh buffalo's milk was obtained from the herd of the Fac. of Agric., Cairo Univ., Egypt and standardised to contain 3% fat by partial skimming of milk.

Streptococcus thermophilus DSMZ 2479 (*S.thermophilus*) and *Lactobacillus delbrueckii* subsp. *bulgaricus* DSMZ 20080 *(L.bulgaricus)* were obtained from the Egyptian Microbial Culture Collection (EMCC) at Cairo Microbiological Resources (Cairo MIRCEN), Fac. of Agric., Ain Shams Univ. Probiotic *Lacticaseibacillus rhamnosus* NRRL B-445 (*L. rhamnosus*) was provided by Northern Regional Research Laboratory, Illinois, USA. Pathogenic bacterial strains including *Bacillus cereus* ATCC 33018 (*B. cereus)*, *Staphylococcus aureus* ATCC 20231(*St. aureus*), *Listeria monocytogenes* V7 (*L. monocytogenes*), *Escherichia coli* O157:H7 ATCC 6933 (*E. coli*), *Salmonella typhimurium* ATCC 14028 (*S. typhimurium*) and *Pseudomonas aeruginosa* ATCC 9027 (*P. aeruginosa*) were kindly donated from the stock cultures of the Agricultural Research Centre, Giza, Egypt. *Yersinia enterocolitica* ATCC 23715 (*Y. enterocolitica*) was supplied by EMCC, Cairo MIRCEN.

Folin-Ciocalteu's phenol reagent and gallic acid (GA) monohydrate were purchased from Fluka (Madrid, Spain), while 1-diphenyl-2-picrylhydrazyl (DPPH) was bought from Sigma Aldrich (Sigma, St. Louis, Mo. USA). Tryptic soy broth or agar supplemented with 0.6% yeast extract (TSB-YE, TSA-YE) was obtained from Difco (Detroit, MI, USA). Other media, antibiotics, solvents and anaerogen sachets for anaerobic conditions were supplied from Oxoid (Ltd, Basingstocke, Hampshire, England), Biolife (Milano, Italy) and/or El Nasr Pharmaceutical Chemicals (Cairo, Egypt).

2.2. Bacterial culture preparation

Streptococcus thermophilus was activated in M17 broth medium (Oxoid) with aerobic incubation at 37°C for overnight, while *L. delbrueckii* and *L. rhamnosus* were cultured into De Man, Rogosa, Sharpe (MRS) broth medium (Oxoid) and anaerobically incubated in BBL anaerobic jar (Becton Dickinson Microbiology Systems, Sparks, MD, USA) provided with disposable anaerogen sachets (oxoid Ltd, Basingstocke, Hampshire, England) at 37°C for 18-24 h. These cultures were then routinely generated in corresponding media and incubation conditions every week and successfully activated twice before use.

For indicator pathogens, each strain was cultured and activated twice in 10 mL TSB-YE at 37 °C for 12-18 h and stored at 4°C. For the experiments, 100 µL aliquot of each strain was transferred to 10 mL of TSB-YE and incubated at 37 °C for 5-6 h to meet the mid-exponential growth phase. One mL of each culture broth was then harvested by Eppendorf centrifugation (Model JA-14, International Equipment Co., USA) for 10 min at 10 000 g at 4 °C. After centrifugation, the pellets were suspended in 0.1% peptone water (Difco) as a culture suspension [22].

2.3. Preparation of ginseng extracts (GE)

Ginseng extracts (GE) were prepared by ginseng water extraction (GWE) and ginseng methanol extraction (GME) involving heating with shaking-assisted equipment, using pure distilled water or 70% (v/v) aqueous methanol as solvents, respectively [23]. For both, 50 g of red ginseng root powder was separately mixed with 1000 mL of each solvent and extracted for 3 h at 100 °C and 60°C, respectively in a water bath. Then, the resultant slurry was filtered, and the solid residue was repeatedly extracted twice under the same conditions. After extraction, the extracts were collected and filtered through filter paper (Whatman No. 2) at room temperature, then evaporated using a rotary evaporator under vacuum filtration at 45°C (Rotavapor® R-300, Flawil, Switzerland) to remove the excess solvents. The ginseng extracts were then freeze-dried and stored at -20°C until used.

2.4. Total phenolic content (TPC) in ginseng extracts

The TPC in both GWE and GME was determined calorimetrically at 725 nm using the Folin-Ciocalteau reagent according to the modified method described by [24]. The ginseng extract powder was dissolved in distilled water (100 mg mL^{-1}). A sample diluted twofold with deionized water (20 ml) was mixed with 0.625 ml of the Folin-Ciocalteau reagent (Sigma-Aldrich, St. Louis, MO), in a 25 ml volumetric flask, after which the mixture was allowed to stand at 25°C for 3 min. Subsequently, 2.5 ml of a saturated solution of Na_2CO_3 (35%) was added to the mixture. The content was mixed and diluted to the full volume with

deionized water. Using a double-beam ultraviolet-visible spectrophotometer (Hitachi, Ltd., Tokyo, Japan), the absorbance was measured at 725 nm against a blank after 60 min for comparison. Gallic acid was used as a standard to prepare the calibration curve and ranged from 2.5 to 20 µg/25 µl of assay solution. TPC was expressed in the form of mg of gallic acid equivalents (GAE) per g of extracts.

2.5. Antioxidant activity of Ginseng extracts by DPPH radical scavenging activity

The antioxidant activity of GE was evaluated with the modified DPPH assay [25] based on the measurement of hydrogen donating or radical scavenging ability using the stable 1-diphenyl-2-picrylhydrazyl (DPPH). In brief, the reaction mixture contained 100 µL of methanolic solution of ginseng (phenol) extract (100 mg mL^{-1}) and 3.9 ml methanolic solution of DPPH (0.0025 g/100 ml CH_3OH). The reaction mixture was then placed in a cuvette and left to stand in a dark place for 30 min, the absorbance at 517 nm was measured against methanol using the ultraviolet–visible spectrophotometer (double-beam, Hitachi U-3210) (Hitachi, Ltd., Tokyo, Japan). Simultaneously, the absorbance at 517 nm of the control sample (0.1 ml methanol + 3.9 ml methanolic solution of DPPH) was measured against methanol. The RSA of the tested samples, expressed as an inhibition percentage of DPPH, was calculated according to the following formula: % Inhibition = 100 X (A – A_0) / A. Where A is the absorbance at 517 nm of the control sample and A_0 is the final absorbance of the test sample at 517 nm.

2.6. Antibacterial activity of ginseng extracts

To determine the antibacterial activities of GWE or GME, agar well diffusion assay was conducted according to CLSI (Clinical and Laboratory Standards Institute) [26] and Mallesha et al., [27] with a few changes. Briefly, one mL of peptone water-suspended pellets of each bacterial pathogen was transferred into TSB-YE broth, incubated at 37 °C for 12 h and diluted to get approximately $5x10^5$ in 0.1% sterile peptone water. Then, 100 µl of each was spread on previously solidified TSA-YE plates. After incubation in the refrigerator for 30 min, 5 mm diameter wells were aseptically made in each plate and filled with 100 µl of GWE or GME. Chloramphenicol (30µg/disk) was used as a positive control against all indicators except for *P. aeruginosa* where nalidixic acid (30µg/disk) was applied, while sterile peptone water was used as a negative control. The plates were incubated for 24 h at 37°C before the diameter (mm) of the clear inhibition zone (IZ) around the wells was measured to determine the antibacterial activity of ginseng extracts. The experiment was repeated.

2.7. Inhibitory activity and bactericidal effect of GME

Based upon the results obtained from the antibacterial activity against the seven test indicators, the GME and the more sensitive strains namely *B. cereus*, *St. aureus*, *E. coli* 0157: H7 and *S. typhimurium*, were selected for this section of the study. The GME was prepared at 0, 0.5, 1.0, 1.5 and 2.0% (w/v) in both TSB-YE broth and sterilized distilled water (DW) to analyze their inhibitory activity on cell growth and their bactericidal effect, respectively [28] against the indicators. The GME-inoculated media with $5x\ 10^5$ CFU mL^{-1} of each bacterial indicator were incubated at 37°C for 24 h. The samples were aliquoted at 0, 4, 8, 12 and 24 h, and serially diluted with 0.1% sterile peptone water and surface plated on TSA-YE. The viable cells were counted as a unit of log CFU mL^{-1}.

2.8. Ginseng-fortified probiotic yoghurt (GFPY)

Five formulations of yoghurt were prepared based on the presence of probiotic *L. rhamnosus* and the concentration of GME, as follows: The first one (CY) is the standard yoghurt which was made with *L. bulgaricus* and *St. thermophilus* at 2% (1:1, v/v), and with neither *L. rhamnosus* nor GME, to serve as the first control, while probiotic yoghurt (CPY) was prepared by both the same starter cultures in the same ratio and 2% (v/v) *L. rhamnosus*, but without GME, to serve as the second control. The other three formulations were made with both starter cultures and probiotic *L. rhamnosus* and fortified with GME (5, 10 and 20 mg ginseng extract/ mL milk, i.e., 0.5, 1 and 2%), to represent $GPY_{0.5}$, GPY_1, and GPY_2, respectively. To be prepared, standardized buffalo's milk (3% fat, 12.2% total solids, 3.5% total protein and 0.70% ash) was heated to 90°C for 10, to eliminate competing microorganisms. GME was added to the corresponding samples after heat treatment of milk to avoid excessive oxidation, glycation and occurrence of the Maillard browning reaction [8]. After immediately cooling the ginseng-fortified milk solutions to 37°C, the starter and/or probiotic *L. rhamnosus* were inoculated into the conformable samples, then incubated at 40°C until its pH reached 4.5 for a proper coagulum (4.5-5 h) and stored in refrigerator at 4°C for 15 days. The samples were assayed for bacterial cell count, pH changes and detection of both coliform and mold and yeast on 1^{st}, 5^{th}, 10^{th} and 15^{th} days, while determination of TPC and antioxidant activity using radical scavenging activity (RSA%), and evaluation of sensory properties were carried out after 1 day of cold storage.

2.8.1. Antioxidant properties of GFPY

2.8.1.1. Preparation of samples

For determining the antioxidant properties of GFPY in terms of TPC and DPPH- RSA, the extract was prepared according to Öztürk et al., [29] with slight modifications. Each sample (10 g) was mixed thoroughly with the aqueous methanolic solution (75%), homogenized by vortex-mixer (VELP Scientific, Italy), and then centrifuged twice at 10,000 rpm for 10 min at 4°C, to remove non-hydrolyzed casein and fat. The obtained supernatants were then filtered (Whatman No.1) and the extracts stayed at 4°C for analysis.

2.8.1.2. Determination of TPC

TPC in the obtained extracts of GFPY was measured using the Folin-Ciocalteu assay as described above by Najafi et al., [24]. The absorbance rate was measured at 725 nm, where gallic acid was used as the reference standard, and the results were expressed as mg GAE/g of the triplicate extracts of yoghurt samples.

2.8.1.3. DPPH radical-scavenging activity

The antioxidant activity of the prepared extracts of GFPY was estimated using the modified DPPH-RSA assay, as described by Harkat et al., [25]. The samples were spectrophotometrically determined at 517 nm in triplicate and the RSA % was calculated as described above.

2.8.2. Viability of *L. rhamnosus* and yoghurt stater cultures in GFPY

Viable cell counts (Log CFUmL^{-1}) of *L. rhamnosus* in GFPY being stored at 4°C was assessed on days 1, 5, 10 and 15. At each measurement point, the samples were tenfold diluted in sterile peptone water (0.1%). One tenth mL of appropriate dilutions was plated in duplicate on Lactobacillus selective MRS agar supplemented with 15 mg/ml fusidic acid [30] and anaerobically incubated at 37°C for 48 hours, after which they were enumerated. *Streptococcus thermophilus* was colonized on M17 agar containing 10% lactose [31] under aerobic conditions for 48 h at 37°C, whilst *L. bulgaricus* was retrieved using acidified MRS agar (pH 5.2), the plates were anaerobically incubated at 43°C for 48 h [32].

2.8.3. Detection of coliform and molds and yeasts in GFPY

Coliforms were detected on violet red bile agar (Oxoid) according to APHA, [33] where the plates were incubated at 37°C for 48 h under aerobic conditions. While molds and yeasts were determined on plate count agar supplemented with chloramphenicol (100 (µg/ ml) (CPCA, pH 6.8), the plates were incubated in an upright position at 25°C for 3 days, after which colonies of yeasts and molds were enumerated [34].

2.9. Survival of *B. cereus* and *E. coli* in GFPY

To determine the effect of antibacterial activities due to ginseng on the pathogens in yoghurt matrix, *B. cereus* and *E. coli* were separately inoculated into all five formulations which were typically prepared as described above. These pathogens were added (approximately 5x10^5 CFU mL^{-1}) to the ginseng-fortified milk solutions at 37°C immediately after adding the starter cultures alone or with probiotic *L. rhamnosus*. After incubation at 40°C until coagulation, all yoghurt formulations samples were stored at 4°C for 15 days. The survival of the two test pathogens represented in viable cell count was determined in all samples when fresh and after 1, 2, 3, 5, 7, 10 and 15 days of cold storage. Ten grams of each sample was transferred to 90 mL 0.1% peptone water (pH 6.3) and homogenized with Stomacher Lab-Blender. Appropriate 10-fold dilutions of the samples were prepared in peptone water and plated in duplicate on selective growth media.

2.9.1. Enumeration of *B. cereus*

Bacillus cereus was determined by the surface plating technique onto *B. cereus* selective agar that was supplemented with egg yolk and polymyxin solution (SR99, Oxoid). Plates were incubated for 24 h at 30°C (BAM, 2001d).The suspected colonies peacock blue-coloured and surrounded by a zone of precipitation of egg yolk[35]were counted as colony-forming units(CFUml^{-1})

2.9.2. Enumeration of *E. coli O157:H7*

For *E. coli* O157:H7, appropriate dilutions of each sample were spread onto plates of Sorbitol MacConkey (SMAC) agar (Oxoid). After incubation for 24 h at 37ºC, sorbitol-fermenting bacteria appeared as pink to red colonies. Typical *E. coli* O157:H7 colonies that are colourless were quantified in log CFUmL^{-1} [36].

2.10. pH measurement

All samples of GFPY were prepared with or without pathogens, and their controls were periodically analyzed for pH values using a digital pH meter (HANNA, Instrument, Portugal) with a glass electrode, when fresh and after 5-day intervals for 15 days of cold storage.

2.11. Sensory evaluation

The effect of GME added in different concentrations on the sensory properties of GFPY in comparison to their controls was assessed according to Karagul-Yuceer and Drake [37] in terms of flavor (60), body and texture (30), appearance and color (10) and (100) for overall acceptability. The assay was carried out by ten trained staff members of the Dairy Dept., Food Industries and Nutrition Research Institute, National Research Centre, Cairo, Egypt, based on their experience in sensory evaluation of yoghurt and fermented dairy products.

2.12. Statistical analysis

Statistical analysis was implemented using SPSS 19.0 (SPSS Ltd., Surrey, UK). Data were presented as the means ± SDs and estimated by one-way or two-way analysis of variance (ANOVA). When $P < 0.05$, the differences were deemed significant.

3. Results and discussion

3.1. Total phenolics, antioxidant activity (DPPH %) and antibacterial activities of ginseng extracts

Extraction procedures utilized to extract functional components from root ginseng, using both different solvents and the process of heating and shaking had a great effect on the obtained content of TPC from ginseng and thus it's antioxidant activity. From Table 1, GME with high temperature (60°C) and shaking was better and produced significantly ($P < 0.05$) higher content of TPC (11.38 mg GAE g^{-1} extract) and more potent antioxidant activity in terms of DPPH (15.195 %) than obtained by (GWE) at 100°C under the same other conditions, 7.34 mg GAE g^{-1} and 7.035%, respectively. The results were compatible with those reported by Zhang et al., [38] where ethanol as a solvent (70%) at 60°C showed a higher extraction yield of TPC and more powerful antioxidant activities than GWE at 100°C. Moreover, high temperature together with shaking might lead ginseng to release more yield of TPC, and accordingly improve the RSA (%), as the antioxidant activities of plant extracts are usually correlated with the levels of TPC. In this regard, Kim et al., [39] and Lee et al., [28] investigated the effect of solvent, time, and temperature on the contents of ginsenosides from ginseng extracted by different methods (heat reflux extraction method (HRE) and subcritical water extraction (SWE), respectively). Their findings indicated that the increasing the temperature used with the solvents, the increasing the diffusivity of the aqueous solvents into the ginseng and the increasing the release of bioactive TP and others during the solid-to-liquid transfer. For antibacterial activity on TSA-YE using well diffusion assay, all indicator pathogens were sensitive to either GWE or GME (70%), with variable susceptibility. Bacillus cereus and E. coli O157:H7 were the most sensitive to GME showing 15.5 and 15 mm of inhibition zone (IZ) around the wells, respectively, followed by S. typhimurium and St. aureus with 13 and 12 mm of IZ, respectively, whereas the least sensitivity was exerted by L. monocytogenes, P. aeruginosa and Y. enterocolitica, with 8, 10 and 11 mm of IZ, respectively (Table 1).

Table 1. Total phenolics, antioxidant activity, and antibacterial activity of ginseng water extract (GWE) and ginseng methanol extract (GME).

Ginseng extracts	Total phenols (mg GAE/g)[1]	Antioxidant activity (DPPH-RSA%)[2]	Inhibition zone diameter (mm)						
			St. aureus	*L. monocytogenes*	*B. cereus*	*E. coli O157:H7*	*S. typhimurium*	*Y. enterocolitica*	*P. aeruginosa*
GME (60°C)	11.38±0.26[a]	15.195±0.45[a]	12±0[b]	8±0[b]	15.5±0.70[b]	15±0[b]	13±0[b]	11±0[b]	10±0[b]
GWE (100°C)	7.34±0.36[b]	7.035±0.65[b]	10±0[c]	8±0[b]	12.5±0.70[c]	12±0[c]	10.5±0.70[c]	10±0[c]	9±0[c]
+control			25±0.08[a]	22±0.022[a]	32±0.05[a]	31±0.0[a]	29±0.04[a]	24±0.032[a]	25±0.02[a]
-control			0.0[d]	0.0[d]	0.0[d]	0.0[d]	0.0[d]	0.0[d]	0.0[d]

[a]Values are mean ± SD. Values with different letters within a column are significantly different (P < 0.05).
[1]Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract based on dry weight.
[2]Antioxidant activity was determined by 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (RSA) as % of inhibition value.
+ Chloramphenicol (30µg/disk) was used as a positive control against all indicators except for P. aeruginosa where nalidixic acid (30µg/disk) was applied. - Sterile peptone water was used as a negative control.

Excluding *L. monocytogenes*, GME elicited a stronger antibacterial effect than that of GWE, with the significant difference in IZ ($P < 0.05$) against the other indicators. The results indicated the higher antibacterial activity of GME may be due to its higher level of TPC than in GWE. Lee et al., [28] investigated the relationship between TPC and the antibacterial activity of GE against *B. cereus* and found the IZ detected was correlated and closely proportional to the concentration of phenolic compounds (R2 = 0.97), reflecting the impact of TPC (flavonoids) in the ginseng extract to inhibit bacterial growth by modulating cellular redox response. In addition, the high temperature during the extraction process could increase the solubility of a less polar compound and thus its release from ginseng to its extract increasing the antibacterial activity against indicator pathogens [28].

3.2. Inhibitory effect of GME on cell growth

Because of higher concentration of TPC and stronger antibacterial activity (Table 1), GME at different concentrations of 0, 0.5, 1, 1.5 and 2% were evaluated for inhibitory effect against the more sensitive indicator pathogens, namely *B. cereus*, *St. aureus*, *E. coli* O157:H7 and *S. typhimurium* in the broth media of TSB-YE (Fig. 1(a), (b), (c) and (d)). Concerning *B. cereus* (Fig. 1(a)), unlike the control and 0.5% extract, the growth of *B. cereus* was decreased by 7.84, 6.9 and 4.59 log CFU ml^{-1} compared to the control, at 2, 1.5 and 1% after 24 h of incubation at 37°C, respectively. The efficacy was statistically significant between all concentrations, as well as between each extract and the control ($P<0.05$). In the case of *St. aureus*, GME at only 2% significantly inhibited the growth of *St. aureus* ($P<0.05$), as the viable cells decreased by 5.66 log CFU ml^{-1} contrasted to the control, after 24 h of incubation (Fig. 1(b)). For *E. coli* O157:H7, the growth was not significantly ($P >0.05$) affected by 0.5 % extract compared to the control. While adding the extract at a rate of 1.5 or 2% inhibited the growth of *E. coli* O157:H7 by 4.42 and 6.12 log CFU mL^{-1}, respectively compared to the control ($P<0.05$), after 24 h (Fig. 1(c)). Similarly, GME had the same effect on *S. typhimurium*. There were no significant differences between 0.5 or 1.0% extract and the control (0%) (Fig. 1(d)), but 1.5 and 2.0% extract exerted a significant effect on the growth of *S. typhimurium*; the viable cell number was decreased by 3.87 and 5.29 log CFU ml^{-1} compared to the control after 24 h of incubation, respectively (Fig. 1(d)). In general, these results indicated that: (1) the bacterial viable cells declined proportionally depending on the concentration of GME after 24 h incubation, (2) there were differences in cell tolerance, depending on the kind of bacteria, towards GME at different concentrations, and (3) the most effective extract was 2% followed by 1%, while the most sensitive indicators were *B. cereus* and *E. coli*, respectively.

3.3. Bactericidal effect of GME on pathogens

The bactericidal effects of GME at 0, 0.5, 1, 1.5 and 2% (w/v) were evaluated against the four most sensitive indicator pathogens during their incubation in sterile distilled water (DW) at 37°C for 24 h (Fig. 2 (a), (b), (c) and (d)). *Bacillus cereus* affected by all concentrations of GME was the most sensitive one, as it was eliminated by 2 and 1.5 % extract after 8 and 24 h of incubation, respectively (Fig. 2 (a)). This pathogen lost 3.11 and 3.4 log CFU ml^{-1} after 24 h of incubation with 0.5 and 1% extract, respectively in comparison to the control, with significant differences between them and the control ($P<0.05$).

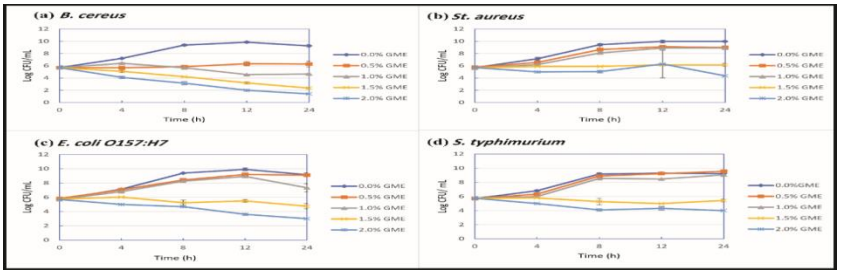

Fig. 1. Inhibitory activity of ginseng methanol extract (GME) at different concentrations (0, 0.5, 1.0, 1.5 and 2%) on the growth of indicator pathogens, in TSB medium at 37° C for 24 h. a: *B. cereus*; b: *St. aureus*; c: *E. coli O157:H7*; d: *S. typhimurium.*

As shown in Fig. 2 (b), *St. aureus* appeared to be fully resistant to 0.5 and 1% extract, whereas the addition of 1.5 and 2% extract had a positive impact (P<0.05) on *St. aureus*, with 0.85 and 1.7 log CFU ml^{-1} reduction compared to the control after 24 h, respectively. While 1.5% extract reduced the numbers of *E. coli* O157:H7 by 3 log CFU ml^{-1} compared to the control after 24 of incubation, 2% GME had a significant bactericidal effect on *E. coli* O157:H7 which was not detected after 12 h of incubation at 37°C (Fig. 2 (c). However, both 0.5 and 1% showed a negative effect on *E. coli* O157:H7, which could increase their numbers by 0.15 and 0.3 log CFU mL^{-1}, respectively compared with the control after incubation end. Fig. 2 (d) illustrated that no cells of *S. typhimurium* were detected by adding GME at 2% after 24 h of incubation. While 1.5% extract reduced the viability of *S. typhimurium* by about 35% (1.99 log CFU mL^{-1}), *S. typhimurium* was not affected by either 0.5 or 1% GME compared to the control, respectively. These results demonstrated that: (1) the most effectively bactericidal activity of GME was 2%, (2) *B. cereus* was the most sensitive strain followed by *E. coli O157:H7* and *S. typhimurium*, respectively, however, *St. aureus* exhibited less sensitivity and more resistance, (3) the bactericidal effect was highly concentration dependent, as it was achieved by 1.5 and 2% in case of *B. cereus*, and by only 2% for both *E. coli* O157:H7 and *S. typhimurium*, (4) the lower concentrations (0.5 and 1%) of GME had negative effect on all strains except for *B. cereus*, and (5) incubation hours also showed a significant impact within the same concentration of the applied extract against the test bacteria. The current results regarding the inhibitory and bactericidal effects of GME were consolidated by findings of Lee et al., [28]. All ginseng extracts, produced by hot water, ethanol 70% and SWE, inhibited the growth of *B. cereus*, *S. enteritidis*, *E. coli* O157:H7 and *L. monocytogenes*. Among them, *B. cereus* exhibited more sensitivity to GE by SWE at 190°C than the other bacteria. They attributed the inhibitory and bactericidal activities of GE to the action of TPC. The mode of bactericidal action of SWE was proved by transmission electron microscopy through the disruption of bacterial cell membranes, with the release of the cellular contents [28] Athari et al., [40] reported that hydro-alcoholic extract of ginseng had a synergistic effect when applied with ampicillin against *L. monocytogenes*. However, the relationship between specific active components and the antimicrobial bioactivity of GE is still indistinct, it is theorized that essential oil, ginsenosides, polysaccharides, proteins, and panaxytriol are all might be responsible for the antimicrobial activities of ginseng [21, 41]. Moreover, these bioactive components, thus their antibacterial activities can be highly affected by both the extraction procedures and processing [28, 42, 38, 21].

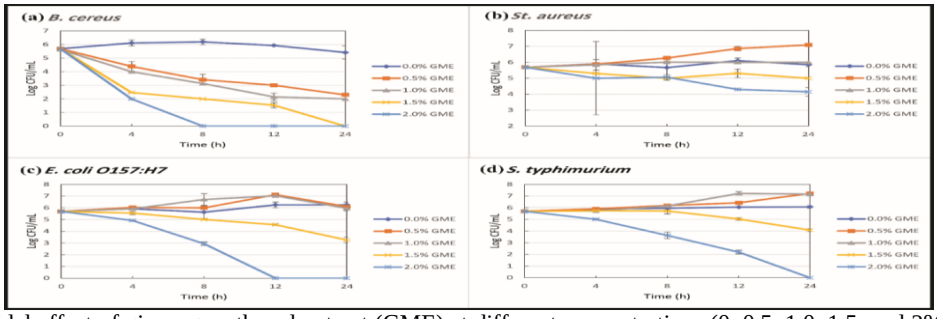

Fig. 2. Bactericidal effect of ginseng methanol extract (GME) at different concentrations (0, 0.5, 1.0, 1.5, and 2%) on the growth of pathogens, in sterile distilled water (DW medium) at 37° C for 24 h. a: *B. cereus*; b: *St. aureus*; c: *E. coli O157:H7*; d: *S. typhimurium*.

3.4. TPC and DPPH-radical scavenging activity of Ginseng-fortified probiotic yoghurt (GFPY)

A conjunction of ginseng and probiotic *L. rhamnosus* was applied to boost the functionality of yoghurt, in particular antioxidant activity, which was assessed in terms of determination of TPC and DPPH RSA. As shown in Table 2, supplementing the yoghurts with GME had a significant effect on their TPC and DPPH RSA, between the treated samples, or between the treated samples and the controls (P<0.05). The inclusion of *L. rhamnosus* increased the TPC of CPY by 12.5% compared to CY, whereas the addition of ginseng at elevating concentrations 0.5, 1 and 2% raised the TPC by 47.8, 100.63 and 219.47% in $GPY_{0.5}$, GPY_1 and GPY_2, respectively compared to CPY. Moreover, the results shown in Table 2 revealed that the DPPH RSA of GFPY were statically higher (P<0.05) than that of classic (CY) and probiotic controls (CPY) at all different concentrations.

Table 2. Total phenolic content (TPC) and DPPH[3]-radical scavenging activity (RSA, %) of probiotic yoghurts fortified with different concentrations of ginseng methanol extract (GME).

Item	Samples[1]				
	CY	CPY	$GPY_{0.5}$	GPY_1	GPY_2
TPC (mg GAE[2]/100g)	8.4 ± 0.45^{e}	9.45 ± 0.31^{d}	13.97 ± 0.14^{c}	18.96 ± 0.30^{b}	30.19 ± 0.31^{a}
DPPH- RSA (%)	46.33 ± 1.52^{e}	47.76 ± 0.15^{d}	56.8 ± 0.34^{c}	67.86 ± 0.23^{b}	78.9 ± 0.17^{a}

[a]Values are mean ± SD. Values with different letters within the same line denote significant differences ($P < 0.05$).
[1]CY= control standard yoghurt (S. thermophilus and L. bulgaricus); CPY= control probiotic yoghurt (S. thermophilus and L. bulgaricus + L. rhamnosus); $GPY_{0.5}$ = ginseng-fortified probiotic yoghurt (S. thermophilus and L. bulgaricus + L. rhamnosus + 0.5% GME); GPY_1 = (S. thermophilus and L. bulgaricus + L. rhamnosus + 1.0% GME); GPY_2 = (S. thermophilus and L. bulgaricus + L. rhamnosus + 2.0% GME).
[2](GAE) = gallic acid equivalents.
[3]Antioxidant activity was determined by 1-diphenyl-2-picryl- hydrazyl (DPPH) radical scavenging activity (RSA) as % of inhibition value.

The results mean that while the antioxidant activity was increased by 1.43% in CPY compared to CY because of adding probiotic *L. rhamnosus*, the addition of ginseng at 0.5, 1 and 2% increased the antioxidant activity by 9.04, 20.1 and 31.14% compared to CPY, respectively. This demonstrated that the addition of ginseng at 1% and 2% increased the antioxidant activity by more than 2- and more than 3-fold higher than that increase resulting from the addition of 0.5%, respectively. Therefore, 1% and 2% of ginseng extract could be deemed to have around a twofold and threefold higher functional potential than 0.5% extract, respectively. Also, the data might imply a roughly positive correlation between TPC and antioxidant activity. The current results were consistent with many previous studies. Park et al., [43] reported that a positive correlation was observed between TPC and antioxidant potential. The TPC of yoghurt supplemented with 2% RGE was 41.1 mg GAE/100g, with highly significant results (P<0.01) against the control product (8.1 mg GAE/100g). Furthermore, they found that all samples of yoghurt supplemented with RGE from 100 to 5000 µg ml^{-1} increased the DPPH RSA (%), with highly significant differences (P<0.01). Jung et al., [18] also reported yoghurt fortified with RGE had higher DPPH-RSA than the control sample, and the antioxidant activity was proportionally improved according to the concentration of RGE; as the treated samples (0.5, 1, 1.5 and 2% extract) achieved 85.9, 90.2, 92.3 and 94.1% in comparison to control (55.9%) on the 31st d of cold storage. Further, the positive action of fermentation of ginseng on TPC and antioxidant activity was previously supported. Lee et al., [28] and Jang et al., [17] demonstrated that fermentation using *L. plantarum* NK181 and the dose of ginseng extract powdered (GEP) added to yoghurt raised the DPPH RSA of the product. Kim et al., [12] stated that fermentation of ginseng by LAB and their enzymes can improve specific compounds and show antioxidative effects. The high potential of antioxidant activity of GFPY may be due to three main effects. Milk represents the first source, as it contains potent antioxidants comprising thiols, ascorbate and whey proteins, and diverse hydrophobic antioxidants including retinol and tocopherol [44]. Ginseng is regarded as a leading provider of different antioxidant constituents. Polyphenols and flavonoids, in particular gentisic, chlorogenic and p-counmaric acids, as well as ginsenosides, are the most important powerful antioxidants of ginseng [45, 41]. Thirdly, the fermentation of yoghurt milk **(**casein and whey proteins) can supply some bioactive peptides and free amino acids through proteolytic degradation, with antioxidant properties [6], especially by yoghurt staters and probiotic *L. rhamnosus*.

3.5. Viability of *S. thermophilus* and *L. bulgaricus* in GFPY

The viability of *S. thermophilus* and *L. bulgaricus* was influenced by the presence of probiotic *L. rhamnosus*, concentration of GME and cold storage period. According to Table 3, both *S. thermophilus* and *L. bulgaricus* had the same pattern of growth and survivability; as their highest viable counts were observed on the 5th day, ranging between 8.63 to 9.06 Log CFU mL^{-1} and 8.81 to 9.07 Log CFU mL^{-1}, respectively, then gradually declined by the effect of storage period, to be ranged from 7.31 to 8.31 Log CFU mL^{-1} for *S. thermophilus* and from 6.15 to 7.63 Log CFU mL^{-1} for *L. bulgaricus*.

For the effect of co-culture of *L. rhamnosus* on viability of yoghurt starter cultures, *S. thermophilus* was positively impacted more than *L. bulgaricus*, as they decreased by 1.83 and 2.57 Log CFU mL^{-1}, respectively in CPY compared to the classic control (CY), in which they decreased by 1.96 and 1.98 Log CFU mL^{-1}, respectively on day 15 of storage. The results in Table 3 indicated that the addition of ginseng kept the viable cell count of *S. thermophilus* and *L. bulgaricus* higher than Log 8 and Log 7 CFU mL^{-1}, respectively at each measurement point during storage, and reduced the rate of viability decline in comparison to both controls.
The viable cell count of *S. thermophilus* was decreased by 0.86, 0.77 and 1.36 Log CFU mL^{-1} in $GPY_{0.5}$, GPY_1 and GPY_2, respectively compared to 1.96 and 1.83 Log CFU mL^{-1} in CY and CPY, whilst *L. bulgaricus* decreased by 1.42, 1.41 and 1.74 Log CFU mL^{-1} compared to 1.98 and 2.57 Log CFU mL^{-1}, respectively in the same samples, after 15 d of storage. Therefore, the best concentration of GME added to yoghurt was 1% followed by 0.5%, with no significant difference between them (P>0.05) for either *S. thermophilus* or *L. bulgaricus*, but there were significant results (P<0.05) between each of them and the other samples, for both two bacteria. These results agreed with those found by Jung et al., [18] who stated the positive effect of RGE added in yoghurt on the viability of *L. acidophilus* and *S. thermophilus*, as their counts were increased proportionally to the increase of RGE added in yoghurt (0.5, 1 and 1.5%), but they were slightly declined in yoghurt containing 2% RGE. Likely, Jang et al., [17] reported that *L. plantarum* NK181 and *S. thermophilus* in GEP added yoghurt were more than 9 Log CFU mL^{-1} and higher than their counts in the control.

Table 3. The viable cell counts (Log CFU mL^{-1}) of *St. thermophilus*, *L. bulgaricus*, and *L. rhamnosus* in probiotic yoghurt fortified with ginseng methanol extract (GME) during cold storage at 4°C.

Bacterial species	Samples[1]	1 d	5 d	10 d	15 d
St. thermophilus	CY	9.27 ± 0.02 ^{Aa}	8.98 ± 0.05^{Ba}	8.14 ± 0.00 ^{Cc}	7.31 ± 0.27 Ed
	CPY	8.96 ± 0.05 ^{Ad}	8.63 ± 0.05^{Bc}	7.77 ± 0.26 ^{Cd}	7.13 ± 0.03 Dd
	$GPY_{0.5}$	9.11 ± 0.00 ^{Ab}	9.0 ± 0.00 ^{Ba}	8.63 ± 0.02 ^{Cb}	8.25 ± 0.06 Db
	GPY_1	9.08 ± 0.05 ^{Ac}	9.06 ± 0.02^{Aa}	8.75 ± 0.04 ^{Bb}	8.31 ± 0.02 Cb
	GPY_2	9.01 ± 0.06 ^{Acd}	8.83 ± 0.11 ^{Ab}	8.07 ± 0.03 ^{Bc}	7.65 ± 0.15 Cc
L. bulgaricus	CY	8.98 ± 0.08 ^{Aa}	9.07 ± 0.00 ^{Aa}	7.89 ± 0.10 ^{Bb}	7.00 ± 0.00 Cc
	CPY	8.72 ± 0.06 ^{Ac}	8.86 ± 0.03 ^{Ac}	7.55 ± 0.12 ^{Bc}	6.15 ± 0.27 Cd
	$GPY_{0.5}$	9.05 ± 0.02 ^{Aa}	8.954 ± 0.0 ^{Bb}	7.94 ± 0.07 ^{Cb}	7.63 ± 0.05 Db
	GPY_1	8.92 ± 0.03 ^{Aa}	9.02 ± 0.04 ^{Ab}	7.98 ± 0.07 ^{Bb}	7.51 ± 0.07 Cb
	GPY2	8.84 ± 0.04 ^{Ab}	8.81 ± 0.07 ^{Ac}	7.51 ± 0.07 ^{Bc}	7.10 ± 0.17 Cc
L. rhamnosus	CPY	8.75 ± 0.04 ^{Bb}	9.10 ± 0.10 ^{Ac}	8.98 ± 0.02 ^{Ac}	8.06 ± 0.0 ^{Cb}
	$GPY_{0.5}$	9.20 ± 0.17 ^{Ba}	9.55 ± 0.12 ^{Ab}	9.25 ± 0.24 ^{Abb}	8.20 ± 0.0 ^{Cb}
	GPY_1	9.30 ± 0.00 ^{Ca}	9.56 ± 0.07 ^{Bb}	10.19 ± 0.03^{Aa}	8.61 ± 0.0 ^{Da}
	GPY_2	9.35 ± 0.10 ^{Ba}	10.13 ± 0.04^{Aa}	10.10 ± 0.02 ^{Aa}	8.0 ± 0.0 ^{Cc}

[1]CY= control standard yoghurt (S. thermophilus and L. bulgaricus); CPY= control probiotic yoghurt (S. thermophilus and L. bulgaricus + L. rhamnosus); $GPY_{0.5}$ = ginseng-fortified probiotic yoghurt (S. thermophilus and L. bulgaricus + L. rhamnosus + 0.5% GME); GPY_1 = (S. thermophilus and L. bulgaricus + L. rhamnosus + 1.0% GME); GPY_2 = (S. thermophilus and L. bulgaricus + L. rhamnosus + 2.0% GME).

$^{a-d}$Means in the same column with different lower-case letters indicate significant differences due to concentrations of GME ($P < 0.05$).

$^{A-E}$Means in the same row with different upper-case letters indicate significant differences due to storage period ($P < 0.05$).

When GEP was raised from 0% to 1%, the viable cell counts in yoghurt rose, otherwise, further concentrations (1.5 and 2%) did not positively affect the viability. The addition of ginseng extracts would provide some nutritional components which enhance the growth of *S. thermophilus* and *L. bulgaricus* [15]. The more survival and stability of *S. thermophilus* versus *L. bulgaricus* in either control or GFPY can be due to that harmony and compatibility between *L. rhamnosus* and *S. thermophilus* seems to be greater than between *L. rhamnosus* and *L. bulgaricus*. The same behavior of the symbiotic interaction between *S. thermophilus* and *L. bulgaricus* during yoghurt fermentation, was reported between *L. rhamnosus GG* and *S. thermophilus* SY-102 as a co-culture system within fermentation process [13].

3.6. Viability of *L. rhamnosus* in GFPY

The results in Table 3 show the mean value of viable cell count of *L. rhamnosus* was significantly higher (P<0.05) in GFPY samples compared to the control (CPY), along the cold storage period. As the GME concentration was raised from 0% to 0.5-1%, the viable cell counts of *L. rhamnosus* in yoghurt became greater and reached their highest value (10.19 Log CFU mL^{-1}) in GPY_1 on the 10^{th} day of storage. However, 2% extract had a lesser effect on the stability of bacteria at the end of the storage. The increase in the storage period of 15 d manifested the decrease in *L. rhamnosus* counts in GFPY and the control. However, the decrease in counts was not much so that *L. rhamnosus* was still higher than 8 Log CFU mL^{-1}, with statistical significance only being recorded for the yoghurt containing 1% GME compared to the control (CPY) or the other two extracts on day 15 of storage (P<0.05). According to Table 3, *L. rhamnosus* was decreased by 0.69 Log CFU mL^{-1} in GPY_1 against 1.0 and 1.35 Log CFU mL^{-1} in $GPY_{0.5}$ and GPY_2, respectively at the end of storage. This confirms the highest positive effect of GME on the viability and stability of *L. rhamnosus* was belong to 1%, followed by 0.5% added yoghurt. The results indicated in Table 3 also declare *L. rhamnosus* was more stable than *S. thermophilus* and *L. bulgaricus*, in all samples. Cimo et al., [8] supported the current results**.** Their study revealed that yoghurt combined with aqueous American GE was significantly (P<0.05) suitable for *L. rhamnosus* GR-1 viability compared to the control yoghurt as the viable cell count was increased in proportion to the level of added ginseng. Throughout the storage period, there existed a synbiotic relationship between the prebiotic components of ginseng and *L. rhamnosus* GR-1. The viable cell counts of *L. rhamnosus* (Table 3) which was higher than 8 log CFU mL^{-1} at the end of cold storage was comparable to those findings reported by Cimo et al., [8] and Hekmat et al., [46], who reported *L. rhamnosus* GR-1 was remained stable and higher than 9 Log CFU mL^{-1} throughout the 28-d cold storage, in ginseng added yoghurts. The positive effect of ginseng on viability has been proven to be due to the fact that it is a prebiotic compound, because it contains ginsenoside which could be absorbed via deglycosylation by gut microbiota, and nine glucose oligosaccharides with a polymerization degree between two and ten [47, 8]. In addition, the results in Table 2 declared GFPY having higher concentrations of GME were found to be higher TPC and higher DPPH RSA, which may reduce the oxidative stress and provide a favorable environment for growth and maintaining the viability of *L. rhamnosus*. The more survival and stability of *L. rhamnosus* than *S. thermophilus* and *L. bulgaricus* in GFPY may be possibly because the degradation and fermentation of prebiotics were found to be greatly varied among different species of probiotics even between strains belonging to the same species [48]. Furthermore, *L. rhamnosus* counts (Table 3) exceeded the minimum acceptable viability (10^6-10^8 CFU mL^{-1}) required for bacteria to be probiotic and exert their health benefits on the host. Therefore, the combination of *L. rhamnosus* with GME in yoghurt as a preferable vehicle could maximize the role of probiotics, ginseng, and yoghurt in enhancing nutritional and health benefits for humans.

3.7. Effect of ginseng extract on pH change in GFPY

The effect of ginseng extract on pH change taken placed in yoghurt represents an important physicochemical property of fermented dairy products in terms of safety and sensory. The results of pH change are shown in Table 4. After fermentation (0 day), the pH value of GFPY and their controls, including samples that were separately inoculated with or without *B. cereus* and *E. coli* O157:H7, decreased from 6.5 to be ranged from 4.5 to 4.55. During storage, a gradual and significant decrease in the pH values of all types and treatments of yoghurt was observed (P<0.05). At the end of storage, the pH decreased to range 4.29-4.35 for yoghurts without pathogens, and 4.3-4.34 for those inoculated with pathogens. The decrease in pH values may be due to metabolic activity of *L. rhamnosus* and yoghurt starter culture as well as test organisms. Moreover, carbohydrates as major ingredients of ginseng can induce growth and enhance the activity of microorganisms in yoghurt [15]. The results were agreeable to those reported by many studies [28, 18, 49]. They confirmed that significant decrease in pH of yoghurts was observed by increasing the storage period (P<0.05), irrespective of added concentrations of ginseng used in different forms.

3.8. Survival of *B. cereus* and *E. coli O157:H7* in GFPY

The growth and survival of *B. cereus* and *E. coli O157:H7 were* determined on cold storage in probiotic yoghurt fortified with different concentrations of ginseng extract ($GPY_{0.5}$, GPY_1 and GPY_2) compared to both classic (CY) and probiotic controls (CPY) are shown in Fig. 3(a) and (b). The results demonstrated that all yoghurt samples had a relatively bactericidal effect against pathogenic bacteria regardless of the presence of ginseng, and proportional to the increasing concentrations of ginseng extract.

Table 4. Changes in pH- values of probiotic yoghurt fortified with ginseng methanol extract (GME), with or without inoculation of *B. cereus* or *E. coli O157:H7*, during cold storage.

Samples[1]	pH- values											
	Without inoculation				With inoculation of *B. cereus*				With inoculation of *E. coli O157:H7*			
	Storage period (day)											
	0	5	10	15	0	5	10	15	0	5	10	15
CY	4.5±0 ^{Ad}	4.39± 0^{Ce}	4.3±0. 0^{Ef}	4.29± 0^{Ee}	4.50±0. 01^{Ac}	4.41±0 ^{Bcd}	4.33±0.0 ^{De}	4.3±0.0^{Ed}	4.5±0.0 2^{Ac}	4.41±0^{Bc}	4.33±0.0 ^{Dd}	4.3±0.0^{Ee}
CPY	4.52± 0^{Ab}	4.39± 0^{Ce}	4.31± 0^{Ee}	4.31± 0^{Ed}	4.50±0. 0^{Bc}	4.39±0.0 ^{Cd}	4.34±0.0 2^{Dbc}	4.30±0.0 11^{Ed}	4.50±0. 0^{Bc}	4.39±0.0 1^{Cd}	4.34±0.0 11^{Dd}	4.30±0^{Ed} e
$GPY_{0.5}$	4.52± 0^{Ab}	4.4±0 ^{Dd}	4.31± 0^{Id}	4.32± 0^{Hd}	4.51±0. 02^{Ac}	4.41±0.0 11^{Cc}	4.35±0^{Gb} c	4.32±0.0 1^{Ha}	4.51±0. 02^{Ac}	4.41±0.0 11^{Cc}	4.36±0.0 ^{Fc}	4.32±0.0 1^{Hcd}
GPY_1	4.51± 0^{Bb}	4.42± 0^{Ec}	4.31± 0^{Hc}	4.33± 0^{Gc}	4.53±0. 01^{Ab}	4.426±0. 0^{Ec}	4.37±0.0 ^{Fb}	4.32±0^{GH} bc	4.52±0. 01^{Ab}	4.43±0.0 1^{Db}	4.37±0.0 ^{Fb}	4.33±0.0 1^{Gb}
GPY_2	4.55 ±0^{Aa}	4.44± 0^{Cb}	4.31± 0^{Gb}	4.35± 0^{Eb}	4.55±0. 0^{Aa}	4.45±0.0 1^{Bb}	4.38±0.0 1Da	4.34±0.0 1^{Fa}	4.54±0. 0^{Aa}	4.45±0.0 ^{Ba}	4.39±0.0 1Da	4.34±0.0 05^{Fa}

[1]CY= control standard yoghurt (*S. thermophilus and L. bulgaricus*); CPY= control probiotic yoghurt (*S. thermophilus and L. bulgaricus* + *L. rhamnosus*); $GPY_{0.5}$ = ginseng-fortified probiotic yoghurt (*S. thermophilus and L. bulgaricus* + *L. rhamnosus* + 0.5% GME); GPY_1 = (*S. thermophilus and L. bulgaricus* + *L. rhamnosus* + 1.0% GME); GPY_2 = (*S. thermophilus and L. bulgaricus* + *L. rhamnosus* + 2.0% GME).

[a-d]Means in the same column with different lower-case letters indicate significant differences by concentrations of GME (P < 0.05).

[A-I]Means in the same row with different upper-case letters indicate significant differences by storage period (P < 0.05).

In respect to *B. cereus*, the viability was statistically decreased in all samples during the cold storage period (P<0.05). The cell counts decreased from 5.69 (initial inoculum) to range between 2.96 and 4.05 Log CFU mL^{-1} on the first day (Fig. 3(a)), with a significant difference between GPY_2 and the others (P<0.05). Interestingly, GPY_2 samples exhibited a highly bactericidal effect on *B. cereus* which was not detected on the second day of storage, while GPY_1 could eliminate the viability of *B. cereus* on the 3rd day on which, however, the pathogen survived in CY, CPY and $GPY_{0.5}$ samples, but it lost 3.21 (56.41%), 2.58 (45.34%) and 2.37 Log CFU mL^{-1} (41.65%) of viability, respectively, (P<0.05). On the fifth day of storage, *B. cereus* lost 100% of viability either in controls or $GPY_{0.5}$ (Fig. 3(a)).

For *E. coli O157:H7*, the storage period had a significant effect in reducing the cell counts in all samples, whether fortified with ginseng or not (controls) (P<0.05). Similarly, to *B. cereus*, *E. coli O157:H7* behaved the same pattern in terms of survival in yoghurt samples. As shown in Fig. 3(b), the counts of *E. coli O157:H7* decreased to range between 3.10 and 4.47 Log CFU mL^{-1} after the 1st day of cold storage. On the 3rd day, *E. coli O157:H7* lost 2.69 (47.23%), 2.39 (42%), 2.21 (38.8%), 2.73 (47.9%) and 4.06 Log CFU mL^{-1} (71.35%) in CY, CPY, $GPY_{0.5}$, GPY_1 and GPY_2 respectively, from the initial inoculation number (5.69 Log CFU mL^{-1}), with significant difference between GPY_2 and the others (P<0.05). While the bactericidal effect of GPY_2 against *E. coli O157:H7* appeared on the 5th day of storage, the GPY_1 showed this effect on the 7th d, to which, however, the pathogen could survive in CY,CPY and $GPY_{0.5}$.On the 10th d of storage, $GPY_{0.5}$,CYand CPY eliminated the viability of *E. coli O157:H7*(Fig.3(b) The results elaborated in Fig. 3(a) and (b) suggested that the higher the concentration of ginseng extract added to yoghurt, the faster the pathogens (*B. cereus* and *E. coli O157:H7*) lost their viability, however, classic and probiotic yoghurts were capable of totally inhibiting the growth and survival of the two pathogens, regardless of the presence of ginseng, but over a longer period. Also, it could be observed that *B. cereus* showed more sensitivity than *E. coli O157:H7* in yoghurts with or without ginseng, and the GPY_2 was the most effective sample against both *B. cereus* and *E. coli O157:H7*, followed by GPY_1, however, $GPY_{0.5}$

showed less effect and was similar in strength with CY and CPY samples. These results were supported by Eom et al., [50] who reported that yoghurt fermented by *L. acidophilus*, *Bif. longum*, and *Str. thermophilus* and enriched by GME (0.5% and 1.0%), had a great power to inhibit the growth of *B. cereus*, *St. aureus*, *L. monocytogenes*, *E. coli*, *S. typhimurium* and *Enterobacter sakazakii* during fermentation and storage. In addition, yoghurt with 1% GME was the most effective compared to either 0.5% GME yoghurt or the control, against all tested pathogen except for *S. typhimurium*. However, *B. cereus KCCM11341* and *E. sakazakii ATCC51329* were the most sensitive pathogens towards yoghurt containing 1% GME. The bactericidal effect of CY, CPY and GFPY against *B. cereus* and *E. coli O157:H7* may be mainly due to direct or indirect action of starter cultures (*Str. thermophilus* and *L. bulgaricus*), probiotic *L. rhamnosus* and ginseng extract. In yoghurt, lactic, formic, acetic and butyric acids that may be produced during fermentation and along storage because of growth and metabolism of starter cultures and *L. rhamnosus*, directly resulted in low pH (~ 4.3), as shown in Table 4.

The antimicrobial effect of organic acids returns to the reduction of pH as well as the undissociated forms of their molecules. However, the effect of low pH and organic acids was found to vary with the strain of bacteria [51], this may explain why *B. cereus* was more sensitive and faster affected in yoghurts than *E. coli O157:H7* which appeared to adapt itself to the yoghurt acidic environment. Further, in addition to organic acids, bacteriocin may be produced by *L. rhamnosus* in the prepared yoghurts. Wei et al., [10] reported that strain XN2, isolated from Chinese yak yoghurt and identified as *L. rhamnosus*, produced bacteriocin XN2 with antibacterial activities against pathogens such as *B. subtilis*, *B. cereus*, *S. aureus* and *E. coli*. Once again, as for the antibacterial effect of ginseng extract against pathogens, its indirect and direct role, and bactericidal activity were detailed in previous parts of this research (3.1, 3.2, 3.3 and 3.4). In general, increasing the concentration of ginseng extract added to yoghurt, the faster the bactericidal effect occurred against the two test pathogens, compared to the controls (Fig. 3). Moreover, the effectively anti-pathogenic effect of GPY_2 followed by GPY_1 may be due to their highest levels of TPC (Table 2) derived from high levels of ginseng extract. The anti-pathogenic effect of GE was found to be correlated and proportional to the TPC **[28]**. Moreover, ginseng extract was reported to have polyacetylene, polysaccharides and panaxytriol which are demonstrated to be responsible for the anti-pathogenic effect of ginseng [21]

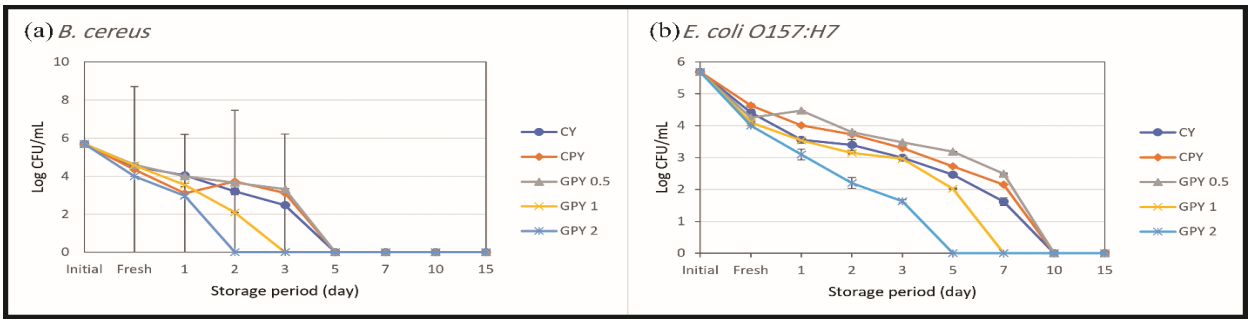

Fig. 3. Survival of *B. cereus* (a) and *E. coli O157:H7* (b) in ginseng-fortified probiotic yoghurt during cold storage. CY= control standard yoghurt (*S. thermophilus and L. bulgaricus*); CPY= control probiotic yoghurt (*S. thermophilus and L. bulgaricus* + *L. rhamnosus*); $GPY_{0.5}$ = ginseng-fortified probiotic yoghurt (*S. thermophilus and L. bulgaricus* + *L. rhamnosus* + 0.5% GME); GPY_1 = (*S. thermophilus and L. bulgaricus* + *L. rhamnosus* + 1.0% GME); GPY_2 = (*S. thermophilus and L. bulgaricus* + *L. rhamnosus* + 2.0% GME).

3.9. Effect of ginseng extract on sensory properties of probiotic yoghurt

The descriptive sensory assessment was performed by a trained panel to quantitatively spot the effect of GME added in varying amounts (0, 0.5, 1 and 2%) on yoghurt organoleptic changes, in terms of flavor (taste and smell), body and texture, appearance and color, and overall acceptability after 1 d of preparation, to determine the extent of consumer acceptance to this newly functional product. As shown in Table 5, Among ginseng-treated yoghurts, $GPY_{0.5}$ received the highest scores ($P<0.05$) compared to GPY_1 and GPY_2, on the level of all tested parameters. Except for color, $GPY_{0.5}$ was not significantly different from the control (CPY) in terms of flavor, texture and overall acceptability ($P>0.05$). At high concentrations, GPY_1 containing 1% extract achieved better scores of sensory properties than those of GPY_2 containing 2% extract ($P<0.05$). However, the classic control (CY) gained the highest scores of overall acceptability, with significant differences ($P<0.05$). Moreover, GPY_2 was ranked last, on all sensory assessments. The results seen in Table 5 indicated that the total quality of yoghurt was not negatively affected by ginseng extract added in low concentration (0.5%), however, the acceptability of GFPY was completely negatively affected at higher concentrations, especially 2%

These results are consistent with those reported by Lee et al., [15] as the scores of sensory properties of yoghurt supplemented with 0.5% or less of HG did not show a significant difference from the control, however, at concentrations higher than 1.0%, the acceptability suffered. Similar findings were demonstrated by Eom et al., [50] as no discernible variation ($P > 0.05$) was observed in any of the sensory properties between yoghurt and yoghurt fortified with 0.5% GME.

The negative effect of a higher concentration above 0.5% ginseng extract, on the sensory properties of yoghurt may be since the higher the concentration of ginseng, the darker the brown colour and the more pronounced the bitter taste. Choi et al., [49] explained that the exposure of ginseng to steaming, drying and heat treatment stimulated non-enzymatic reactions, which led the color to dark brown.

Table 5. Sensory evaluation of ginseng-fortified probiotic yoghurt (GFPY).

Items	Treatments[1]				
	CY	CPY	$GPY_{0.5}$	GPY_1	GPY_2
Flavor (taste and smell) (60)	56.25 ± 0.8579^{a}	$55.15\pm0.57b^{c}$	55.5 ± 0.52^{b}	54.9 ± 0.56^{c}	54.25 ± 0.35^{d}
Body and texture (30)	27.75 ± 0.35^{b}	28 ± 0.40^{ab}	28.4 ± 0.65^{a}	28 ± 0.40^{ab}	26.8 ± 0.42^{c}
Appearance and color (10)	8.25 ± 0.26^{a}	7.95 ± 0.28^{b}	7.65 ± 0.33^{c}	7.25 ± 0.26^{d}	7.05 ± 0.15^{d}
Over acceptability (100)	92.35 ± 0.88^{a}	91.2 ± 0.71^{b}	91.55 ± 0.36^{b}	90.05 ± 0.64^{c}	88 ± 0.62^{d}

[1]CY= control standard yoghurt (S. thermophilus and L. bulgaricus); CPY= control probiotic yoghurt (S. thermophilus and L. bulgaricus + L. rhamnosus); $GPY_{0.5}$ = ginseng-fortified probiotic yoghurt (S. thermophilus and L. bulgaricus + L. rhamnosus + 0.5% GME); GPY_1 = (S. thermophilus and L. bulgaricus + L. rhamnosus + 1.0% GME); GPY_2 = (S. thermophilus and L. bulgaricus+ L. rhamnosus +2.0% GME).Different lowercase letters (a-d) in the same row indicate significant differences ($P < 0.05$).

Moreover, Eom et al., [50] and Lee et al., [15] attributed the negative sensory scores of ginseng-added yoghurts to the bitterness arising from some compounds in ginseng, such as triterpenoid peptides and propylene glycol. However, the effect of the storage period on sensory properties, as well as not adding sweeteners to reduce bitterness, were not investigated and thus is a shortage of this research. In general, the current study revealed that fortifying probiotic yoghurt with ginseng extract at low concentration did not significantly affect the overall acceptability.

4. Conclusion

This research was designed to produce a functional ginseng-fortified probiotic yoghurt to play a dual role in antioxidant effect and antibacterial activity via the conjunction of *L. rhamnosus* with ginseng extract in yoghurt as a preferable vehicle that could maximise the role of probiotics, ginseng and yoghurt in providing nutritional benefits and maintaining gut health, to provide Egyptian consumers and local probiotics market with a healthy, safe and functional dairy product, as a diet-oriented prevention strategy that might contribute to decreasing both the onset of oxidative stress-related chronic diseases and bacterial-associated infectious ones.

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