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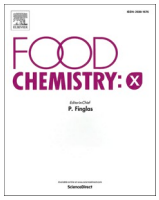

# Enhancing the texture of fat-free yogurt with *Panax ginseng* leaf-stem extract and casein: Focusing on their softening effect

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ABSTRACT

Fat-free yogurt often undergoes syneresis because it lacks fat. Although casein prevents syneresis, it induces protein aggregation and lumpy texture. Surfactants are commonly employed to mitigate these quality challenges. Saponins, abundant in *Panax ginseng* by-products like leaves and stems, possess surfactant activity, thereby preventing protein aggregation. In this study, ginseng leaf-stem extract (GE) was assessed to prevent lumpy and grainy yogurt texture. The fermentative, bioactive, physical, and sensory properties of GE-supplemented yogurt were evaluated. GE accelerated yogurt fermentation by promoting the growth of lactic acid bacteria and demonstrated higher antioxidant activity than unsupplemented yogurt. GE stabilized the yogurt matrix, and GE-supplemented yogurt exhibited smaller protein particles and reduced aggregation. Casein-induced lumpy texture was minimized by GE without compromising the syneresis-preventing ability of casein. Sensory evaluation confirmed the soft texture and acceptable taste of the GE-supplemented yogurt. Collectively, GE is a cost-effective surfactant option for improving the texture of fat-free yogurt.

## 1. Introduction

Yogurt, the most popular fermented dairy product, is a viscous gel fermented using a starter culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The consumption of yogurt and the demand for healthier options, such as low-fat products, has increased owing to the growing interest in healthy foods (Zhang et al., 2019). However, low-fat yogurt, especially the set and fat-free types, undergoes syneresis due to the release of whey and the absence of fat (Gharibzahedi & Altintas, 2024; Qing et al., 2023; Wu, Dai, et al., 2023). This negatively affects the structural and textural properties of yogurt, especially because fat also plays a crucial role in yogurt organoleptic properties by providing desirable texture, appearance, and sensory attributes (Zhao et al., 2023). Therefore, identifying fat substitutes that do not compromise yogurt quality is crucial.

Hydrocolloids, including polysaccharides and proteins, are well-known fat substitutes in the yogurt industry (Sandoval-Castilla et al., 2004; Zhao et al., 2023). Whey syneresis is effectively prevented by increasing the total solids content (Qing et al., 2023), and milk-derived proteins, such as casein and whey protein, are commonly used as fat substitutes (Sandoval-Castilla et al., 2004). Casein has highly hydrophilic properties and effectively prevents syneresis by increasing viscosity compared to other milk-derived proteins (Arab et al., 2023). However, casein causes protein aggregation, resulting in a lumpy yogurt texture (Amalfitano et al., 2019; Li et al., 2023). At the recommended concentration of 1–2 %, casein causes a grainy texture in yogurt due to protein aggregation (Gao et al., 2023). Therefore, the discovery of food additives that prevent protein aggregation, while maintaining the syneresis-preventing properties of casein, remains challenging.

Saponin, which comprises hydrophilic sugar chains and hydrophobic sapogenins, prevents protein aggregation by acting as a surfactant and emulsifier (Goral & Wojciechowski, 2020). Saponins reduce protein aggregation by interacting with proteins *via* electrostatic and hydrophobic interactions or hydrogen bonding (Ban et al., 2023). Furthermore, saponin hydrophilic sugar residues stabilize proteins by interacting with their hydrophobic amino acid regions, thereby increasing interfacial activity (Bottcher & Drusch, 2017). *Panax ginseng*, a plant renowned for its medicinal properties, contains a high concentration of saponins, including the bioactive compounds known as ginsenosides (Ralla et al., 2017). Similar to ginseng roots, ginseng leaves and stems contain higher amounts of bioactive compounds, including ginsenosides (Kang & Kim, 2016; Yip et al., 1985). To maximize the utilization of ginseng leaves and stems, which are often discarded in substantial amounts during root production, we explored their potential

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to enhance fat-free yogurt texture. Given their significantly lower cost compared to ginseng roots, this research also aimed to investigate ginseng leaves and stems as a promising alternative source of saponins.

Therefore, we examined the surfactant properties of GE by evaluating its ability to prevent protein aggregation in fat-free yogurt. Additionally, we assessed the fermentative, bioactive, and other physical characteristics of fat-free yogurt incorporating GE.

## 2. Materials and methods

### 2.1. Materials

Fresh ginseng leaves and stems (5-year-old) were obtained from a ginseng farm in Jeonbuk, South Korea. Skimmed milk powder was purchased from the Seoul Dairy Cooperative (Seoul, Korea). The starter culture was obtained from Sacco Srl (Lyofast YAB 450 AB, Codaragok, Italy) and included *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus*, and *Bifidobacterium animalis* subsp. *lactis*. Casein and citric acid were purchased from ESfood Co. (Gyeonggi, South Korea). Lactobacilli MRS broth was supplied by Becton, Dickinson and Company (Sparks, MD, USA). Agar powder and 0.1 N NaOH solution were purchased from Duksan Science (Seoul, Korea). All other chemicals, including saponin, were of analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

### 2.2. Preparation of ginseng leaf-stem extract (GE)

Ginseng leaves and stems were washed with running water for 2 h before being air-dried at 60 °C until reaching a constant weight. The dried leaves and stems were then finely ground into a powder. Each dried leaf and stem powder was mixed at a ratio of 1:1 (*w*/w) and extracted with 60 % ethanol (1:20, *w/v*) at 80 °C for 2 h using a boiling pot (OCOO, Boryeong, Korea), as previously described (Jung et al., 2006). The extracts were filtered through Whatman No. 2 filter paper (Cytiva, Marlborough, MA, USA), and the residue was rinsed with 60 % ethanol (1:10, *w/v*) for further extraction and filtration under the same conditions. After the filtrate was collected, the samples were concentrated using a rotary evaporator (Tokyo Rikakikai Co., Tokyo, Japan). The concentrate was lyophilized and stored at −20 °C until further use.

### 2.3. Preparation of yogurt and yogurt supernatant

The yogurt and supernatant were prepared as previously described (Han et al., 2023; Kim et al., 2023). Skimmed milk powder (12 % w/v) was mixed with distilled water, and casein, GE, and saponin were added to the skim milk at specified ratios (*w/v*). The yogurts were classified as follows: Y-CON, control yogurt; Y-CAS, yogurt with 1 % casein; Y-GE 0.1, yogurt with 1 % casein and 0.1 % GE; Y-GE 0.2, yogurt with 1 % casein and 0.2 % GE; and Y-SAP, yogurt with 1 % casein and 0.2 % saponin. The prepared mixture was pasteurized at 85 °C for 30 min, cooled to 42 °C, and inoculated with the starter culture (7.32 log CFU/mL; 1 % *v*/v). After inoculation, the yogurt samples were incubated at 42 °C until they reached pH 4.6, and then stored at 4 °C for further studies. To prepare the supernatant, yogurt samples (10 *g*) were centrifuged at 3000 *g* for 10 min at 4 °C. The supernatants were re-centrifuged under the same conditions, filtered using a 0.45-μm filter, and then stored at −20 °C until use.

### 2.4. Viable lactic acid bacteria (LAB) count, pH, acidification kinetics, and titratable acidity (TA) of yogurt

Viable LAB were counted on MRS agar plates every 90 min, as previously described (Bock et al., 2024). The pH was measured every 20 min using a LAQUA pH meter (Horiba, Kyoto, Japan), and the acidification kinetics were calculated as previously described (Kim et al., 2023). The acidification kinetics included the maximum acidification rate ($V_{max}$), time for $V_{max}$ ($T_{max}$), time for pH 5.5 ($T_{pH5.5}$), and time for complete fermentation ($T_f$). To measure TA, yogurt (10 g) was mixed with distilled water (10 g) every hour and titrated using 0.1 N NaOH until the pH reached 8.3. The TA was calculated using the following equation:

$$\text{TA}\ (\%) = \text{used NaOH}\ (\text{mL})/\text{sample weight}\ (\text{g}) \times 0.009 \times 100 \quad (1)$$

where 0.009 is the conversion factor of the lactic acid.

### 2.5. Total phenol content (TPC) and total flavonoid content (TFC) of yogurt

The TPC and TFC were measured as previously described (Zhang et al., 2019). Briefly, for TPC, the supernatant (30 μL) was mixed with distilled water (120 μL) and Folin-Ciocalteu reagent (20 μL). Sodium carbonate solution (30 μL) was added to the mixture and incubated in the dark for 90 min. The absorbance was measured at 725 nm and expressed as gallic acid equivalents (g GAE/mL). For TFC, the supernatant (1 mL) was vortexed with 10 % aluminum chloride (100 μL) and 1 M potassium acetate (100 μL). Distilled water (2.8 mL) was added to the solution, which was then incubated in the dark for 40 min. TFC was expressed as quercetin equivalent (μg of QCE/mL), by measuring the absorbance at 415 nm.

### 2.6. Radical scavenging activity and reducing power capacity of yogurt

Radical scavenging activity was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (ABTS). For the DPPH radical scavenging assay, 0.01 mM DPPH reagent was mixed with the supernatant and incubated for 30 min in the dark. After incubation, absorbance was measured at 515 nm and calculated as follows:

$$\text{DPPH}\ (\%) = \left(1 - \text{OD}_{\text{sample}}/\text{OD}_{\text{control}}\right) \times 100 \quad (2)$$

For the ABTS radical scavenging assay, 14.8 mM ABTS reagent and 5.0 mM potassium persulfate were mixed at 1:1 and diluted with distilled water to an absorbance of 0.700 ± 0.005 at 734 nm. The supernatant was mixed with the $ABTS^{+}$ solution and incubated for 15 min in the dark. Absorbance was measured at 734 nm and calculated as follows:

$$\text{ABTS}\ (\%) = \left(1 - \text{OD}_{\text{sample}}/\text{OD}_{\text{control}}\right) \times 100 \quad (3)$$

The reducing power capacity was measured using the ferric ion-reducing antioxidant potential (FRAP) method, as previously described (Cho et al., 2023). The FRAP solution was prepared by mixing 300 mM acetate buffer, 10 mM 2,4,6-Tris(2-pyridyl)-*s*-triazine, and 20 mM ferric chloride (10:1:1), and incubated at 37 °C for 15 min at 100 rpm. The solution was added to the supernatant and incubated in the dark for 30 min. The results were reported as absorbance at 593 nm.

### 2.7. Color and total soluble solid content of yogurt

Yogurt color was measured using a CR-400 colorimeter (Konica Minolta, Ltd., Osaka, Japan). Calibration was conducted using a white plate before measurement, and the values were represented as lightness ($L^*$), redness ($a^*$), and yellowness ($b^*$). The total soluble solid content of yogurt was determined using a refractometer (RX-5000α, ATAGO Co., Tokyo, Japan) and expressed as °Brix (%).

### 2.8. Water holding capacity (WHC) and syneresis of yogurt

WHC was measured as previously described (Kim et al., 2023). The yogurt (20 *g*) was centrifuged at 3000 *g* for 10 min at 4 °C. After centrifugation, supernatants were collected and weighed. WHC was calculated using the following equation:

$$\text{WHC}\ (\%) = (1–(W_1/W_2)) \times 100 \quad (4)$$

where $W_1$ is the weight of the supernatant and $W_2$ is the weight of the yogurt before centrifugation.

Syneresis was performed as previously described, with minor modifications (Brüls et al., 2024). After inoculation, the yogurt was transferred to a sterilized plastic cup and incubated at 42 °C until complete fermentation. The serum was isolated by pipetting without applying force to the curd. Syneresis was calculated as follows:

$$\text{Syneresis}\ (\%) = (W_1/W_2) \times 100 \quad (5)$$

where $W_1$ is the weight of the serum after fermentation and $W_2$ is the weight of the yogurt before serum removal.

### 2.9. Microstructure of yogurt

The protein microstructure of yogurt was evaluated using fast green FCF staining, as previously reported, with slight modifications (Gantumur et al., 2024; Keum et al., 2023). Before staining, the yogurt was diluted with distilled water. Each yogurt (1 g) was stained with fast green FCF (20 μL) dissolved in distilled water (0.1 % *w/v*) and spread on a glass slide. After covering with a coverslip, the samples were completely stained for 15 min in the dark. Images were observed using a fluorescence microscope (Eclipse Ti2-U; Nikon Co., Ltd., Tokyo, Japan) and captured using a Nikon Eclipse Ts2R camera (Nilon Co., Ltd., Tokyo, Japan).

### 2.10. Particle size measurement of yogurt

The particle size distribution and mean particle diameter were evaluated using a laser diffraction instrument (Mastersizer 3000E, Malvern, UK). The refractive index was set at 1.33, and the yogurt was dispersed in the water phase.

### 2.11. Protein solubility of yogurt

The protein solubility of yogurt was determined as described previously (Wu, Deng, et al., 2023), with modifications. The yogurts under the same pH conditions were vortexed and centrifuged at 10,000 *g* for 20 min at 4 °C. After centrifugation, the supernatant was collected for the bicinchoninic acid (BCA) assay. The supernatant protein content was measured using a BCA protein assay kit (Sigma-Aldrich, St. Louis, MO, USA) and calculated as follows:

$$\text{Solubility}\ (\%) = C_s/C_0 \times 100 \quad (6)$$

where Cs is the supernatant protein concentration and C0 is the initial protein concentration.

### 2.12. Texture profile analysis (TPA) of yogurt

For TPA, a backward extrusion test was performed using a texture analyzer (TA.XT plusC, Stable Micro Systems, Surrey, UK), as previously described (Wu, Dai, et al., 2023). The yogurt was transferred to a cylindrical container and the tests were conducted under the following conditions: disc diameter (35 mm); distance (30 mm); test speed (1.0 mm/s). The TPA measurements included firmness, consistency index, cohesiveness, and viscosity index.

### 2.13. Rheological properties of yogurt

Yogurt viscosity was measured using a DV-E viscometer (Brookfield, Toronto, ON, Canada), as previously described (Zhang et al., 2019). The yogurt (40 g) was transferred to a 50-mL conical tube, and the viscosity was measured at 50 rpm with a 63 spindle every minute from 5 to 8 min.

A frequency sweep test was conducted for viscoelasticity using a rheometer (MCR 92, Anton Paar, Graz, Austria) with a 50 mm-diameter parallel plate and a gap of 1 mm. The linear viscoelasticity region (LVR) was determined, and the test was then performed within the LVR ranging from 0.1 to 100 rad/s at 25 °C.

A three-interval thixotropy test (3ITT) of the yogurt was performed using a rheometer, and the results were recorded as viscosity. A 50 mm-diameter parallel plate was used, and the gap was set to 1 mm at 25 °C. Each stage was set as follows: pre-shear (2 $s^{-1}$, 2 min); first stage (1 $s^{-1}$, 5 min); second stage (100 $s^{-1}$, 1 min); final stage (1 $s^{-1}$, 5 min). Recovery and deformation rates were calculated using the following equations (Yılmaz et al., 2016; Zhang et al., 2022):

$$\text{Recovery rate}\ (\%) = V_{60}/V_1 \times 100 \quad (7)$$

$$\text{Deformation rate}\ (\%) = (V_1–V_2)/V_1 \times 100 \quad (8)$$

where $V_{60}$ is the viscosity of the yogurt 60 s after deformation, $V_1$ is the initial viscosity of the yogurt, and $V_2$ is the viscosity after deformation.

### 2.14. Fourier transform infrared spectroscopy (FT-IR) of yogurt powder

For FT-IR analysis, yogurt was freeze-dried and powdered. The FT-IR spectra of the yogurt powders were recorded using an FT-IR spectrophotometer (FT/IR-4100 type A; JASCO, Tokyo, Japan). The wavelength range was 600–4000 $cm^{-1}$ and the resolution was 4 $cm^{-1}$.

### 2.15. In vitro digestion of yogurt powder

Yogurt powder was digested *in vitro* to evaluate digestibility, following the method of Gharibzahedi and Altintas (2024) with minor modifications. The oral phase was not included in the process to observe protein digestibility. To simulate the gastric phase, yogurt powder (200 mg) was dissolved in 33 mM glycine buffer under gastric conditions (pH 2). Pepsin (10 U/mg) was added to the solution and incubated at 37 °C for 2 h with gentle shaking. After incubation, the digestion was terminated by heating at 95 °C for 5 min and cooling on ice for 10 min. The mixture was centrifuged at 2000 *g* for 10 min at 4 °C, and the supernatant was removed. For the intestinal phase, 0.05 mM sodium dihydrogen phosphate (2 mL) and trypsin (6.6 U/mg) were added to the gastric phase precipitate under intestinal conditions (pH 6.8). The mixture was incubated at 37 °C for 1 h with gentle shaking. The digestion was terminated under the same conditions as in the gastric phase, and the precipitate was collected by centrifugation at 5000 *g* for 10 min at 4 °C. The hydrolysates from each phase were diluted in phosphate buffered saline and stored at −20 °C for sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) analysis.

### 2.16. SDS-PAGE of yogurt hydrolysate

SDS-PAGE was performed using hydrolysates from the gastric and intestinal phases as previously described (Gharibzahedi & Altintas, 2024). Briefly, the protein concentration in the hydrolysate samples was measured using a BCA assay and mixed with sample buffer. The protein content in each well was standardized and loaded onto a 4–20 % gradient gel (Bio-Rad, Hercules, CA, USA). The gel was stained with a staining solution for 2 h, and then soaked in a destaining solution until the protein profiles developed.

### 2.17. Sensory evaluation of yogurt

Sensory evaluation of yogurt was conducted by a group of trained panelists (eight males and eight females; age, 25–33 years). Y-SAP was excluded from the sensory evaluation because the purchased saponin standard was not food-grade. GE has been reported to be safe for human consumption in previous studies (Cheon et al., 2014; Wang et al., 2009). Each sample was prepared in a plastic cup, served with a spoon and

assigned a random three-digit number. The panelists were provided water to clean their palates between samples. Five attributes (lumpiness, sourness, smoothness, creaminess, and bitterness) were recorded on a nine-point scale as follows: 1 = lowest intensity; 9 = highest intensity. Sensory attributes and their definitions were selected based on previous studies (Desai et al., 2013; Gantumur et al., 2024; Laiho et al., 2017; Lin et al., 2024), as shown in Supplementary Table 1. For the sourness and bitterness attributes, standard scores of 3.0 were assigned using the following reference solutions: sourness, 0.08 % citric acid solution; bitterness, 0.25 % caffeine solution. This study was approved by the Institutional Review Board of Konkuk University (7001355–202,404-HR-781).

### 2.18. Statistical analysis

Data are presented as the mean ± standard deviation. Statistical analyses were performed using SPSS-PASW (version 22.0; SPSS Inc., Chicago, IL, USA) using a two-way analysis of variance. Significant differences were determined using Duncan's multiple range test with a significance level of $p < 0.05$. All experiments were conducted at least thrice. Pearson's correlation coefficient was used to illustrate the relationships between the variables in yogurt using MetaboAnalyst 6.0 (https://www.metaboanalyst.ca). To avoid data distortion, outliers were removed, and normalization was performed. Positive correlations are marked in red, and negative correlations are marked in blue.

## 3. Results and discussion

### 3.1. Viable LAB count, pH, acidification kinetics, and TA of yogurt

The fermentative properties of GE in yogurt were evaluated by measuring viable LAB count, pH, acidification kinetics, and TA. Viable LAB counts were measured on MRS agar over a 6-h period (Fig. 1A). The addition of GE to yogurt significantly increased LAB growth throughout the fermentation period compared with that in yogurt without GE ($p < 0.05$). At the end of the measurement period, the LAB counts in Y-GE 0.1 and Y-GE 0.2 were 9.21 and 9.31 log CFU/mL, respectively, which were higher than that in other yogurt samples. Given its bioactive compounds, such as ginsenosides and phenolic compounds, ginseng is recognized as a potential agent for promoting LAB growth (Song et al., 2021). Considering that GE most likely contains similar compounds, it is reasonable to assume that GE supports LAB growth (Kowalska et al., 2017). The yogurt pH decreased as the LAB grew; the pH variations and kinetic parameters of the GE-supplemented yogurt are presented in Fig. 1B and Supplementary Table 2. The pH decreased more rapidly in the GE-supplemented yogurt in a dose-dependent manner (Fig. 1B). Consequently, the time to complete fermentation was shorter in the GE-supplemented yogurt than in the other samples without GE (Supplementary Table 2). During yogurt fermentation, *S. thermophilus* initiates the process by lowering the pH to 5.5 through lactose fermentation, which subsequently stimulates *L. bulgaricus* (Gharibzahedi & Altintas, 2024). The rapid pH decrease to 5.5 in GE-supplemented yogurt implies that GE promoted LAB growth, particularly *S. thermophilus*. This was supported by the higher LAB counts during the initial fermentation period (Fig. 1A). Furthermore, GE-supplemented yogurts exhibited higher TA levels than those in the other yogurts at all time points ($p < 0.05$; Fig. 1C). This increase was related to an increase in LAB and a decrease in pH (Bock et al., 2024; Kim et al., 2023). In addition to the LAB growth-promoting ability of GE, the added proteins, such as casein, enhance probiotic viability (Gharibzahedi & Altintas, 2024). Thus, Y-CAS and Y-SAP required a shorter time than Y-CON ($p < 0.05$) required to complete fermentation. Collectively, GE stimulated fermentation and accelerated yogurt production.

### 3.2. TPC, TFC, and antioxidant activities of yogurt

The bioactive properties of GE-supplemented yogurt are presented in Table 1. Y-GE 0.2 showed higher TPC values than Y-CON ($p < 0.05$), followed by Y-GE 0.1. The trends in TFC were similar to those in TPC. Y-CON and Y-CAS had the lowest TPC and TFC values ($p < 0.05$). High levels of phenolic and flavonoid compounds have been found in ginseng byproducts, especially in leaves (Chung et al., 2016; Jung et al., 2006). Thus, the high TPC and TFC in the GE-supplemented yogurt may have resulted from the abundance of bioactive compounds in GE. Importantly, Y-CON, Y-CAS, and Y-SAP do not contain any inherent phenolic or flavonoid compounds. The observed TPC and TFC values in these yogurts are likely artifacts arising from limitations of the analytical methods. For instance, the hydrolysis of milk lactose during fermentation can generate reducing sugars that may react with the reagents used to detect phenolic compounds (Rashwan et al., 2024; Wang et al., 2020). Furthermore, Y-SAP exhibited a significantly higher TFC value

**Table 1**
TPC, TFC, radical scavenging activities, and reducing power of yogurts.

| Measurements | Yogurt groups | | | | |
|---|---|---|---|---|---|
| | Y-CON | Y-CAS | Y-GE 0.1 | Y-GE 0.2 | Y-SAP |
| TPC (μg of GAE/mL) | 45.6 ± 0.3$^{c}$ | 47.6 ± 1.0$^{c}$ | 54.8 ± 1.5$^{b}$ | 61.0 ± 1.0$^{a}$ | 53.3 ± 2.0$^{b}$ |
| TFC (μg of QCE/mL) | 1.9 ± 0.3$^{d}$ | 2.2 ± 0.1$^{d}$ | 5.1 ± 0.1$^{c}$ | 7.8 ± 0.1$^{b}$ | 8.8 ± 0.1$^{a}$ |
| ABTS (%) | 49.7 ± 0.7$^{d}$ | 46.2 ± 0.6$^{e}$ | 68.6 ± 0.5$^{b}$ | 73.8 ± 1.8$^{a}$ | 65.8 ± 0.7$^{c}$ |
| DPPH (%) | 32.1 ± 0.1$^{d}$ | 28.3 ± 0.3$^{e}$ | 47.3 ± 0.7$^{b}$ | 49.1 ± 1.6$^{a}$ | 42.0 ± 0.7$^{c}$ |
| FRAP | 1.7 ± 0.1$^{bc}$ | 2.0 ± 0.3$^{b}$ | 2.0 ± 0.0$^{b}$ | 2.7 ± 0.1$^{a}$ | 1.5 ± 0.1$^{c}$ |

TPC: total phenol content, TFC: total flavonoid content, ABTS: 2,2′-azino-bis, DPPH: 2,2-diphenyl-1-picrylhydrazyl, FRAP: ferric ion-reducing antioxidant potential, GAE: gallic acid equivalent, QCE: quercetin equivalent, Y-CON: control yogurt, Y-CAS: yogurt with 1 % casein, Y-GE 0.1: yogurt with 1 % casein and 0.1 % GE, Y-GE 0.2: yogurt with 1 % casein and 0.2 % GE, Y-SAP: yogurt with 1 % casein and 0.2 % saponin. $^{a–e}$ indicate significant differences within the same row ($p < 0.05$).

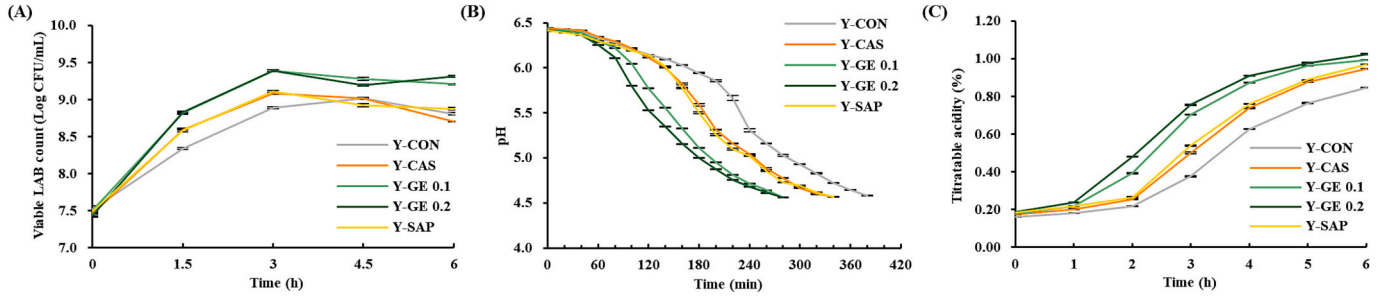


**Fig. 1.** (A) Viable LAB count, (B) pH, and (C) titratable acidity of yogurts, measured every 90, 20, and 60 min, respectively. Y-CON: control yogurt, Y-CAS: yogurt with 1 % casein, Y-GE 0.1: yogurt with 1 % casein and 0.1 % GE, Y-GE 0.2: yogurt with 1 % casein and 0.2 % GE, Y-SAP: yogurt with 1 % casein and 0.2 % saponin.

compared to Y-GE 0.2. This data might be attributable to the influence of the inherent yellow-orange color of saponin on the spectrometric TFC measurement. The absorbance of saponin at the measurement wavelength may have contributed to an artificially elevated TFC reading. Yogurts fortified with GE exhibited significantly higher antioxidant properties than those of the other yogurts, as measured by the ABTS, DPPH, and FRAP assays ($p < 0.05$). Among the non-GE yogurts, Y-SAP displayed the highest antioxidant activity ($p < 0.05$). The higher radical scavenging activities observed in Y-SAP than in Y-CON and Y-CAS may be attributed to the potential antioxidant activities of saponins (Hu et al., 2012). Antioxidant activities, including ABTS, DPPH, and FRAP activities, are closely related to the phenolic and flavonoid compound content (Rumpf et al., 2023; Zhang et al., 2019). Ginsenosides have superior antioxidant capacity (Song et al., 2021); thus, the enhanced antioxidant activity observed in the GE-supplemented yogurt may be attributed to the abundance of bioactive compounds in GE. Further analytical studies are required to identify the active compounds in GE that contribute to its antioxidant activity.

### 3.3. Color and total soluble solid content of yogurt

For yogurt color, the $L^*$, $a^*$, and $b^*$ values were measured, and the visual appearance was displayed (Table 2 and Fig. 2A). The $L^*$ values of Y-CAS and Y-SAP were higher than those of Y-CON. This may be attributed to the white color of added casein. However, GE-supplemented yogurt showed a decrease in $L^*$ and $a^*$ values and an increase in $b^*$ values despite the addition of casein. These color changes may be attributed to the yellowish color of GE, as indicated by the visual appearance of yogurt (Fig. 2A).

The addition of GE increased the Brix value in a concentration-dependent manner (Table 2). Y-CAS also showed a higher Brix value than that of Y-CON ($p < 0.05$). The Brix value indicates the total soluble solid content, which corresponds to the amounts of exopolysaccharides (EPS) (Kim et al., 2023). EPSs are metabolites produced by LAB that improve the rheological and textural properties of yogurt (Brüls et al., 2024). Thus, GE may have stimulated EPS production by LAB through its growth-promoting activity. Interestingly, Y-SAP exhibited the highest Brix value, which was comparable with that of Y-GE 0.2 ($p < 0.05$). Although Y-SAP and Y-CAS displayed similar LAB counts, the elevated Brix value in Y-SAP was most likely not attributable to EPS production by LAB. Saponins, known to stabilize bubbles in solutions particularly in protein-surfactant interactions (Li et al., 2023), may have contributed to the increased Brix values by forming and maintaining bubbles during yogurt processing, thereby affecting light refraction.

**Table 2**
Color, total soluble solid content, WHC, syneresis, protein solubility, and TPA of yogurts.

| Parameters | Yogurt groups | | | | |
|---|---|---|---|---|---|
| | Y-CON | Y-CAS | Y-GE 0.1 | Y-GE 0.2 | Y-SAP |
| $L^*$ | 90.8 ± 0.3$^{b}$ | 91.4 ± 0.4$^{a}$ | 90.1 ± 0.0$^{c}$ | 88.4 ± 0.1$^{d}$ | 91.2 ± 0.5$^{ab}$ |
| $a^*$ | −3.4 ± 0.1$^{a}$ | −3.3 ± 0.4$^{a}$ | −5.1 ± 0.0$^{b}$ | −5.6 ± 0.0$^{c}$ | −3.1 ± 0.1$^{a}$ |
| $b^*$ | 5.5 ± 0.4$^{e}$ | 6.3 ± 0.5$^{d}$ | 14.2 ± 0.0$^{b}$ | 18.1 ± 0.1$^{a}$ | 7.1 ± 0.7$^{c}$ |
| °Brix (%) | 7.3 ± 0.0$^{d}$ | 7.3 ± 0.0$^{c}$ | 7.4 ± 0.0$^{b}$ | 7.5 ± 0.0$^{a}$ | 7.5 ± 0.0$^{a}$ |
| WHC (%) | 29.4 ± 0.7$^{d}$ | 37.3 ± 1.3$^{bc}$ | 39.7 ± 0.2$^{a}$ | 38.2 ± 0.3$^{b}$ | 36.0 ± 0.4$^{c}$ |
| Syneresis (%) | 8.2 ± 0.4$^{a}$ | 5.9 ± 0.2$^{c}$ | 5.3 ± 0.2$^{d}$ | 5.0 ± 0.2$^{d}$ | 6.5 ± 0.1$^{b}$ |
| Protein solubility (%) | 90.0 ± 3.2$^{a}$ | 62.0 ± 1.9$^{d}$ | 66.5 ± 2.6$^{c}$ | 74.5 ± 2.1$^{b}$ | 72.2 ± 2.3$^{b}$ |
| Firmness (g) | 22.3 ± 0.4$^{c}$ | 45.1 ± 3.8$^{a}$ | 35.9 ± 1.0$^{b}$ | 35.7 ± 1.4$^{b}$ | 36.9 ± 2.3$^{b}$ |
| Consistency index (g·s) | 495.6 ± 6.4$^{c}$ | 1010.9 ± 74.5$^{a}$ | 828.5 ± 11.3$^{b}$ | 810.5 ± 26.2$^{b}$ | 884.6 ± 46.4$^{b}$ |
| Cohesiveness (g) | 13.6 ± 0.2$^{c}$ | 35.4 ± 4.4$^{a}$ | 26.8 ± 0.9$^{b}$ | 28.0 ± 1.6$^{b}$ | 28.9 ± 2.6$^{b}$ |
| Viscosity index (g·s) | 22.5 ± 1.2$^{c}$ | 95.6 ± 12.2$^{a}$ | 72.0 ± 3.4$^{b}$ | 75.4 ± 4.7$^{b}$ | 77.1 ± 6.7$^{b}$ |

WHC: water holding capacity, TPA: texture profile analysis, Y-CON: control yogurt, Y-CAS: yogurt with 1 % casein, Y-GE 0.1: yogurt with 1 % casein and 0.1 % GE, Y-GE 0.2: yogurt with 1 % casein and 0.2 % GE, Y-SAP: yogurt with 1 % casein and 0.2 % saponin. $^{a–e}$ indicate significant differences within the same row ($p < 0.05$).

### 3.4. WHC and syneresis of yogurt

WHC and syneresis significantly affect the stability, texture, and overall sensory properties of yogurt (Arab et al., 2023). These two factors are inversely related, with syneresis decreasing with increasing WHC (Wu, Deng, et al., 2023). The WHC and syneresis results are presented in Table 2. Y-CAS yogurt fortified with casein demonstrated a marked WHC increase and a decrease in syneresis compared with that of the Y-CON ($p < 0.05$). This quality improvement is attributed to hydrophilic properties of casein, which effectively trap water and contribute to a strong and rigid gel structure (Arab et al., 2023). Among the casein-supplemented yogurt samples, Y-SAP exhibited the least stability, likely due to an excessive amount of saponin exceeding its optimal concentration. This may have weakened the gel structure and increased whey release compared to Y-CAS. GE-supplemented yogurt exhibited enhanced stability by increasing WHC and reducing syneresis. Several factors contributed to the improved stability. First, the saponins present in GE formed stable emulsion systems; thus, a crucial balance between casein and saponins was achieved. Second, the polyphenols in GE strengthened the yogurt gel structure. Polyphenols coat proteins and create cross-linking interactions, thereby decreasing syneresis by forming aggregates (Qing et al., 2023; von Staszewski et al., 2011). As evidenced by the TPC results (Table 1), GE-supplemented yogurt had a higher phenol content than that of the other groups. Consequently, the WHC of GE-supplemented yogurt was higher than that of the control yogurt. Finally, the increased Brix value in GE-supplemented yogurt, which is associated with EPS production, may also play a role. EPSs generated during yogurt fermentation act as bio-thickening agents with excellent water-binding capabilities (Arab et al., 2023; Brüls et al., 2024). Their viscous and robust properties hinder the mobility of water within the yogurt matrix, leading to reduced syneresis. Collectively, casein increased the WHC of fat-free yogurt, thereby reducing syneresis, whereas GE complemented these effects owing to its versatile characteristics.

### 3.5. Microstructure, particle size distribution, and protein solubility of yogurt

Protein particle size in yogurt plays a crucial role in determining its textural and sensory properties (Gantumur et al., 2024). Large protein particles cause a grainy texture, which reduces consumer preference (Gao et al., 2023). To investigate the relationship between protein particle size and yogurt properties, protein microstructure was visualized using fast green FCF staining (Fig. 2B). Compared with that of Y-CON, Y-CAS had larger protein particles that formed aggregates. Notably, GE-supplemented yogurt displayed smaller protein particles, greater uniformity, and reduced aggregation than that of casein-supplemented yogurt (Y-CAS). Saponins, possessing both hydrophilic and hydrophobic regions, decrease surface tension by rapidly adsorbing at oil-water and air-water interfaces (Li et al., 2023). Moreover, in emulsion systems, such as yogurt, the electrostatic and steric repulsion properties of saponins contribute to a creamier texture by preventing aggregation (Bottcher & Drusch, 2017). Proteins also exhibit both hydrophilic and hydrophobic characteristics, thereby enabling them to interact with themselves or with other components that possess similar properties (Gao et al., 2023). Therefore, the observed reduction in protein size and aggregation is most likely a result of protein-saponin

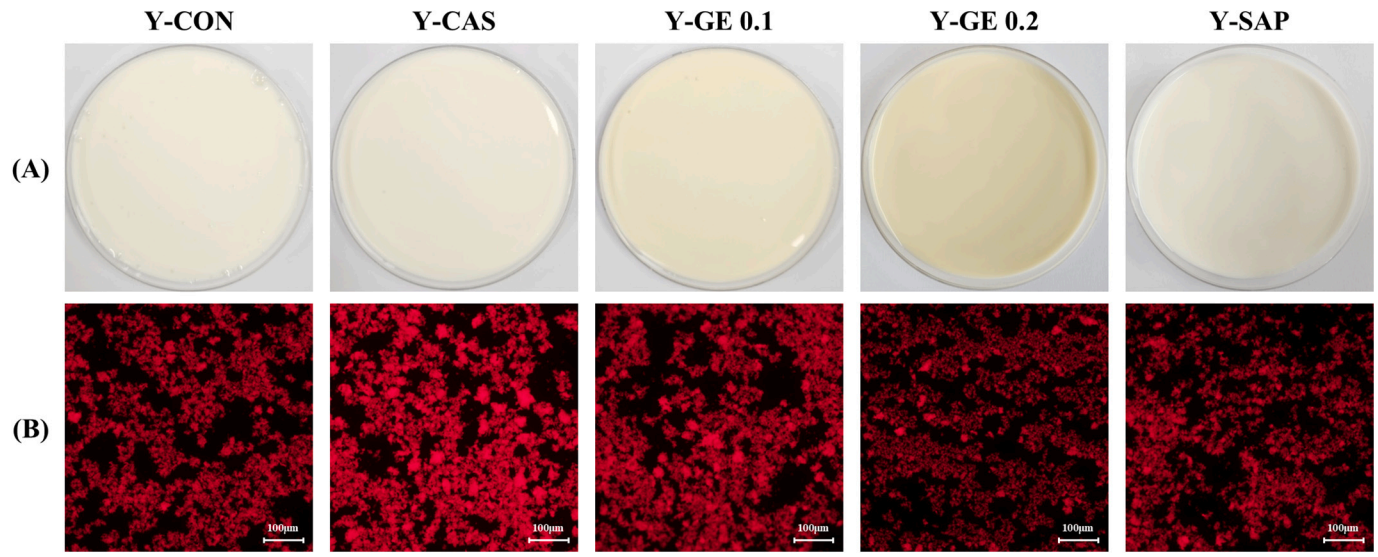


**Fig. 2.** (A) Visual appearance and (B) microstructure of yogurts stained with fast green FCF. Y-CON: control yogurt, Y-CAS: yogurt with 1 % casein, Y-GE 0.1: yogurt with 1 % casein and 0.1 % GE, Y-GE 0.2: yogurt with 1 % casein and 0.2 % GE, Y-SAP: yogurt with 1 % casein and 0.2 % saponin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

interactions facilitated by GE acting as a surfactant. This trend is also evident in the particle size distribution (Fig. 3). Specifically, when comparing the particle size distributions of the yogurts, addition of casein shifted the peak towards larger particle sizes compared with that of Y-CON. Additionally, a peak at over 100 μm was observed, indicating protein aggregation within the yogurt. Conversely, GE shifted the peak towards smaller particle sizes in a concentration-dependent manner. Given its surfactant properties, GE prevents protein aggregation and reduces particle size, as evidenced by the microstructural data (Fig. 2B). Although Y-SAP shifted the peak towards smaller particle sizes than those of Y-CAS, a peak at over 100 μm was still observed, resembling Y-CAS. Protein aggregation in Y-CAS and Y-SAP was also confirmed by the $D_{90}$ values, which were 129.50 and 102.48 μm, respectively, and significantly larger than the others ($p < 0.05$) (Supplementary Table 3). Excess surfactants paradoxically cause aggregation by combining with proteins in the bulk phase (Li et al., 2023). This suggests that excessive saponin may have caused protein aggregation in the yogurt. The diverse particle size distributions of Y-SAP reflect both the dispersion effect of saponins as surfactant and the aggregation caused by their excessive addition.

Given that decreased protein solubility typically correlates with increased protein aggregation (von Staszewski et al., 2011; Wu, Dai, et al., 2023), the yogurt protein solubility was also measured (Table 2). As supported by the microstructure and particle size distribution results (Fig. 2B and 3), casein addition decreased protein solubility by aggregating protein. In agreement with previous studies, the GE or saponin addition enhanced protein solubility. This may be attributed to the ability of saponins to prevent protein aggregation. In conclusion, GE demonstrated its effectiveness as a natural surfactant in casein-supplemented yogurt by preventing protein aggregation and improving protein solubility.

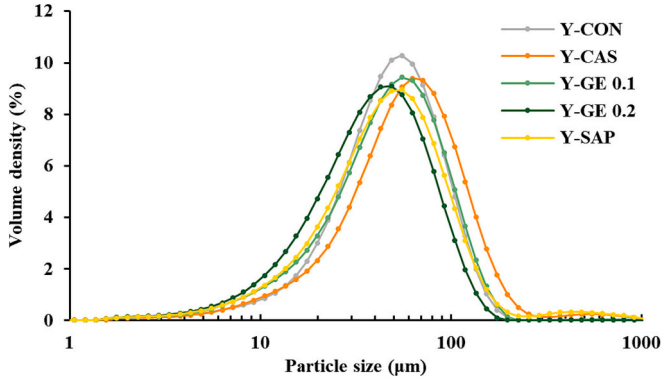


**Fig. 3.** Particle size distribution of yogurts analyzed using mastersizer. Y-CON: control yogurt, Y-CAS: yogurt with 1 % casein, Y-GE 0.1: yogurt with 1 % casein and 0.1 % GE, Y-GE 0.2: yogurt with 1 % casein and 0.2 % GE, Y-SAP: yogurt with 1 % casein and 0.2 % saponin.

### 3.6. TPA and rheological properties of yogurt

Yogurt TPA results are shown in Table 2. Y-CON showed the lowest values for all the parameters, while Y-CAS exhibited the highest values ($p < 0.05$). GE-supplemented yogurt and Y-SAP had values lower than Y-CAS but higher than Y-CON ($p < 0.05$). Our data indicate that casein improved the quality of fat-free yogurt by elevating all TPA parameters. The higher TPA values of GE-supplemented yogurts and Y-SAP suggest that GE and saponins supported casein function. Although increasing protein levels generally enhances yogurt firmness, excessive protein causes aggregation, leading to a grainy texture (Arab et al., 2023; Hovjecki et al., 2023; Qing et al., 2023). The Y-CAS results were consistent with these findings, that the yogurt exhibited both high firmness and protein aggregation (Fig. 2B and 3, Table 2). In summary, although casein strengthens yogurt, it may cause lumpiness. However, the addition of GE, a natural surfactant, counteracts this undesirable effect.

Viscosity and viscoelasticity were measured to determine rheological properties. As shown in Fig. 4A, both GE-supplemented yogurts had higher viscosity values than those of the other yogurts ($p < 0.05$). Increased viscosity could be a result of GE-stimulated EPS production by LAB. The lowest viscosity was observed for Y-SAP ($p < 0.05$), most likely because of the formation of an unstable gel matrix caused by excessive saponin content. Interestingly, Y-CAS had a viscosity similar to that of Y-CON ($p > 0.05$), even though the addition of casein increases viscosity due to its superior hydrophilic residues (Arab et al., 2023). Yogurt with a flexible texture tends to have higher WHC and lower firmness (Graca et al., 2022). In contrast, the rigid and inflexible structure of yogurt is caused by increased entanglement of protein polymers in the gel matrix (Wu, Deng, et al., 2023). Subsequently, the high viscosity of GE-supplemented yogurt appeared to be a result of the formation of a

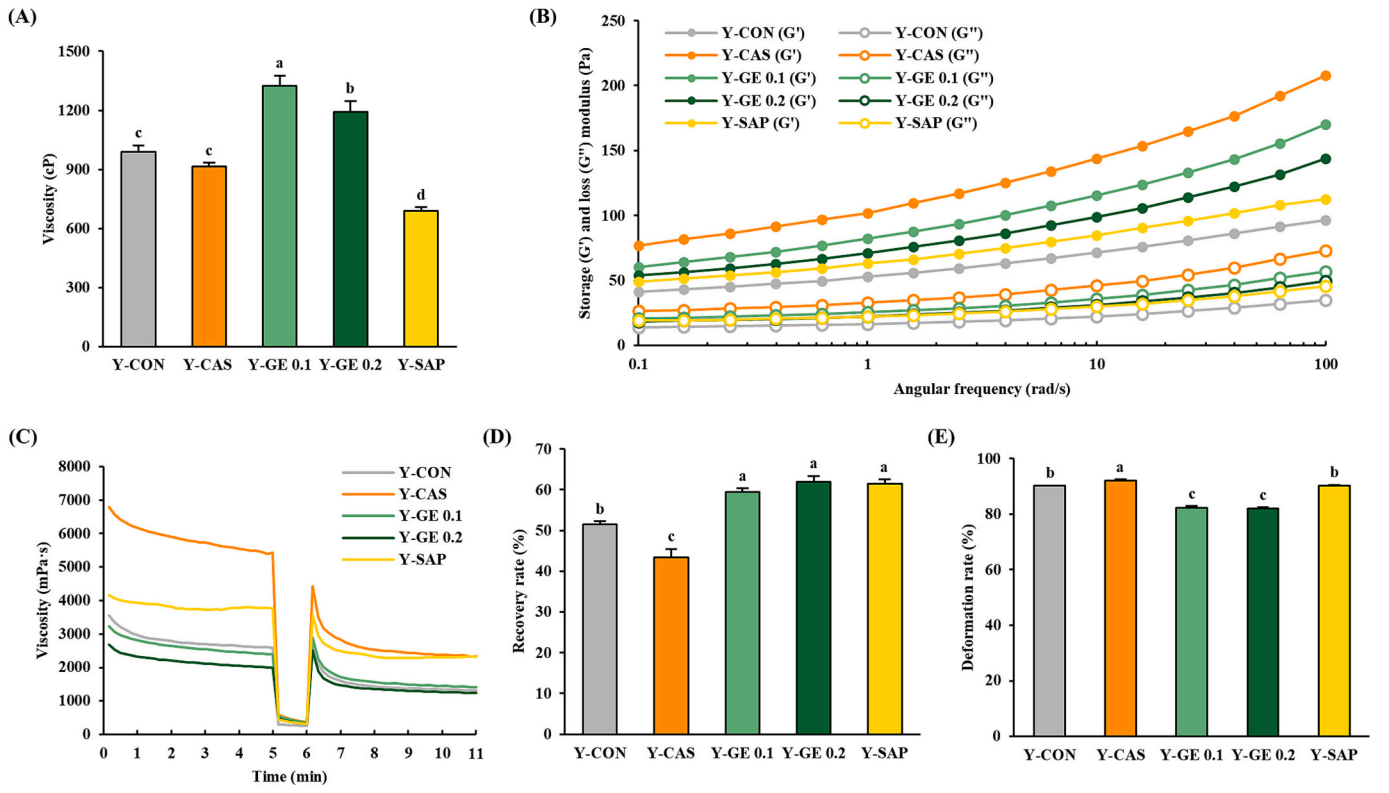


**Fig. 4.** Rheological properties of yogurts. (A) Viscosity, (B) frequency sweep for viscoelasticity, (C) viscosity in the 3ITT, (D) recovery rate, and (E) deformation rate of yogurts. Viscosity was measured using a viscometer, whereas the other parameters were assessed with a rheometer. Y-CON: control yogurt, Y-CAS: yogurt with 1 % casein, Y-GE 0.1: yogurt with 1 % casein and 0.1 % GE, Y-GE 0.2: yogurt with 1 % casein and 0.2 % GE, Y-SAP: yogurt with 1 % casein and 0.2 % saponin. $^{a-c}$ indicates significant difference ($p < 0.05$).

flexible structure with higher WHC and lower firmness. Unlike the GE-supplemented yogurt, Y-CAS exhibited protein aggregation and formed large protein particles through entanglement, leading to an inflexible structure. This inflexibility resulted in decreased yogurt viscosity. These viscosity results align with the fact that GE contributes to a flexible yogurt structure and improves its rheological properties.

In viscoelasticity, G' values are related to firmness (Lin et al., 2024) and contribute to a firmer texture (Laiho et al., 2017). Thus, in line with the firmness results in Table 2, the G' values were observed in the following order: Y-CAS, Y-GE 0.1, Y-GE 0.2, Y-SAP, and Y-CON (Fig. 4B). Given that Y-CAS had higher G' values ($p < 0.05$), GE may be responsible for softening the yogurt texture, as evidenced by the decreased G' values in GE-supplemented yogurt. Viscoelasticity data also reflects the gel strength and stability, and low G' values suggest a weaker yogurt gel (Wu, Deng, et al., 2023). Our data showed that Y-CON formed a weak gel matrix, as indicated by the lowest G' value, which is consistent with our WHC data (Table 2). The results of the viscoelasticity analysis were consistent with that of the TPA data, indicating that GE contributes to a softer yogurt texture.

The recovery and deformation rates were calculated using the viscosity data from the 3ITT experiments (Fig. 4C–E). High recovery and low deformation rates in yogurt generally signify its resistance to structural degradation (Yılmaz et al., 2016), which is indicative of gel stability from a rheological perspective (Lin et al., 2024). Our data showed that GE-supplemented yogurt exhibited the highest recovery and lowest deformation rates among all the yogurt samples ($p < 0.05$). In contrast, Y-CAS showed the lowest recovery and highest deformation rate ($p < 0.05$). Y-CAS exhibited high firmness and low viscosity (Table 2 and Fig. 4A). The combination of low viscosity and high firmness suggests low flexibility (Graca et al., 2022). This may explain why the lumpy texture of the Y-CAS was less resilient to the deformation caused by high shear stress. Therefore, Y-CAS demonstrated a lower thixotropic behavior and formed an unstable gel when subjected to external stress. The increased recovery rate of GE-supplemented yogurt can be partly attributed to EPS production by LAB (Wang et al., 2023). The viscosity-enhancing properties of EPS contributes to the stability and resilience of yogurt against external forces. Overall, our analysis of textural and rheological properties showed that casein made fat-free yogurt firmer, potentially compromising its sensory quality. However, the addition of GE addressed this issue by softening the texture, reducing firmness, and improving the viscosity and stability of the gel structure.

### 3.7. FT-IR spectroscopy of yogurt powder

FT-IR spectroscopy was conducted to identify the GE components in yogurt (Fig. S1). Lyophilized yogurt was used to minimize interference from water. The FT-IR spectra revealed that Y-GE 0.1 and Y-GE 0.2 exhibited the most similar peak patterns to Y-SAP, indicating a strong resemblance in their components. Characteristic peaks at 2870 $cm^{-1}$, 1517 $cm^{-1}$, and 1640 $cm^{-1}$ were attributed to -CH stretching, amide I, and amide II, respectively, suggesting protein-saponin interactions (Liu et al., 2003). Casein addition increased the intensity of these peaks, further supporting the protein-saponin interactions. A saponin-specific peak at 2300–2500 $cm^{-1}$, associated with methylene groups (Cho et al., 2010), was observed in Y-GE 0.1, Y-GE 0.2, and Y-SAP, with the highest intensity in Y-SAP, reflecting its higher saponin content. Peaks at 1040 $cm^{-1}$, indicative of C-O-C bonds in sugar residues (Schreiner et al., 2021), were more prominent in Y-SAP and Y-GE 0.2 than in Y-CON and Y-CAS. Overall, FT-IR analysis confirmed the presence of saponin-structured ginsenosides as the primary components of GE and highlighted the significant protein-saponin interactions in GE-supplemented yogurt.

### 3.8. Digestibility of yogurt

Although previous studies have focused on the fermentative, bioactive, and physical properties of yogurt, the digestive ability is another crucial factor to consider. Digestive function affects both digestive health and nutritional value (Sensoy, 2021). To examine yogurt proteins modifications during digestion, *in vitro* digestion experiments were conducted, followed by SDS-PAGE (Fig. S2). Di Marzo et al. (2021) found that all digested yogurt samples contained protein fragments smaller than 20 kDa, which were primarily whey proteins, thereby suggesting complete gastric-phase pepsin degradation of casein. In this study, we observed bands at approximately 14 kDa, indicative of α-lactalbumin, in all yogurt samples during the gastric phase (Fig. S2). Furthermore, all yogurt samples were degraded into small peptides (< 10 kDa) during the intestinal phase (Fig. S2). Therefore, casein addition during the gastric phase made it more difficult for enzymes to degrade the protein. This was particularly evident in Y-CAS, which showed an increased α-lactalbumin band. However, Y-GE 0.2 and Y-SAP exhibited lower α-lactalbumin levels than those of Y-CAS and Y-CON, suggesting that GE and saponin may decrease protein aggregation, as previously observed (Fig. 2B). These findings highlight the ability of GE to prevent protein aggregation and improve digestion.

### 3.9. Sensory evaluation of yogurt

To evaluate the effect of GE on yogurt taste and texture, sensory analysis was conducted using a nine-point scale intensity test (Fig. 5). Owing to its saponin content, GE has a bitter taste (Bottcher & Drusch, 2017). Consequently, Y-CON and Y-CAS, which lacked bitterness-inducing substances, had the lowest bitterness scores ($p < 0.05$; Fig. 5). Conversely, Y-GE 0.2 had the highest bitterness score ($p < 0.05$), followed by Y-GE 0.1. As bitterness negatively affects consumer preference (Wu, Deng, et al., 2023), Y-GE 0.2 may be less favored than Y-GE 0.1. The bitterness score of Y-GE 0.1 was determined to be approximately 2.94. In our sensory evaluation, a bitterness score of 3.0 was assigned to a 0.25 % caffeine reference solution. Considering that tea generally contains 1.0–2.0 % caffeine (Boros et al., 2016; Khokhar & Magnusdottir, 2022), the bitterness of Y-GE 0.1 is substantially lower (4–8 times) than that of tea, suggesting it would be acceptable to most consumers. Although GE-supplemented yogurts exhibited higher LAB counts and lower pH (Fig. 1A-B), which are expected to increase sourness, the bitter taste of GE most likely masked any differences in sourness.

In addition, to assess texture, lumpiness, smoothness, and creaminess were evaluated. Y-CAS exhibited the highest lumpiness ($p < 0.05$), whereas GE addition reduced this effect ($p < 0.05$). Lumpiness is associated with particle size (Laiho et al., 2017); hence, Y-CAS aggregated proteins to form larger particles, whereas GE prevented aggregation (Fig. 3). Lumpiness is inversely related to smoothness and creaminess and is related to particle size; thus, the lumpy texture of Y-CAS resulted from protein aggregation. Therefore, Y-CAS scored the lowest in smoothness and creaminess ($p < 0.05$), whereas the other yogurts scored higher because of less aggregation. Y-GE 0.2 scored the highest in creaminess ($p < 0.05$), followed by Y-GE 0.1, and Y-CON, differing from the smoothness data. Creaminess is particularly influenced by small particle size and grainy texture (Cayot et al., 2008). Thus, the small, non-grainy Y-GE 0.2 particles contributed to its highest creaminess score. In addition to aggregation, smoothness and creaminess are related to viscosity and syneresis (Lin et al., 2024; Wu, Dai, et al., 2023). This is consistent with our viscosity and syneresis data (Fig. 4A and Table 2). The higher viscosity and lower syneresis of the GE-supplemented yogurt contributed to improved smoothness and creaminess. Sensory evaluation confirmed the effectiveness of GE in preventing protein aggregation and lumpiness. Generally, creaminess and smoothness significantly influence consumer acceptance (Zhao et al., 2023). Additionally, smaller particle sizes and a well-distributed gel matrix enhance texture (Gao et al., 2023; Gharibzahedi & Altintas, 2024). Moreover, improved physical properties, such as texture and reduced whey separation, positively impact consumer perceptions (Brüls et al., 2024). Although our sensory evaluation did not include preferences, we concluded that GE-supplemented yogurts with improved texture would be preferred over Y-CON and Y-CAS, unless consumers were sensitive to bitterness. Considering both taste and overall consumer preference, Y-GE 0.1 emerges as the most promising GE-supplemented yogurt, offering enhanced quality and reduced bitterness.

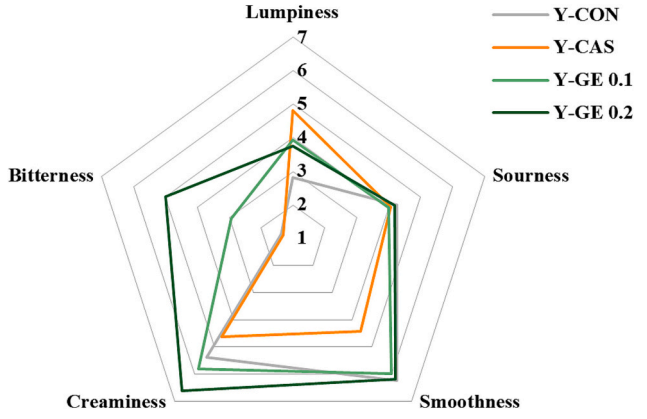


**Fig. 5.** Sensory evaluation of yogurts. Y-CON: control yogurt, Y-CAS: yogurt with 1 % casein, Y-GE 0.1: yogurt with 1 % casein and 0.1 % GE, Y-GE 0.2: yogurt with 1 % casein and 0.2 % GE, Y-SAP: yogurt with 1 % casein and 0.2 % saponin.

### 3.10. Correlation analysis

GE and saponins prevented protein aggregation. Paradoxically, as shown by particle size distribution (Fig. 3), saponins also contributed to protein aggregation. The presence of saponins prompted us to investigate the well-dispersed and small protein particles exhibited by GE-supplemented yogurt without protein aggregation. Thus, to identify other factors that influence the prevention of protein aggregation, Pearson's correlation test was performed and visualized as a heatmap. Interestingly, negative correlations were observed between LAB and indicators of protein aggregation (*e.g.*, deformation rate, $D_{32}$, and $D_{43}$) (Fig. 6). From this negative correlation, LAB were concluded to degrade proteins into smaller particles during fermentation, thereby preventing protein aggregation. Thus, the absence of aggregation peaks in GE-supplemented yogurt was attributed to the LAB growth-promoting function of GE (Fig. 3). In addition, TPC was positively correlated with LAB and negatively correlated with $T_f$ (h). Therefore, phenolic compounds in GE significantly influenced LAB growth, as shown in Fig. 1 and Table 1. Additionally, WHC and syneresis showed positive and negative correlations, respectively, with $V_{max}$ and $T_f$ (h), which are related to LAB growth. Therefore, increased LAB enhances EPS production, leading to a stable yogurt gel matrix. This is consistent with the results presented in Fig. 1 and Table 2. In summary, the soft and creamy texture of yogurt, resulting from the prevention of protein aggregation, can be attributed to the LAB growth-promoting and surfactant functions of GE.

## 4. Conclusion

In this study, we assessed the fermentative, bioactive, physical, and sensory characteristics of GE- and casein-supplemented fat-free yogurt. GE addition boosted fermentation rates and increased the total LAB count in yogurt. Moreover, GE enhanced antioxidant activity by increasing phenol and flavonoid contents. In terms of physical properties, GE stabilized the yogurt matrix by reducing syneresis and

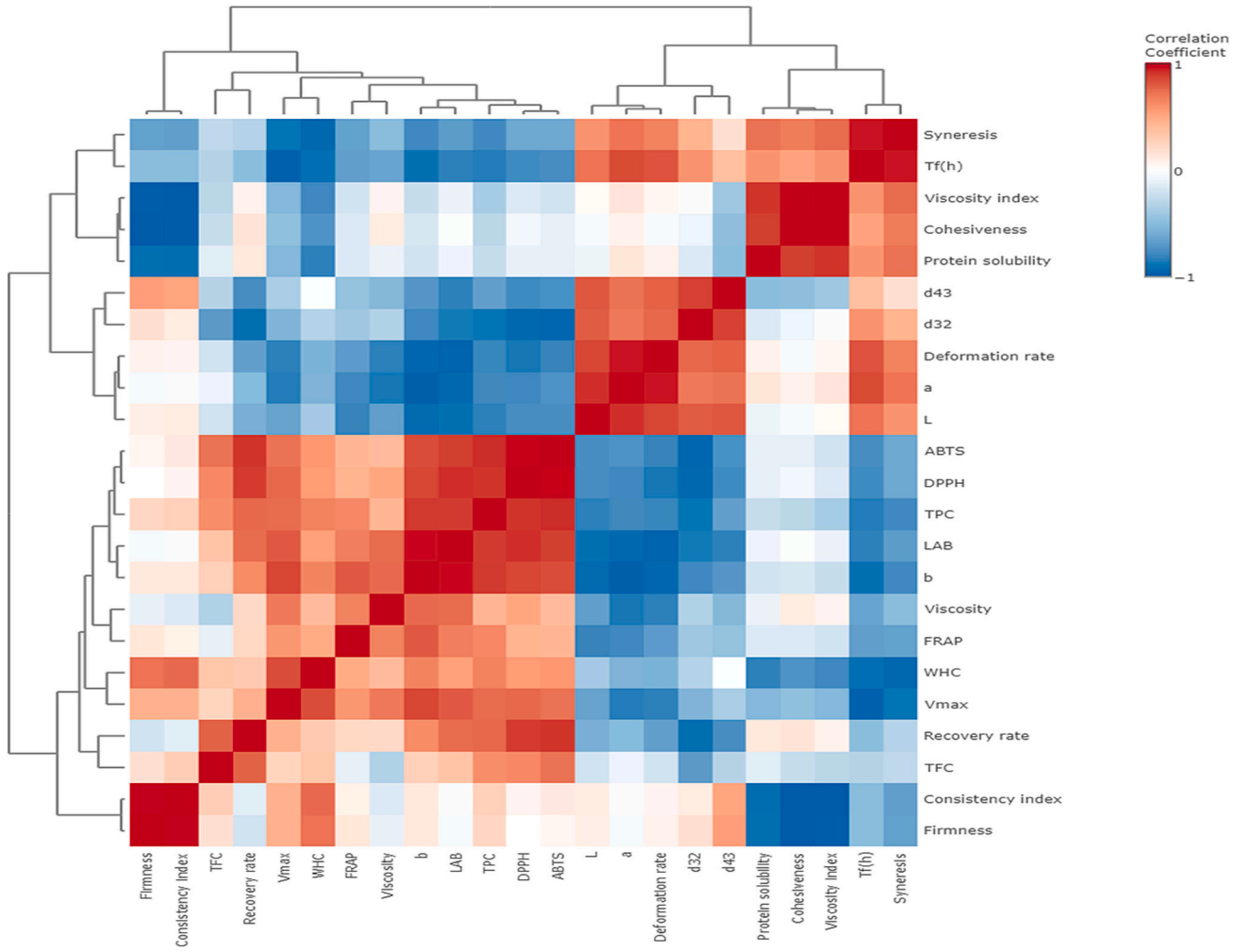


**Fig. 6.** Heatmap for pearson correlation analysis of yogurt experiments.

improving WHC. Furthermore, GE minimized protein particle size, prevented aggregation, and improved protein solubility. TPA and rheological experiments showed that GE-supplemented yogurt exhibited decreased firmness and increased viscosity compared with the lumpy-textured Y-CAS. Sensory evaluation revealed that Y-GE 0.1 had the most desirable characteristics, with a soft and creamy texture, among all yogurt samples. Overall, GE is a promising additive for improving lumpy texture, functioning as a natural surfactant without negatively affecting the benefits of casein. Considering the significantly lower cost of ginseng leaves and stems compared to roots, our findings suggest that GE presents a cost-effective and natural solution for enhancing fat-free yogurt texture by acting as a surfactant and softening agent. To further investigate the potential of GE as a natural surfactant, future studies should explore its applications in various dairy products and emulsion systems.

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None.

## CRediT authorship contribution statement

**Dong Hyun Keum:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Hyun Ju Lee:** Writing – review & editing, Investigation. **Ji Hwan Ryoo:** Writing – review & editing, Investigation. **Sung Gu Han:** Writing – review & editing, Writing – original draft, Supervision.

## Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this study.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.102242.

## Data availability

Data will be made available on request.

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