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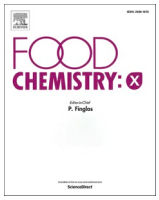

Enhancing emulsion, texture, rheological and sensory properties of plant-based meat analogs with green tea extracts

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ABSTRACT

Plant-based meat analogs require improvements in taste and texture to better replicate traditional meat. L-theanine and tannin, abundant in green tea, influence food taste and physicochemical properties. This study evaluated the quality characteristics of green tea extract (GE)-supplemented plant-based patties (PP) and the mechanisms affecting taste and texture. Green tea was extracted with water (GWE) or 70 % ethanol (GEE). GEE contained higher tannin and lower L-theanine levels than GWE. Both GWE and GEE reduced protein deterioration and lipid oxidation in PP throughout the 28-day storage period. PP with 1.0 % GEE (PP-GEE1.0) showed improved emulsion stability and texture due to non-covalent interactions including hydrophobic interaction and hydrogen bonds, and increased β-sheet structures between tannin and pea protein. PP-GEE1.0 also had superior sensory characteristics due to an optimal balance of L-theanine and tannin. Overall, the incorporation of GE, particularly GEE significantly improved physicochemical properties, sensory quality, and storage stability of PP.

1. Introduction

Meat products are consumed worldwide due to their nutritional value, taste, and affordability. However, the growth of meat production is unsustainable, leading to environmental problems, such as water depletion, climate change, and loss of biodiversity (Fiorentini et al., 2020). Furthermore, the sustainability of meat as a primary food source is a concern regarding human health problems, including cardiovascular diseases and cancers, and animal welfare (Giromini & Givens, 2022). Therefore, there is a demand for meat analogs to alleviate concerns associated with meat consumption.

Plant-based meat alternatives are conventional meat analogs made from plant proteins, polysaccharides, water, and oil. Numerous studies have aimed to replicate the properties of meat. For example, an oleogel comprising over 75 % (*v*/v) oil encapsulated by protein microgels was incorporated into plant-based meat analogs to regulate oil release, thereby enhancing the desired juiciness of the meat (Han, Wang, Guo, Wang and Yang, 2023). Additionally, a Pickering emulsion formulated from various polysaccharides and pea protein significantly improved the texture of plant-based patties (Keum et al., 2024). While research on flavors in plant-based meat analog is underway, it currently lags behind efforts concentrated on replicating meat texture. Thus, it is necessary to not only establish the physical framework of plant-based meat but also explore various substances to make meat-like flavors and effectively mask any beany odor.

Green tea (*Camellia sinensis*) is one of the most widely consumed beverages worldwide because of its elevated polyphenol and amino acid contents, which enhance its anti-inflammatory, anticancer, and cardioprotective properties (Prasanth et al., 2019). Furthermore, green tea and its extracts serve as versatile food additives, enriching the creation of unique flavors, improving the physicochemical properties of food, and contributing to food preservation (Passos et al., 2022). L-theanine, an amino acid abundant in green tea leaves, is structurally similar to glutamate, a well-established contributor to the umami taste (Williams et al., 2022). In addition, a previous study reported that L-theanine activated the umami receptors T1R1 + T1R3, resulting in the perception of an umami taste sensation, providing a savory or meaty taste (Dermiki et al., 2013; Narukawa et al., 2014). However, excessive amounts of L-theanine can diminish the umami taste while enhancing sweetness (Yamamoto et al., 2008). Tannin, which are also commonly found in green tea leaves, can be extracted using a combination of water and organic solvents (Ivanova et al., 2021). In the food industry, the beneficial effects of tannin, such as its antioxidant and antibacterial properties, are well established. Tannin creates substantial protein-tannin

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complexes through hydrogen bonds and hydrophobic interactions, which subsequently influence the physicochemical properties of food (Molino et al., 2019). However, high tannin content in food may impart a bitter flavor due to its astringency, leading to decreased appetite (Hung et al., 2010). Previous studies have investigated the effects of green tea extract (GE) with their different extraction solvents and concentrations on the physicochemical properties and sensory attributes during storage in various food products (Dönmez et al., 2017; Passos et al., 2022). However, the effects of GE, particularly on the physicochemical and sensory properties in the plant-based meat analogs, have not been well investigated.

Thus, we hypothesized that incorporating GE could enhance the physicochemical properties, sensory characteristics, and storage stability of plant-based patties (PP) owing to the presence of L-theanine and tannin. In this study, green tea was extracted using different extraction solvents (water or 70 % ethanol), and GE was incorporated into PP (0.5 %, 1.0 %, or 1.5 %, *w*/w). Particularly, chemical reactions and rheological properties were measured to specifically identify the interaction between isolated pea protein (IPP) and GE.

2. Materials and methods

2.1. Materials

Textured pea protein (TPP) was purchased from Sotexpro Co. (Paris, France). κ-Carrageenan, salt and beet powder were purchased from ESfood Co. (Gyeonggi, Korea). Potato starch was purchased from Daehan Flour Mills Co. (Seoul, Korea). IPP was purchased from Hyangrim Co. (Seoul, Korea). Methyl cellulose was purchased from LOTTE Fine Chemical Co. (Ulsan, Korea). Canola and coconut oils were purchased from CJ Cheiljedang (Seoul, Korea) and Palmtop Vegeoil Products Sdn. Bhd. (Johor, Malaysia), respectively. Green tea leaf powder was purchased from Edentown F&B Co. (Incheon, Korea). L-theanine was purchased from Aladdin (Shanghai, China). Tannin and polyvinylpolypyrrolidone (PVPP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antifoam agent was purchased from Shin-Etsu Silicone Co. (Seoul, Korea). Total viable count (TVC) plate, *Escherichia coli* count plate, coliforms count plate, and yeasts and molds count plate were purchased from 3 M (Minnesota, USA). Folin–Ciocalteu reagent, thiobarbituric acid (TBA), bromocresol green, methyl red, boric acid, and acetic acid were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Preparation of GE

Green tea leaf powder was extracted as previously described with minor modifications (Ivanova et al., 2021; Perini et al., 2021). Green tea leaf powder (30 g) was extracted with 400 mL of water (GWE) or 70 % (*v*/v) ethanol (GEE). The samples were stirred for 1 h, followed by sonication for an additional 1 h. The extracts were then filtered through Whatman No. 1 filter paper (GE Healthcare Life Sciences, Buckinghamshire, UK). The filtered solvents were evaporated at 48 °C under reduced pressure using a rotary evaporator (Tokyo Rikakikai Co., Ltd., Tokyo, Japan). Finally, GWE and GEE were freeze-dried and stored at −80 °C until use.

2.3. L-theanine content of GE using high-performance liquid chromatography (HPLC) analysis

HPLC was performed using an Agilent 1260 Infinity HPLC system equipped with an ultraviolet (UV) detector fixed at 190 nm. Before HPLC analysis, GE (5 mg/mL) was filtered through a 0.2 μm polyethersulfone membrane filter (CHMLAB Group, Barcelona, Spain). The analysis was conducted on an Agilent Eclipse plus C18 column (5 μm × 4.6 mm × 250 mm, Agilent, Wilmington, USA) maintained at 28 °C. (A) water and (B) acetonitrile were used as mobile phases. The gradient elution was programmed as follows: 0–10 min, 100 % A; 10–12 min, 20 % A; 12–20 min, 20 % A; 20–22 min, 100 % A; 22–40 min, 100 % A. The injected sample volume was 10 μL with a flow rate of 1 mL/min. Eluted compounds were identified by comparing their retention times. The calibration curves of the L-theanine standard were used to quantify the same compound in the GE (mg/g dry weight) by comparing the retention times and calculating the corresponding areas. Samples were measured triplicate, respectively.

2.4. Total tannin content of GE

Total tannin content (TTC) was determined by PVPP/Folin–Ciocalteu method, as described previously, with slight modifications (Palacios et al., 2021). Total phenolic content (TPC) was determined by combining 30 μL of the GE, 120 μL of distilled water, 20 μL of 1 M Folin–Ciocalteu reagent, and 30 μL of 5 % sodium carbonate. The mixture was vortexed and kept in the dark at room temperature for 90 min. Tannin (20, 40, 60, 80, 100 μg/mL) was prepared in the same manner as standard solutions. The absorbance was measured at 725 nm using a spectrophotometer (BioTek Instruments, Winooski, VT, USA). To determine the remnant phenolic content (RPC) after the precipitation of tannins, 100 mg of PVPP was dissolved in 1.0 mL of water and 750 μL of GE. The mixture was stored at 4 °C for 15 min and then centrifuged at 3000 ×*g* for 10 min at 4 °C. The supernatant was used for Folin–Ciocalteu analysis following the same procedure. The TTC was calculated by using the eq. (1) and expressed as mg tannin equivalent (TAE)/g dry weight. Measurements were performed in triplicate.

$$\text{TTC (mg TAE/g dry weight)} = \text{TPC (mg TAE/g dry weight)} - \text{RPC (mg TAE/g dry weight)} \quad (1)$$

2.5. Preparation of plant-based patties

To evaluate the characteristics of PP supplemented with GWE and GEE, seven formulations of patties were prepared based on their different concentrations (Table S1): PP supplemented with GWE 0.5 % (*w*/w) (PP-GWE0.5); PP supplemented with GWE 1.0 % (w/w) (PP-GWE1.0); PP supplemented with GWE 1.5 % (w/w) (PP-GWE1.5); PP supplemented with GEE 0.5 % (w/w) (PP-GEE0.5); PP supplemented with GEE 1.0 % (w/w) (PP-GEE1.0); PP supplemented with GEE 1.5 % (w/w) (PP-GEE1.5). TPP was hydrated for 1 h at 4 °C using water dissolved in different concentrations of GWE and GEE. After 1 h, TPP was mixed with the ingredients for 6 min. A control group was manufactured without GE. Subsequently, 110 g of the mixture was formed into patties using a patty presser (manual burger press 4″, Spikomat Ltd., Nottingham, UK), and then it was stored at 4 °C for 1, 7, 14, and 28 days, respectively. To evaluate the cooked patties, samples were cooked using an electric pan (DW-1530, Daewon Home Electric Co., Ltd., Gyeonggi, Korea) at 150 °C for 3 min per side until the internal temperature of patties reached 80 °C. Due to the hygiene considerations of PP, sensory

evaluation was only performed on day 1. The cooked patties were used immediately for sensory evaluation and allowed to equilibrate at room temperature for 30 min before measuring physicochemical properties and storage stability. All experiments were repeated twice. Within each replicate, four patties were prepared for each formulation.

2.6. pH and color measurement

The pH was measured on storage days 1, 7, 14, and 28. To measure pH, 5 g of the patty was mixed with 20 mL of distilled water and homogenized at 10,000 rpm for 1 min using a homogenizer (HG-15 A, DAIHAN Scientific Co., Ltd., Gangwon, Korea). The pH of the samples was measured six times before and after cooking using a pH meter (LAQUA, Horiba, Kyoto, Japan) during each storage period.

Color measurement was conducted on storage days 1, 7, 14, and 28. The color of the PP surface of the raw and cooked patties was measured using a colorimeter (CR-210; Konica Minolta, Ltd., Osaka, Japan). Calibration of the colorimeter was adjusted using a white plate ($L^* = +97.27$, $a^* = +5.21$, $b^* = -3.40$) followed by the color was presented as L^* (lightness), a^* (redness), and b^* (yellowness). The samples were measured eight times during each storage period, respectively.

2.7. Microbiological evaluation of PP

The microbiological evaluation of PP was conducted on days 1, 7, 14, and 28 of storage. Raw patty (20 g) was mixed with 18 mL of 0.1 % peptone water for 2 min using a stomacher (Masticator Paddle Blender; IUL Instrument, Barcelona, Spain). The mixture was serially diluted with 0.1 % peptone water, and 100 μL of the diluted mixture was spread onto TVC, *E. coli*, coliform, and yeast and mold count plate. TVC, *E. coli,* and coliforms count plates were incubated at 37 °C for 24 h, and molds and yeasts count plate was incubated at 25 °C for 5 days. The results are expressed as log CFU/g. Samples were measured three times during each storage period, respectively.

2.8. Volatile basic nitrogen (VBN) of PP

The VBN (mg%) content of PP was determined on storage days 1, 7, 14, and 28 using the Conway microdiffusion methods as described previously (Cho et al., 2023). The cooked patty (5 g) was mixed with distilled water (20 mL) and homogenized at 10,000 rpm for 1 min using a homogenizer (HG-15 A; DAIHAN Scientific Co., Ltd., Gangwon, Korea). After homogenization, distilled water was added to the mixture until the volume reached 50 mL. The mixture was centrifuged at 3000 ×*g* for 10 min at 10 °C, and the supernatant was filtered using Whatman No. 1 filter paper. One milliliter of the filtered sample and 1 mL of a 50 % K_2CO_3 solution were added to the outer section of the Conway microdiffusion cells. Then, in the inner section of the Conway microdiffusion cells, 1 mL of 0.01 N H_3BO_3 and 100 μL of Conway reagent (1:1 = 0.066 % bromocresol green in ethanol:0.066 % methyl red in ethanol) were added. The cells were incubated at 37 °C for 90 min. After 90 min, the solution in the inner section was titrated with 0.02 N H_2SO_4 solution. Samples were measured three times during each storage period, respectively.

2.9. Lipid oxidation of PP

Lipid oxidation in the PP on days 1, 7, 14, and 28 of storage was quantified the production of thiobarbituric acid-reactive substances (TBARS) as previously described (Kim et al., 2023). Cooked patty (10 g) was added to distilled water (50 mL) with the addition of 0.2 mL of 0.3 % butylated hydroxytoluene and homogenized at 10,000 rpm for 1 min using a homogenizer (Nissei Co., Ltd., Tokyo, Japan). Then, the mixture was transferred to a distillation flask and was added to 47.5 mL of distilled water, 2.5 mL of 4 N HCl solution, and 1 mL of antifoam agent. The mixture was distilled until 30 mL of distillate was collected. Five milliliters of distillate was mixed with 5 mL of TBA reagent (0.02 M in 90 % acetic acid) in a tube and heated at 95 °C for 30 min. After cooling for 10 min, the absorbance was measured at 538 nm using a UV/VIS spectrophotometer (Optizen 2120 UV Plus, Mecasys Co., Ltd., Daejeon, Korea) to quantify the TBARS production. Samples were measured thrice on days 1, 7, 14, and 28.

2.10. Water holding capacity (WHC), cooking yield, and emulsion stability of PP

WHC was determined on days 1, 7, 14, and 28 of storage using a previously established method (Han, Keum, Hong, Kim and Han, 2023). Ten grams of the raw patty were placed in a conical tube and centrifuged at 6000 ×*g* for 15 min at 10 °C. Then, the samples were heated in a water bath for 15 min at 85 °C and cooled to room temperature for 1 h. Subsequently, the samples were centrifuged at 6000 ×*g* for 15 min at 10 °C, and the supernatant was extracted using a pipette. Samples were measured thrice on days 1, 7, 14, and 28, and WHC was calculated using the eq. (2).

$$\text{WHC}\,(\%) = [1 - (\text{weight of the empty tube}\,(g) + \text{weight of the patty}\,(g) - \text{weight of the patty and tube after heating and centrifugation}\,(g))/\text{water content in the raw patty})] \times 100 \quad (2)$$

The cooking yield was measured on days 1, 7, 14, and 28 of storage by calculating the weight difference between the raw and cooked patties. The patty was cooked following the same procedure mentioned in section 2.5 and cooled to room temperature for 30 min. Samples were measured thrice on days 1, 7, 14, and 28, and the percentage of cooking yield was calculated using the eq. (3).

$$\text{Cooking yield}\,(\%) = [\text{weight of a cooked patty}/\text{weight of a raw patty}] \times 100 \quad (3)$$

Emulsion stability was measured on storage days 1, 7, 14, and 28. Twenty-five grams of raw patty were filled in a 50 mL conical tube followed by centrifugation at 5000 ×*g* for 5 min at 10 °C. Samples were heated in a water bath at 80 °C for 30 min, and then cooled to room temperature for 30 min. The fluid exudated from the patty was transferred to an aluminum-weighing dish. The dishes containing the fluid were weighed, and water was removed by drying in an oven at 105 °C for 24 h. The dishes were cooled to room temperature for 30 min and weighed again. Samples were measured three times on days 1, 7, 14, and 28, and the total fluid, water, and fat exudations were calculated using the eq. (4), (5), and (6).

$$\text{Total fluid exudation}\,(\%) = (\text{weight of initial released fluid}\,(g)/\text{weight of raw patty}\,(g)) \times 100 \quad (4)$$

$$\text{Water exudation (\%)} = [(\text{weight of initial released liquid (g)} + \text{weight of dish (g)} - \text{weight of the dish after drying (g)})/\text{weight of raw patty (g)}] \times 100 \quad (5)$$

$$\text{Fat exudation (\%)} = [(\text{weight of the dish after drying (g)} - \text{weight of the empty dish (g)})/\text{weight of raw patty (g)}] \times 100 \quad (6)$$

2.11. Texture profile analysis (TPA) of PP

The TPA of PP was performed on days 1, 7, 14, and 28 of storage using a texture analyzer (TA-XT plus, Stable Micro Systems Ltd., Surrey, Goldaming, UK) equipped with a 40 mm cylinder probe (P/40). The cooked patty was cooled to room temperature for 30 min and cut into 1.5 × 1.5 × 1 cm pieces. The texture parameters (hardness, springiness, cohesiveness, chewiness, and gumminess) of the sample were measured under the following conditions: pre-test speed 2.0 mm/s; test speed 1.0 mm/s; post-test speed 1.0 mm/s; force 5 g. Samples were measured eight times on days 1, 7, 14, and 28.

2.12. Rheological properties of IPP gel supplemented with GE

To prepare the IPP gel samples, the ingredients were blended according to the specified formulation (Table S2) and stirred for 30 min. Subsequently, the samples were heated in a water bath at 90 °C until the internal temperature reached 80 °C and cooled to room temperature for 1 h. The IPP gel samples were divided into the following groups: IPP gel supplemented with 1.0 % GWE (*w*/w) (IG-GWE), IPP gel supplemented with 1.0 % GEE (w/w) (IG-GEE), and IPP gel supplemented with 1.0 % tannin (w/w) (IG-TA). The rheology of samples was measured using a rheometer (MCR 92, Anton Paar, Graz, Austria) equipped with a 25 mm-diameter parallel plate. The sample was loaded onto a static plate, and a 1 mm gap was maintained between the plates. The apparent viscosity was measured at 25 °C with shear rates of 0.1–100 1/s. The linear viscoelasticity region was determined by conducting strain sweep tests under the following conditions: strain ranging from 0.01 % to 10 % and a frequency of 1 Hz. The frequency sweep test was performed under the following conditions: temperature, 25 °C; frequency, 0.1–100 rad/s; strain, 1.0 %. At each test, the storage modulus (G') and loss modulus (G'') were measured. Samples were measured triplicate, respectively.

2.13. Fourier transform-infrared (FT-IR) spectroscopy of IPP gel supplemented with GE

The functional groups of the IPP gels were measured using an FT-IR spectrophotometer (FT/IR-4700; JASCO, Tokyo, Japan). The spectral data were characterized under the following conditions: wavelength, 500–4000 cm^{-1}; resolution, 1 cm^{-1}. To analyze the secondary structure, the band at 1600–1700 cm^{-1} was deconvoluted using PeakFit 4.12 software (SeaSolve Software Inc., Framingham, USA). The secondary structure content of IPP gels was quantified as the Gaussian area under the curve of each associated peak [β-sheet (1615–1640 cm^{-1}, 1690–1700 cm^{-1}), α-helix (1650–1665 cm^{-1}), β-turn (1665–1690 cm^{-1}), and random coil (1640–1650 cm^{-1})] according to Rolandelli et al. (2024) and Salgado et al. (2023).

2.14. Sensory evaluation

Sensory evaluation of PP was conducted on day 1 of storage following our previously described method (Han, Keum, Hong, Kim and Han, 2023). Twelve trained panelists (eight males and four females, aged 26–31 years) participated in the sensory evaluation of the PP. The training, accompanied by introducing patties in the initial sessions to acquaint the panelists with specific characteristics, including umami and off-flavor, needed to be evaluated. The panelists received training using identical PP in three sessions lasting 20–30 min each over the course of two weeks before the formal sensory evaluations. The sample was cut

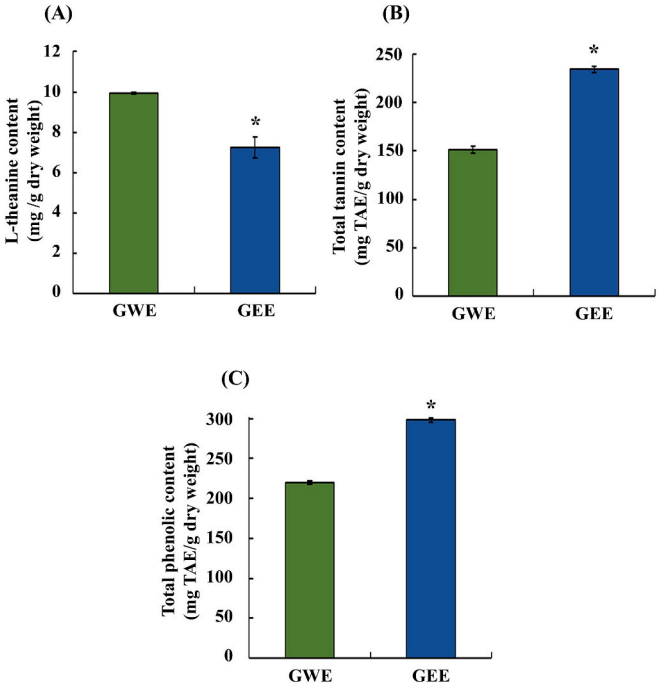

Fig. 1. (A) L-theanine content (mg/g dry weight), (B) total tannin content (mg TAE/g dry weight), and (C) total phenolic content (mg TAE/g dry weight) of green tea extracts. The L-theanine content was measured using HPLC, while the total tannin and total phenolic contents were determined using the PVPP/Folin–Ciocalteu method. GWE: green tea water extract, GEE: green tea 70 % ethanol extract. * indicates a significant difference compared with GWE ($P < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
pH of plant-based patties supplemented with green tea extracts during 28 days of refrigerated storage.

Parameter	Treatment	Storage period (day)			
		1	7	14	28
pH of raw patties	Control	6.66 ± 0.00^{Ba}	6.65 ± 0.01^{Ba}	6.67 ± 0.03^{Ba}	6.70 ± 0.02^{Aa}
	PP-GWE0.5	6.59 ± 0.01^{Bb}	6.59 ± 0.01^{Bc}	6.59 ± 0.01^{Bc}	6.63 ± 0.01^{Abc}
	PP-GWE1.0	6.54 ± 0.00^{Cd}	6.58 ± 0.01^{Bd}	6.59 ± 0.01^{Ac}	6.59 ± 0.01^{Ad}
	PP-GWE1.5	6.47 ± 0.00^{Bg}	6.49 ± 0.01^{Bf}	6.53 ± 0.02^{Ad}	6.54 ± 0.02^{Ae}
	PP-GEE0.5	6.57 ± 0.00^{Dc}	6.60 ± 0.01^{Cb}	6.62 ± 0.01^{Bb}	6.64 ± 0.02^{Ab}
	PP-GEE1.0	6.53 ± 0.00^{De}	6.55 ± 0.00^{Ce}	6.58 ± 0.00^{Bc}	6.62 ± 0.02^{Ac}
	PP-GEE1.5	6.49 ± 0.00^{Bf}	6.49 ± 0.00^{Bf}	6.48 ± 0.01^{Be}	6.51 ± 0.01^{Af}
pH of cooked patties	Control	6.50 ± 0.04$^{Ca2)}$	6.55 ± 0.03$^{Ba3)}$	6.55 ± 0.02^{Ba}	6.63 ± 0.01^{Aa}
	PP-GWE0.5	6.38 ± 0.00^{Db}	6.44 ± 0.01^{Cb}	6.51 ± 0.02^{Bc}	6.55 ± 0.01^{Ac}
	PP-GWE1.0	6.31 ± 0.03^{Dd}	6.42 ± 0.00^{Cbc}	6.47 ± 0.00^{Bd}	6.53 ± 0.01^{Ad}
	PP-GWE1.5	6.29 ± 0.00^{Cde}	6.37 ± 0.03^{Be}	6.42 ± 0.01^{Af}	6.43 ± 0.01^{Af}
	PP-GEE0.5	6.35 ± 0.00^{Dc}	6.44 ± 0.00^{Cb}	6.53 ± 0.01^{Bb}	6.58 ± 0.02^{Ab}
	PP-GEE1.0	6.29 ± 0.00^{Dde}	6.41 ± 0.02^{Cd}	6.48 ± 0.01^{Bd}	6.52 ± 0.01^{Ad}
	PP-GEE1.5	6.27 ± 0.00^{De}	6.34 ± 0.00^{Cf}	6.44 ± 0.00^{Be}	6.50 ± 0.01^{Ae}

Control: plant-based patty without green tea extract, PP-GWE0.5: plant-based patty supplemented with green tea water extract 0.5 %, PP-GWE1.0: plant-based patty supplemented with green tea water extract 1.0 %, PP-GWE1.5: plant-based patty supplemented with green tea water extract 1.5 %, PP-GEE0.5: plant-based patty supplemented with green tea 70 % ethanol extract 0.5 %, PP-GEE1.0: plant-based patty supplemented with green tea 70 % ethanol extract 1.0 %, PP-GEE1.5: plant-based patty supplemented with green tea 70 % ethanol extract 1.5 %.
A–D Means values in the same row are significantly different ($P < 0.05$).
a–f Means values in the same column are significantly different ($P < 0.05$).
Data are presented as mean ± standard deviation ($n = 8$).

into 1.5 × 1.5 × 1 cm pieces, and a 3-digit random number was assigned to each sample. After evaluating each sample, panelists cleaned their palates with water. Subsequently, the samples were assessed using a 9-point scale for off-flavor intensity (1 = not at all, 9 = extremely strong), umami intensity (1 = not at all, 9 = extremely strong), appearance, taste, flavor, firmness, and overall acceptability (1 = extremely dislike, 9 = extremely like). The Institutional Review Board approved the procedure for sensory evaluation (7001355–202,309-HR-694). All participants provided informed consent at the beginning of the study for their data to be used and analyzed.

2.15. Statistical analysis

All data are presented as mean ± standard deviation. Statistical analyses were performed using the SPSS-PASW statistics software (version 22.0; SPSS Inc., Chicago, IL, USA). Except for the measurement of L-theanine and tannin content and sensory evaluation, which were conducted using one-way analysis of variance (ANOVA), statistical significance was analyzed using two-way ANOVA. Duncan's multiple range post-hoc test or independent two-sample *t*-test was used to identify significant differences ($P < 0.05$) between groups.

Table 2
Microorganisms in plant-based patties supplemented with green tea extracts during 28 days of refrigerated storage.

Parameter (Log CFU/g)	Treatment	Storage period (day)			
		1	7	14	28
TVC	Control	2.98 ± 0.05^{Ca}	2.95 ± 0.05^{Ca}	3.87 ± 0.03^{Ba}	4.02 ± 0.03^{Aa}
	PP-GWE0.5	2.86 ± 0.05^{Cb}	2.85 ± 0.05^{Cb}	3.83 ± 0.01^{Ba}	3.96 ± 0.01^{Ab}
	PP-GWE1.0	2.85 ± 0.01^{Cb}	2.86 ± 0.01^{Cb}	3.68 ± 0.03^{Bb}	3.91 ± 0.01^{Ac}
	PP-GWE1.5	2.83 ± 0.03^{Cb}	2.85 ± 0.03^{Cbc}	3.64 ± 0.03^{Bb}	3.87 ± 0.01^{Ad}
	PP-GEE0.5	2.78 ± 0.01^{Cc}	2.79 ± 0.01^{Cc}	3.86 ± 0.03^{Ba}	3.94 ± 0.04^{Ab}
	PP-GEE1.0	2.73 ± 0.02^{Cc}	2.75 ± 0.02^{Cc}	3.45 ± 0.03^{Bc}	3.62 ± 0.07^{Ae}
	PP-GEE1.5	2.78 ± 0.02^{Cc}	2.80 ± 0.02^{Cc}	3.48 ± 0.03^{Bc}	3.66 ± 0.03^{Ae}
E. coli	Control	N.D.	N.D.	N.D.	N.D.
	PP-GWE0.5	N.D.	N.D.	N.D.	N.D.
	PP-GWE1.0	N.D.	N.D.	N.D.	N.D.
	PP-GWE1.5	N.D.	N.D.	N.D.	N.D.
	PP-GEE0.5	N.D.	N.D.	N.D.	N.D.
	PP-GEE1.0	N.D.	N.D.	N.D.	N.D.
	PP-GEE1.5	N.D.	N.D.	N.D.	N.D.
Coliform	Control	N.D.	N.D.	N.D.	N.D.
	PP-GWE0.5	N.D.	N.D.	N.D.	N.D.
	PP-GWE1.0	N.D.	N.D.	N.D.	N.D.
	PP-GWE1.5	N.D.	N.D.	N.D.	N.D.
	PP-GEE0.5	N.D.	N.D.	N.D.	N.D.
	PP-GEE1.0	N.D.	N.D.	N.D.	N.D.
	PP-GEE1.5	N.D.	N.D.	N.D.	N.D.
Yeast and mold	Control	N.D.	N.D.	0.82 ± 0.58	0.82 ± 0.58
	PP-GWE0.5	N.D.	N.D.	0.82 ± 0.58	0.82 ± 0.58
	PP-GWE1.0	N.D.	N.D.	N.D.	0.52 ± 0.58
	PP-GWE1.5	N.D.	N.D.	0.52 ± 0.58	0.52 ± 0.58
	PP-GEE0.5	N.D.	N.D.	0.52 ± 0.58	1.00 ± 0.00
	PP-GEE1.0	N.D.	N.D.	N.D.	N.D.
	PP-GEE1.5	N.D.	N.D.	N.D.	N.D.

Control: plant-based patty without green tea extract, PP-GWE0.5: plant-based patty supplemented with green tea water extract 0.5 %, PP-GWE1.0: plant-based patty supplemented with green tea water extract 1.0 %, PP-GWE1.5: plant-based patty supplemented with green tea water extract 1.5 %, PP-GEE0.5: plant-based patty supplemented with green tea 70 % ethanol extract 0.5 %, PP-GEE1.0: plant-based patty supplemented with green tea 70 % ethanol extract 1.0 %, PP-GEE1.5: plant-based patty supplemented with green tea 70 % ethanol extract 1.5 %.
A–C Means values in the same row are significantly different ($P < 0.05$).
a–e Means values in the same column are significantly different ($P < 0.05$).
N.D. means non-detection of microorganisms.
Data are presented as mean ± standard deviation ($n = 3$).

3. Results and discussion

3.1. L-theanine and tannin contents of GE

The L-theanine and tannin contents of GE may contribute to the unique flavors and overall physicochemical properties in the PP. The L-theanine, total tannin, and total phenolic content of GE are presented in Fig. 1. GWE had a significantly higher L-theanine content (9.94 mg/g dry weight) than GEE (7.23 mg/g dry weight) ($P < 0.05$, Fig. 1A). However, as shown in Fig. 1B and C, GEE exhibited higher tannin

Table 3
Volatile basic nitrogen (VBN) and thiobarbituric acid-reactive substances (TBARS) values of plant-based patties supplemented with green tea extracts during 28 days of refrigerated storage.

Parameter	Treatment	Storage period (day)			
		1	7	14	28
VBN (mg%)	Control	3.74 ± 0.65^{C}	5.04 ± 0.00^{Ba}	5.79 ± 0.32^{ABa}	6.35 ± 0.65^{Aa}
	PP-GWE0.5	3.74 ± 0.65^{B}	4.86 ± 0.32^{Aa}	5.23 ± 0.65^{Aab}	5.60 ± 0.00^{Ab}
	PP-GWE1.0	3.36 ± 0.00^{C}	4.67 ± 0.32^{Ba}	5.04 ± 0.00^{ABbc}	5.42 ± 0.32^{Ab}
	PP-GWE1.5	3.74 ± 0.65^{B}	4.48 ± 0.00^{Aa}	4.86 ± 0.32^{Abc}	5.23 ± 0.32^{Ab}
	PP-GEE0.5	3.36 ± 0.00^{B}	4.30 ± 0.65^{ABab}	4.48 ± 0.56^{Acd}	5.04 ± 0.56^{Ac}
	PP-GEE1.0	2.99 ± 0.65^{B}	3.74 ± 0.32^{Abc}	4.11 ± 0.32^{Ad}	4.48 ± 0.00^{Ac}
	PP-GEE1.5	3.36 ± 0.00^{B}	3.55 ± 0.65^{Bc}	3.92 ± 0.00^{Bd}	4.67 ± 0.32^{Ac}
TBARS value (mg MDA/kg)	Control	0.95 ± 0.01$^{Da2)}$	1.05 ± 0.00$^{Ca3)}$	1.24 ± 0.00^{Ba}	1.45 ± 0.02^{Aa}
	PP-GWE0.5	0.85 ± 0.00^{Db}	1.04 ± 0.00^{Ca}	1.14 ± 0.00^{Bb}	1.33 ± 0.01^{Ab}
	PP-GWE1.0	0.79 ± 0.01^{Dc}	1.00 ± 0.01^{Cb}	1.05 ± 0.00^{Bc}	1.24 ± 0.02^{Ac}
	PP-GWE1.5	0.76 ± 0.00^{Dd}	1.00 ± 0.00^{Cb}	1.04 ± 0.00^{Bc}	1.24 ± 0.02^{Ac}
	PP-GEE0.5	0.76 ± 0.01^{Dd}	1.01 ± 0.01^{Cb}	1.05 ± 0.01^{Bc}	1.22 ± 0.02^{Ac}
	PP-GEE1.0	0.72 ± 0.01^{De}	0.95 ± 0.00^{Cc}	0.98 ± 0.01^{Bd}	1.19 ± 0.01^{Ad}
	PP-GEE1.5	0.68 ± 0.00^{Df}	0.95 ± 0.01^{Cc}	0.98 ± 0.00^{Bd}	1.19 ± 0.02^{Ad}

Control: plant-based patty without green tea extract, PP-GWE0.5: plant-based patty supplemented with green tea water extract 0.5 %, PP-GWE1.0: plant-based patty supplemented with green tea water extract 1.0 %, PP-GWE1.5: plant-based patty supplemented with green tea water extract 1.5 %, PP-GEE0.5: plant-based patty supplemented with green tea 70 % ethanol extract 0.5 %, PP-GEE1.0: plant-based patty supplemented with green tea 70 % ethanol extract 1.0 %, PP-GEE1.5: plant-based patty supplemented with green tea 70 % ethanol extract 1.5 %.
A–C Means values in the same row are significantly different ($P < 0.05$).
a–e Means values in the same column are significantly different ($P < 0.05$).
Data are presented as mean ± standard deviation (n = 3).

content (234.63 mg/g dry weight) than GWE (151.22 mg/g dry weight). TPC was also higher in GEE than GWE. The proportion of tannins in total polyphenols was 78.67 % in GEE, while the proportion was 68.73 % in GWE. Since L-theanine is a water-soluble compound, it was present in high concentrations in GWE. Conversely, plant polyphenols are more effectively extracted with organic solvents, resulting in higher concentrations in GEE. Consistent with our data, a previous study showed that green tea water extract exhibited higher theanine content and lower TPC than green tea 70 % ethanol extract (Lin et al., 2012). Taken together, green tea leaves extracted with water showed higher L-theanine content, whereas those extracted with 70 % ethanol displayed higher tannin and total phenolic content.

3.2. pH and color of PP

The pH of PP was shown in Table 1. The addition of GE resulted in a decrease in the pH of the patties before and after cooking ($P < 0.05$). The pH values of GWE and GEE were 5.48 and 5.31, respectively. It appears that the addition of GE to patty led to a decrease in pH value. Upon incorporating polyphenols into food, the pH decreased and the growth of microorganisms was suppressed, thereby inhibiting the formation of basic nitrogen compounds (Cao et al., 2013). During the storage period, the pH of both raw and cooked PP increased ($P < 0.05$). The rise in pH can be attributed to the proliferation of microorganisms that break down proteins into alkaline ammonia and utilize low molecular weight compounds (Masniyom et al., 2002).

The color and visual appearance of PP were exhibited in Table S3, Table S4, and Fig. S1. Before cooking, the L^* and a^* values of PP decreased with the addition of GE, whereas the b^* values increased ($P < 0.05$, Table S3). In addition, there was an increase in L^* values for all raw patties on days 14 and 28 ($P < 0.05$). In cooked patties, the GE addition showed decreased L^*, a^*, and b^* values (Table S4). The color of meat was changed during cooking process, and in the case of beef, all of the L^*, a^*, and b^* values were generally decreased (Ball et al., 2021). Hence, our data indicated that the incorporation of GE improved the appearance of cooked PP by imitating a meat-like color change during the cooking process.

3.3. Microbiological evaluation of PP

The TVC, *E. coli* count, coliforms count, and yeasts and molds were shown in Table 2. The TVC of all samples increased as the storage period progressed ($P < 0.05$). Compared with the control, GE-supplemented PP exhibited a decreased TVC ($P < 0.05$). For all storage periods, PP-GEE1.0 and PP-GEE1.5 showed significantly lower TVC levels than those of PP-GWE1.0 and PP-GWE1.5 ($P < 0.05$). GEE has a high concentration of polyphenols, which are known for their antibacterial properties (Haminiuk, Plata-Oviedo, de Mattos, Carpes and Branco, 2014). These compounds are primarily nonpolar substances. Throughout the storage period, *E. coli* and coliforms, which are pivotal indicators of food hygiene, were absent in PP. However, it is noteworthy that yeasts and molds, employed to evaluate the food safety of plant-derived products (Tóth et al., 2021), were observed in small quantities in several PP groups on day 14. However, yeasts and molds were not detected in PP-GEE1.0 and PP-GEE1.5 during the storage periods. Taken together, the addition of GE appeared to reduce microbial contamination in PP by inhibiting the growth of microorganisms, with PP-GEE1.0 and PP-GEE1.5 demonstrating the most promising results.

3.4. VBN and TBARS value of PP

VBN was generated by protein deterioration during food processing or storage; thus, it was considered an indicator of the freshness of protein-based food products. As shown in Table 3, the VBN of PP during storage was significantly influenced by the GE concentration and extraction solvent ($P < 0.05$). GE did not change the VBN content on day 1 ($P > 0.05$); however, there was an increase in VBN content after 28 days, which was attenuated by GE, particularly in GEE-supplemented patties. There was no marked difference between PP-GEE1.0 and PP-GEE1.5 during the storage periods. Protein degradation occurs primarily due to the growth of microorganisms (Jin et al., 2018). As described in Table 2, the polyphenols in GE prevented the growth of microorganisms. The type and content of extracted polyphenols can vary depending on the choice of extraction solvent (Pasrija & Anandharamakrishnan, 2015). Therefore, PP-GEE1.0 and PP-GEE1.5 might have a suitable type and content of polyphenols to effectively control the VBN of PP during refrigerated storage.

Lipid oxidation in food may occur because of compromised oxidative stability, resulting in the degradation of nutritional value, flavor, texture, and appearance and concurrently diminishing the shelf life of products (Woo et al., 2023). TBARS values were determined to quantify the formation of secondary oxidation products, such as malondialdehyde, alkenals, and alkadienals (Shin et al., 2021). Throughout the storage periods, there was a notable increase in the TBARS values (Table 3, $P < 0.05$). However, it was noteworthy that PP-GEE1.0 and PP-GEE1.5 showed significantly lower TBARS values than the other groups throughout the storage periods ($P < 0.05$). GE, renowned for its high polyphenol content and antioxidant properties, has been reported to decrease the TBARS value in pork sausage (Jayawardana et al., 2019). Moreover, tannin demonstrated a more significant decrease in the

Table 4
Water holding capacity (WHC), cooking yield, and emulsion stability of plant-based patties supplemented with green tea extracts during 28 days of refrigerated storage.

Parameter	Treatment	Storage period (day)			
		1	7	14	28
WHC (%)	Control	89.38 ± 0.81^{bc}	89.61 ± 1.24^{b}	89.79 ± 0.33^{b}	88.58 ± 0.34^{b}
	PP-GWE0.5	89.35 ± 1.13^{bc}	89.93 ± 1.24^{b}	89.92 ± 0.66^{b}	88.73 ± 0.52^{b}
	PP-GWE1.0	90.98 ± 1.03^{ABa}	91.68 ± 0.15^{Aa}	91.45 ± 0.29^{ABa}	90.30 ± 0.77^{Ba}
	PP-GWE1.5	88.92 ± 1.14^{c}	89.12 ± 0.53^{b}	89.53 ± 0.41^{b}	88.45 ± 0.47^{b}
	PP-GEE0.5	89.34 ± 1.23^{bc}	90.64 ± 0.74^{ab}	89.98 ± 0.28^{b}	89.28 ± 1.05^{ab}
	PP-GEE1.0	91.29 ± 1.03^{a}	92.03 ± 0.63^{a}	91.85 ± 0.75^{a}	90.58 ± 1.00^{a}
	PP-GEE1.5	88.88 ± 1.43^{c}	89.50 ± 1.41^{b}	90.05 ± 0.26^{b}	88.67 ± 0.50^{b}
Cooking yield (%)	Control	85.08 ± 0.42^{Ad}	84.72 ± 0.64^{Ab}	82.84 ± 0.82^{Bc}	83.34 ± 0.83^{Bc}
	PP-GWE0.5	86.33 ± 0.60^{Abc}	85.38 ± 0.29^{ABb}	84.74 ± 0.96^{Bab}	84.49 ± 0.79^{Babc}
	PP-GWE1.0	87.31 ± 1.50^{Aa}	86.75 ± 1.01^{ABa}	85.10 ± 0.39^{Bab}	85.53 ± 0.60^{ABa}
	PP-GWE1.5	85.74 ± 1.40^{c}	85.89 ± 0.96^{ab}	84.95 ± 0.73^{ab}	85.08 ± 0.82^{ab}
	PP-GEE0.5	86.16 ± 0.70^{Abc}	85.84 ± 0.37^{Aab}	83.82 ± 0.40^{Bbc}	83.99 ± 0.62^{Bbc}
	PP-GEE1.0	87.10 ± 0.73^{a}	86.77 ± 0.50^{a}	85.41 ± 1.44^{a}	85.57 ± 0.46^{a}
	PP-GEE1.5	86.21 ± 1.03^{Abc}	84.90 ± 0.42^{Bb}	84.20 ± 0.28^{Bb}	84.16 ± 0.36^{Bbc}
Total fluid exudation (%)	Control	0.62 ± 0.10^{Ba}	2.41 ± 0.02^{Aa}	2.36 ± 0.26^{Aa}	2.62 ± 0.08^{Aa}
	PP-GWE0.5	0.40 ± 0.03^{Cb}	1.53 ± 0.10^{Bb}	1.47 ± 0.22^{Bbc}	1.96 ± 0.28^{Ab}
	PP-GWE1.0	0.27 ± 0.02^{Cc}	0.80 ± 0.29^{BCe}	1.23 ± 0.55^{ABbc}	1.70 ± 0.34^{Ab}
	PP-GWE1.5	0.44 ± 0.04^{Db}	0.99 ± 0.03^{Cde}	2.21 ± 0.19^{Ba}	2.45 ± 0.18^{Aa}
	PP-GEE0.5	0.46 ± 0.03^{Ba}	1.29 ± 0.07^{Ac}	1.51 ± 0.42^{Abc}	1.59 ± 0.37^{Ab}
	PP-GEE1.0	0.24 ± 0.03^{Cc}	0.26 ± 0.07^{Cf}	0.91 ± 0.10^{Bc}	1.46 ± 0.34^{Ab}
	PP-GEE1.5	0.40 ± 0.04^{Cb}	1.08 ± 0.10^{Bcd}	1.77 ± 0.34^{Aab}	1.90 ± 0.05^{Ab}
Water exudation (%)	Control	0.25 ± 0.05^{a}	0.27 ± 0.07^{a}	0.30 ± 0.04^{a}	0.37 ± 0.08^{a}
	PP-GWE0.5	0.07 ± 0.03^{Bb}	0.05 ± 0.01^{Bb}	0.06 ± 0.05^{Bbc}	0.27 ± 0.04^{Aab}
	PP-GWE1.0	0.05 ± 0.02^{Bb}	0.03 ± 0.01^{Bb}	0.03 ± 0.01^{Bc}	0.19 ± 0.09^{Abcd}
	PP-GWE1.5	0.07 ± 0.02^{Bb}	0.04 ± 0.01^{Bb}	0.08 ± 0.01^{Bbc}	0.21 ± 0.06^{Abc}
	PP-GEE0.5	0.09 ± 0.01^{b}	0.08 ± 0.03^{b}	0.12 ± 0.07^{b}	0.12 ± 0.05^{cd}
	PP-GEE1.0	0.04 ± 0.02^{Bb}	0.02 ± 0.01^{Bb}	0.03 ± 0.01^{Bc}	0.09 ± 0.03^{Ad}
	PP-GEE1.5	0.07 ± 0.03^{Bb}	0.04 ± 0.02^{Bb}	0.04 ± 0.02^{Bc}	0.13 ± 0.02^{Acd}
Fat exudation (%)	Control	0.37 ± 0.05^{Ba}	2.15 ± 0.09^{Aa}	2.09 ± 0.27^{Aa}	2.25 ± 0.16^{Aa}
	PP-GWE0.5	0.33 ± 0.01^{Ba}	1.47 ± 0.05^{Ab}	1.41 ± 0.19^{Abc}	1.68 ± 0.24^{Ab}
	PP-GWE1.0	0.22 ± 0.04^{Cb}	0.76 ± 0.30^{BCe}	1.18 ± 0.54^{ABbc}	1.52 ± 0.30^{Ab}
	PP-GWE1.5	0.38 ± 0.04^{Ca}	0.94 ± 0.03^{Bde}	2.13 ± 0.19^{Aa}	2.24 ± 0.24^{Aa}
	PP-GEE0.5	0.37 ± 0.03^{Ba}	1.21 ± 0.05^{Ac}	1.39 ± 0.35^{Abc}	1.47 ± 0.32^{Ab}
	PP-GEE1.0	0.20 ± 0.02^{Cb}	0.24 ± 0.01^{Cf}	0.88 ± 0.11^{Bc}	1.37 ± 0.31^{Ab}
	PP-GEE1.5	0.33 ± 0.06^{Da}	1.04 ± 0.09^{Ccd}	1.48 ± 0.23^{Bb}	1.77 ± 0.03^{Ab}

Control: plant-based patty without green tea extract, PP-GWE0.5: plant-based patty supplemented with green tea water extract 0.5 %, PP-GWE1.0: plant-based patty supplemented with green tea water extract 1.0 %, PP-GWE1.5: plant-based patty supplemented with green tea water extract 1.5 %, PP-GEE0.5: plant-based patty supplemented with green tea 70 % ethanol extract 0.5 %, PP-GEE1.0: plant-based patty supplemented with green tea 70 % ethanol extract 1.0 %, PP-GEE1.5: plant-based patty supplemented with green tea 70 % ethanol extract 1.5 %.
A–D Means values in the same row are significantly different ($P < 0.05$).
a–f Means values in the same column are significantly different ($P < 0.05$).
Data are presented as mean ± standard deviation (n = 3).

peroxide value (primary oxidation products) and the TBARS value than catechin, attributed to the interaction between tannin and pea protein (Soendjaja & Girard, 2024). The ethanol extract of green tea exhibited a higher tannin content compared to the water extract of green tea. Consequently, GEE demonstrated increased resistance to lipid oxidation, potentially attributable to its elevated tannin levels compared with GWE. This suggests that GEE could be a more suitable choice for PP during refrigerated storage.

3.5. WHC, cooking yield, and emulsion stability of PP

WHC, cooking yield, and emulsion stability (total fluid, water, and fat exudation) of PP were shown in Table 4. In the WHC results of day 1, the addition of 1.0 % GWE or GEE to patties induced a significant increase in WHC compared with the control ($P < 0.05$). Meanwhile, the 1.5 % addition of GWE or GEE negatively affected the WHC. During storage, WHC decreased slightly. The increase in WHC by the 1.0 % GE resulted from the phenolic compounds in the GE (Passos et al., 2022). Polyphenols can interact with proteins via hydrogen bond, hydrophobic interaction, and covalent bonds, which can improve their thermal stability (Quan et al., 2019). Nevertheless, the decrease in WHC by 1.5 % GWE or GEE was assumed to be due to the hindrance of protein-protein interactions by the excessive polyphenol content in the extract. Protein-protein interaction is an important factor for the stable formation of heat-induced gel. In a previous study, the addition of high concentration of GE disturbed disulfide bond formation between proteins in meat emulsion during heating, resulted in reduced WHC and emulsion stability (Jongberg et al., 2015).

The cooking yield of PP was increased with GE addition, and 1.0 % addition resulted in a significantly higher cooking yield than the other concentrations ($P < 0.05$). After 14 day of storage, the cooking yield of PP without GE and with 0.5 % GE were a significant decrease, compared to the on day 1. However, the cooking yield of patties treated with 1.0 % GEE was retained for 28 days ($P > 0.05$). The superior cooking yield of PP with 1.0 % GEE appeared to be associated with higher WHC.

The total fluid exudation of PP was significantly reduced by the GE addition, except for PP-GEE0.5 ($P < 0.05$). Meanwhile, fat exudation was decreased only in the 1.0 % GE-added patties on day 1. During the storage periods, the total fluid exudation of the PP increased. The addition of 1.0 % GEE to the patties led to the lowest total fluid exudation for 28 days, which was highly related to the increased WHC and cooking yield. As described above, this might have resulted from increased protein-polyphenol interaction and especially polyphenols in the GEE had a superior effect. Therefore, 1.0 % GEE in PP was adequate to enhance the WHC, cooking yield, and emulsion stability.

3.6. Texture profile analysis of PP

TPA is necessary for the development of PP in terms of imitating the texture characteristics of meat. Table 5 showed the texture profiles of PP supplemented with GWE or GEE. During the storage periods, the texture parameters generally increased, except for cohesiveness ($P < 0.05$). On days 14 and 28, the GEE-added patty groups had significantly higher hardness values than the GWE group ($P < 0.05$). In a previous study, the

Table 5
Texture profile analysis of plant-based patties supplemented with green tea extracts during 28 days of refrigerated storage.

Parameter	Treatment	Storage period (day)			
		1	7	14	28
Hardness (N)	Control	7.63 ± 0.90^{Bd}	7.69 ± 0.39^{Bd}	10.87 ± 1.05^{Ad}	10.16 ± 1.14^{Ac}
	PP-GWE0.5	7.97 ± 0.55^{Bcd}	7.84 ± 1.26^{Bd}	12.10 ± 0.84^{Acd}	12.35 ± 0.92^{Ab}
	PP-GWE1.0	8.63 ± 0.37^{Bbc}	9.34 ± 0.78^{Bc}	12.68 ± 0.55^{Abc}	12.41 ± 0.83^{Ab}
	PP-GWE1.5	8.01 ± 0.58^{Bcd}	7.93 ± 0.81^{Bd}	12.05 ± 1.48^{Acd}	12.50 ± 0.89^{Ab}
	PP-GEE0.5	9.00 ± 1.74^{Cab}	11.59 ± 0.77^{Bb}	13.62 ± 1.39^{Aab}	13.78 ± 0.85^{Aa}
	PP-GEE1.0	9.60 ± 0.52^{Ba}	13.87 ± 0.97^{Aa}	14.71 ± 2.03^{Aa}	14.24 ± 1.12^{Aa}
	PP-GEE1.5	8.03 ± 0.43^{Bcd}	13.52 ± 1.46^{Aa}	14.56 ± 1.68^{Aa}	14.09 ± 1.45^{Aa}
Springiness	Control	0.69 ± 0.02^{Bc}	0.67 ± 0.02^{Bd}	0.75 ± 0.01^{Acd}	0.74 ± 0.02^{Ab}
	PP-GWE0.5	0.72 ± 0.01^{Bab}	0.71 ± 0.02^{Bc}	0.75 ± 0.02^{Ad}	0.74 ± 0.01^{Ab}
	PP-GWE1.0	0.74 ± 0.01^{a}	0.76 ± 0.03^{b}	0.74 ± 0.03^{d}	0.75 ± 0.04^{b}
	PP-GWE1.5	0.73 ± 0.02^{Ba}	0.76 ± 0.03^{Ab}	0.75 ± 0.01^{Ad}	0.75 ± 0.02^{ABb}
	PP-GEE0.5	0.73 ± 0.02^{Bab}	0.78 ± 0.03^{Aab}	0.78 ± 0.01^{Aab}	0.79 ± 0.02^{Aa}
	PP-GEE1.0	0.73 ± 0.01^{Bab}	0.79 ± 0.01^{Aa}	0.81 ± 0.04^{Aa}	0.80 ± 0.03^{Aa}
	PP-GEE1.5	0.72 ± 0.01^{Bb}	0.77 ± 0.01^{Aab}	0.78 ± 0.01^{Abc}	0.78 ± 0.02^{Aa}
Cohesiveness	Control	0.46 ± 0.02^{Ad}	0.44 ± 0.02^{ABc}	0.41 ± 0.03^{Bc}	0.43 ± 0.04^{ABcd}
	PP-GWE0.5	0.50 ± 0.02^{c}	0.51 ± 0.03^{ab}	0.51 ± 0.03^{ab}	0.50 ± 0.03^{c}
	PP-GWE1.0	0.54 ± 0.02^{ab}	0.53 ± 0.03^{a}	0.55 ± 0.02^{a}	0.54 ± 0.02^{ab}
	PP-GWE1.5	0.50 ± 0.04^{c}	0.50 ± 0.02^{b}	0.50 ± 0.02^{b}	0.51 ± 0.02^{c}
	PP-GEE0.5	0.54 ± 0.03^{ab}	0.52 ± 0.04^{ab}	0.53 ± 0.04^{ab}	0.52 ± 0.02^{bc}
	PP-GEE1.0	0.55 ± 0.02^{a}	0.54 ± 0.03^{a}	0.55 ± 0.04^{a}	0.56 ± 0.02^{a}
	PP-GEE1.5	0.52 ± 0.01^{c}	0.50 ± 0.03^{b}	0.50 ± 0.04^{b}	0.51 ± 0.03^{c}
Chewiness (N)	Control	2.83 ± 0.43^{Bd}	1.98 ± 0.23^{Ce}	3.52 ± 0.11^{Ad}	3.61 ± 0.33^{Ad}
	PP-GWE0.5	3.22 ± 0.25^{Bbcd}	2.67 ± 0.71^{Bd}	4.43 ± 0.58^{Ac}	4.43 ± 0.62^{Ac}
	PP-GWE1.0	3.68 ± 0.51^{Bab}	3.66 ± 0.56^{Bc}	4.94 ± 0.64^{Ac}	4.95 ± 0.62^{Ac}
	PP-GWE1.5	3.13 ± 0.72^{Bcd}	3.17 ± 0.72^{Bcd}	4.55 ± 0.68^{Ac}	4.52 ± 0.28^{Ac}
	PP-GEE0.5	3.49 ± 0.33^{Cabc}	4.94 ± 0.97^{Bb}	5.82 ± 0.88^{Aa}	5.83 ± 0.51^{Ab}
	PP-GEE1.0	3.81 ± 0.68^{Ca}	5.84 ± 0.70^{Ba}	6.59 ± 0.71^{Aa}	6.46 ± 0.63^{ABa}
	PP-GEE1.5	3.24 ± 0.31^{Bbcd}	5.52 ± 0.64^{Ab}	5.71 ± 0.67^{Ab}	5.52 ± 0.77^{Ab}
Gumminess (N)	Control	3.65 ± 0.56^{Bb}	3.21 ± 0.35^{Bd}	4.62 ± 0.29^{Ae}	4.73 ± 0.69^{Ac}
	PP-GWE0.5	4.30 ± 0.36^{Bab}	3.80 ± 0.62^{Cd}	6.06 ± 0.31^{Acd}	6.32 ± 0.50^{Ac}
	PP-GWE1.0	4.74 ± 0.76^{Ba}	4.65 ± 0.71^{Bc}	6.79 ± 0.47^{Abc}	6.66 ± 0.48^{Ac}
	PP-GWE1.5	4.42 ± 0.96^{Ba}	3.76 ± 0.44^{Bd}	5.92 ± 0.46^{Ad}	6.31 ± 0.42^{Ac}
	PP-GEE0.5	4.49 ± 0.73^{Ca}	5.45 ± 0.79^{Bb}	7.23 ± 0.88^{Ab}	7.45 ± 0.46^{Ab}
	PP-GEE1.0	4.93 ± 0.77^{Ca}	7.44 ± 0.77^{Ba}	8.25 ± 0.99^{Aa}	8.31 ± 0.81^{Aa}
	PP-GEE1.5	4.28 ± 0.19^{Bab}	7.21 ± 0.81^{Aa}	7.36 ± 1.40^{Ab}	7.37 ± 1.01^{Ab}

Control: plant-based patty without green tea extract, PP-GWE0.5: plant-based patty supplemented with green tea water extract 0.5 %, PP-GWE1.0: plant-based patty supplemented with green tea water extract 1.0 %, PP-GWE1.5: plant-based patty supplemented with green tea water extract 1.5 %, PP-GEE0.5: plant-based patty supplemented with green tea 70 % ethanol extract 0.5 %, PP-GEE1.0: plant-based patty supplemented with green tea 70 % ethanol extract 1.0 %, PP-GEE1.5: plant-based patty supplemented with green tea 70 % ethanol extract 1.5 %.
A–C Means values in the same row are significantly different ($P < 0.05$).
a–e Means values in the same column are significantly different ($P < 0.05$).
Data are presented as mean ± standard deviation (n = 8).

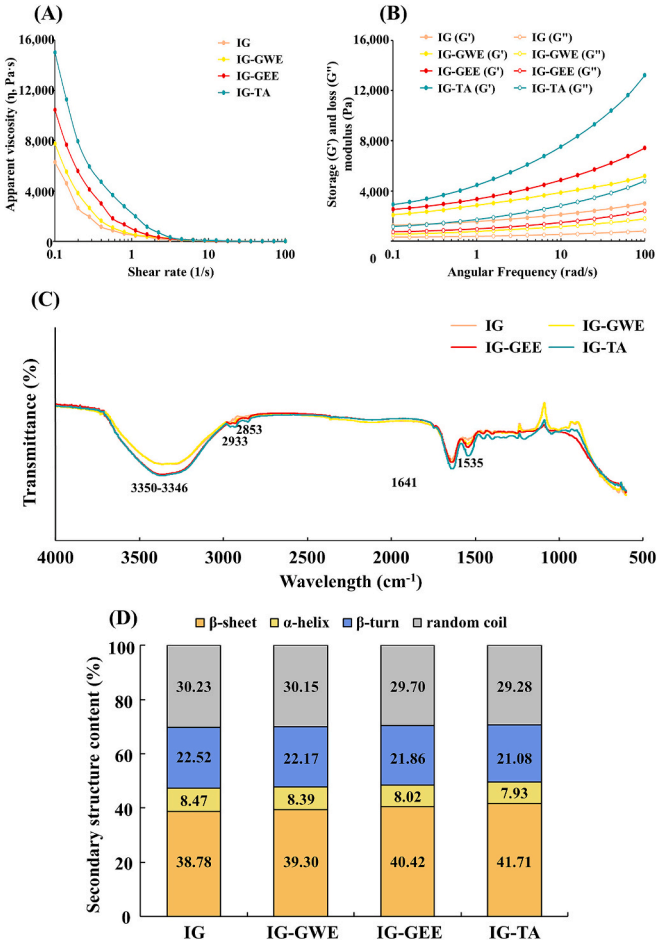

Fig. 2. Rheological properties and Fourier transform-infrared (FT-IR) spectra of isolated pea protein (IPP) gels supplemented with different green tea extracts. (A) Apparent viscosity at 25 °C, (B) viscoelasticity as a function of frequency in the range of 0.1–100 rad/s at 25 °C and 1 % strain, (C) FT-IR spectra, and (D) secondary structure content of IPP gels. Each colored line indicates a different sample. IG: isolated pea protein emulsion gel without green tea extract, IG-GWE: isolated pea protein emulsion gel supplemented with green tea water extract 1.0 %, IG-GEE: isolated pea protein emulsion gel supplemented with green tea 70 % ethanol extract 1.0 %, IG-TA: isolated pea protein emulsion gel supplemented with tannin 1.0 %. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

addition of tannin to camel meat sausage increased its hardness compared with the addition of catechin, a polyphenol abundant in green tea water extract (Maqsood et al., 2016). Hence, the elevated tannin content in GEE resulted in greater hardness of GEE-added PP during the storage period. On day 1, both PP-GWE1.0 and PP-GEE1.0 elevated texture parameters, including hardness, cohesiveness, chewiness, and gumminess, compared with the control group. Particularly, PP-GEE1.0 exhibited a significantly higher hardness value than PP-GWE1.0 ($P < 0.05$). It was assumed that the higher hardness value of PP-GEE1.0 resulted from the effect of tannin, which enhanced the physical

properties compared with other polyphenols. In a previous study, myofibrillar protein and meatballs containing tannins consistently exhibited higher hardness than those containing other polyphenols, including catechin, quercetin, gallic acid, and quercitrin (Xu et al., 2021). Our data also demonstrated that PP-GWE1.5 and PP-GEE1.5 exhibited lower overall texture parameters than the patty groups added to 1.0 % GWE or GEE ($P < 0.05$). Along with our WHC, cooking yield, and emulsion stability data, this decrease might be attributed to the excessively high polyphenol content, which hindered protein-protein interaction, consequently leading to lower overall texture parameters. Taken together, the incorporation of 1.0 % GE improved the texture profiles of PP, of which GEE was superior as it contained the optimal amount of tannins.

3.7. Rheological properties, FT-IR spectra, and secondary structure content of protein gel supplemented with GE

GEE had a high tannin content and played a crucial role in enhancing the texture properties of PP. In addition, previous studies have demonstrated that tannin imparted positive physical properties to food products (Maqsood et al., 2016; Xu et al., 2021). Thus, we assumed that the tannin within GE may contribute to enhancing the physical properties of PP. To identify the interaction of GE with IPP, a major protein ingredient in our PP, the rheological properties of protein gels supplemented with either GE or tannin were investigated (Fig. 2A and B). Apparent viscosity refered to the viscosity that varied with shear rate, and all protein gels exhibited the characteristics of a non-Newtonian fluid whose viscosity decreased with shear (Fig. 2A). The addition of GE and tannin increased the apparent viscosity of the protein gels. GEE had a stronger influence on increasing apparent viscosity than GWE, whereas IG-TA showed the highest apparent viscosity. Regarding the non-destructively measured viscoelasticity, storage modulus (G′) was higher than loss modulus (G") in all frequency ranges (Fig. 2B). These rheological properties of the protein gels were enhanced by GE and tannin supplementation, and the protein gel with GEE showed the highest viscoelasticity among the GE-added groups, which was similar to the result of apparent viscosity. Similar interactions have been previously reported. For example, the interactions between proteins and polyphenols could improve the rheological properties of hydrogel (Ding et al., 2019). Furthermore, similar to our data for IG-TA, tannin enhanced the rheological behavior of soy protein gel (Hu et al., 2023). Consequently, GE can interact with the IPP in PP, and polyphenols, including tannin can enhance the rheological properties of the protein gel.

Through FT-IR analysis, structural changes can be inferred by observing variations in the transmission intensity and shifts in the peak band wavelengths. The structural changes resulting from the chemical interaction between IPP and either GE or tannin are shown in Fig. 2C. The IPP gels exhibited notable peaks at specific wavelengths: 3346–3350 cm^{-1}, 2933–2940 cm^{-1}, 2853 cm^{-1}, 1641 cm^{-1}, and 1535 cm^{-1}. At the broad absorption peak from 3100 to 3600 cm^{-1}, IG-GEE and IG-TA showed higher intensity than IG and IG-GWE. These bands correspond to the O—H and N—H stretching vibrations of phenolic or hydroxyl groups, suggesting the formation of intramolecular or intermolecular hydrogen bonds within the protein gel (Ran & Yang, 2022). Additionally, the incorporation of GE and tannin resulted in a peak shift from 3350 cm^{-1} (IG) to 3348 cm^{-1} (IG-GWE), 3347 cm^{-1} (IG-GEE), and 3346 cm^{-1} (IG-TA), respectively. The peak shift to a lower wavelength (i.e., blue shift) within the range of 3100–3600 cm^{-1} indicates an increase in the hydrogen bonding content, implying that the addition of GEE, rich in tannin, had the potential to significantly enhance the hydrogen bonding content compared with GWE (Li et al., 2024). In contrast to IG and IG-GWE, IG-GEE and IG-TA exhibited slightly higher peak intensities at the wavelengths 2933–2940 cm^{-1} and 2853 cm^{-1} (C—H stretching of the $–CH_3$ and $–CH_2$ groups), indicating hydrophobic interactions. In the wavelength range of 2933–2940 cm^{-1}, the peaks of the IG gels showed a red shift at 2940 cm^{-1} (IG), 2937 cm^{-1} (IG-GWE), and 2933 cm^{-1} (IG-GEE, IG-TA), suggesting an increase in the strength of hydrophobic interactions upon the addition of GE and tannin (Yang et al., 2020). A previous study revealed that tannin contained dihydroxy-phenol and trihydroxy-phenol groups, enabling them to form potent non-covalent interactions (hydrogen bonding, hydrophobic interaction) with glycinin and β-conglycinin proteins (Hu et al., 2023). Glycinin and β-conglycinin, serving as the primary subunits of soy protein, were classified as legumin (11S globulin) and vicilin (7S globulin), and they represent the main subunits found in pea protein (Hammond et al., 2016). It was assumed that tannin also formed a non-covalent interaction with IPP due to the structural similarity between soy protein and pea protein. Hence, GEE, which was enriched with tannin, might contribute to establishing a more stable structure via non-covalent interactions than GWE. The addition of GE and tannin to IPP gel resulted in observable changes in the peak intensity around the wavelengths of 1641 cm^{-1} and 1535 cm^{-1}, which belong to the amide I region (1700 cm^{-1} to 1600 cm^{-1}, primarily associated with C═O stretching vibration in the protein backbone) and the amide II region (1500 cm^{-1} to 1600 cm^{-1}, related to C—N stretching vibration and N—H bending vibration), respectively. Alterations in the amide I and amide II bands could reflect modifications in the secondary structure of the protein (Hu et al., 2023).

The protein secondary structure content of IPP gels, quantified through deconvoluted FT-IR spectra around the amide I region (1700 cm^{-1} to 1600 cm^{-1}), was illustrated in Fig. 2D. In terms of the secondary structure of the pea protein gel, the β-sheet content was the primary constituent, accounting for approximately 38.78 %. With the incorporation of GE and tannin, a decrease was observed in α-helix, β-turn, and random coil content, while β-sheet content showed an increase compared with IG. Specifically, within IG-TA, these trends were notably observed at 7.93 % for α-helix, 21.08 % for β-turn, 29.28 % for random coil, and 41.71 % for β-sheet content, respectively. The decrease in the random coil content indicates a shift toward a more ordered secondary structure within the IG-TA. This suggests that the interaction between IPP and tannin could potentially facilitate the formation of more stable

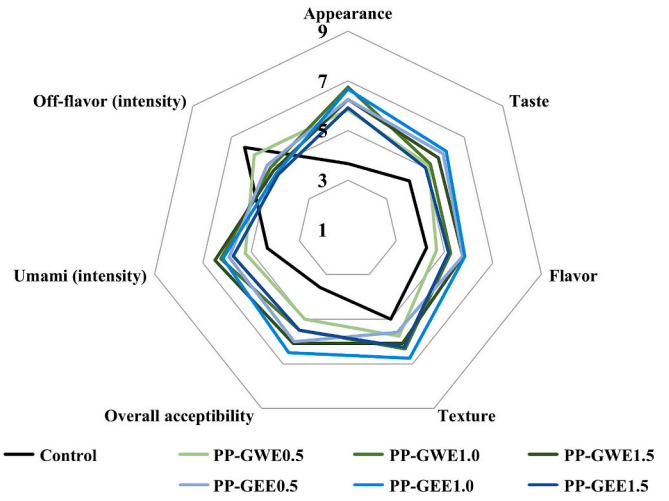

Fig. 3. Sensory properties of plant-based patties supplemented with different concentrations of green tea extracts (day 1). Each colored line indicates a different sample. Control: plant-based patty without green tea extract, PP-GWE0.5: plant-based patty added with green tea water extract 0.5 %, PP-GWE1.0: plant-based patty supplemented with green tea water extract 1.0 %, PP-GWE1.5: plant-based patty supplemented with green tea water extract 1.5 %, PP-GEE0.5: plant-based patty supplemented with green tea 70 % ethanol extract, PP-GEE1.0: plant-based patty supplemented with green tea 70 % ethanol extract 1.0 %, PP-GEE1.5: plant-based patty supplemented with green tea 70 % ethanol extract 1.5 %. The experiment was conducted with twelve panelists ($n = 12$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

structures (Hu et al., 2023). Meanwhile, during the gelation process, protein gel transitioned from α-helical to β-sheet structure, facilitating the formation of unfolded proteins that contribute to an organized network (Kobayashi et al., 2017). Consequently, the binding of tannin-IPP might promote disruption of the intermolecular hydrogen bonding of helical structure via a conformational change in the polypeptide chain and then convert to β-sheet structure (Li et al., 2021; Mozafarpour et al., 2022). The β-sheet structure possesses a weaker hydration strength and a larger surface area than the α-helix, which made favorable protein-protein interactions and gel network formation, thereby contributing to the reinforcement of gel strength (Wang et al., 2023). Indeed, enhancements in texture properties and viscoelasticity of pea protein-based blends concurrent with the transition from α-helix to β-sheet conformation (Ozturk et al., 2023).

Collectively, our data demonstrate that the interaction between tannin and IPP led to the formation of a non-covalent bond and a reduction of the α-helix and random coil content while increasing the presence of the β-sheet within the secondary protein structure. Consequently, owing to its high tannin content, GEE displayed structural changes resembling those of tannin, which might influence the improved texture and rheological properties of PP-GEE and IG-GEE.

3.8. Sensory evaluation of PP

Sensory evaluation was performed to evaluate the quality characteristics of PP (Fig. 3). The color data (Table S3, Table S4, and Fig. S1) revealed that the addition of GE reduced the L^*, a^*, and b^* values of PP after cooking. These color changes gave a meat patty-like appearance to the panels, resulting in significantly higher appearance scores for the groups with GE addition, than the control group ($P < 0.05$). In the taste and flavor categories, PP-GWE1.5, PP-GEE0.5, and PP-GEE1.0 received significantly higher scores than the control group. The taste and flavor scores for PP-GEE1.5 were 5.00 and 5.17, respectively, which were lower than those of PP-GEE0.5 and PP-GEE1.0. Tannin can enhance the physicochemical properties and storage conditions of food; however, excessive amounts can impart a bitter taste to food (Hung et al., 2010). Therefore, the incorporation of GEE1.5 % into PP received low scores from the panels, primarily because of the bitter taste of the tannin. PP-GEE1.0 received the highest texture score. On day 1, the texture properties of PP-GEE1.0 were higher than those of the other groups (Table 5), as stable cross-linking was formed by the combination of an appropriate amount of tannin and IPP. Although texture parameter values do not always have a positive effect on foods, the texture parameter values of PP-GEE1.0 were preferred by the panelists in our sensory evaluation. Umami intensity received the highest score of 6.50 for PP-GWE1.5. This may be because L-theanine was more enriched in GWE than in GEE. L-theanine imparts a unique flavor to food products. However, there was no significant difference in umami intensity between PP-GWE1.5 and all GEE-added groups ($P > 0.05$). The off-flavor intensity was significantly lower in PP-GEE1.0 and PP-GEE1.5 than the control ($P < 0.05$). Lipid oxidation can induce the production of undesirable odorous compounds, such as hexanal, hexanol, and heptanone (Soendjaja & Girard, 2024). The low TBARS values observed for PP-GEE1.0 and PP-GEE1.5 suggest the suppression of lipid oxidation, resulting in fewer off-flavor compounds. This was likely the reason for the low off-flavor intensity scores of the panels. Taken together, even though GWE has a higher L-theanine content than GEE, it does not seem to substantially affect the sensory characteristics. PP-GEE1.0, which received outstanding evaluations in terms of texture, off-flavor, and umami, was considered the most suitable option for sensory appeal.

4. Conclusions

This study aimed to evaluate the quality characteristics of GE-supplemented PP and the chemical reactions associated with taste and texture. Green tea was extracted with water or 70 % ethanol, and the effect of GE supplementation on the physicochemical characteristics, sensory properties, and storage stability of PP during refrigerated storage was investigated. GWE had a higher L-theanine content and lower tannin content than GEE. After adding GE, the color of PP decreased in terms of lightness, redness, and yellowness after cooking ($P < 0.05$). During 28 days of storage, PP-GEE1.0 and PP-GEE1.5 effectively inhibited the growth of microorganisms. Furthermore, the decreased microbial growth observed in PP-GEE1.0 and PP-GEE1.5 corresponded to a reduction in protein deterioration (VBN value). Lipid oxidation (TBARS value) in PP was attenuated by the addition of GE, with the GEE-added groups being particularly noteworthy. Incorporating 1.0 % GE into PP improved WHC, cooking yield, and emulsion stability. The addition of 1.0 % GE also improved the texture properties of PP, with PP-GEE1.0 exhibiting a higher increase in texture parameters than PP-GWE1.0 ($P < 0.05$). This effect might be attributed to the non-covalent interaction between tannins and IPP and the transition from α-helical to β-sheet structure. In the sensory evaluation, PP-GEE1.0 showed most suitable overall sensory characteristics. Our data suggest that incorporating GEE, which contains an optimal balance of L-theanine and tannins, could enhance the overall quality of plant-based patties, particularly texture and sensory properties.

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Institutional review board statement

The Institutional Review Board approved the sensory evaluation procedure (7001355–202,309-HR-694).

CRediT authorship contribution statement

Jong Hyeon Han: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Dong Hyun Keum:** Writing – review & editing, Methodology, Investigation. **Vahinika Kothuri:** Writing – review & editing, Methodology. **Yea-Ji Kim:** Writing – review & editing, Methodology, Investigation. **Hyuk Cheol Kwon:** Writing – review & editing, Methodology. **Do Hyun Kim:** Writing – review & editing, Methodology. **Hyun Su Jung:** Writing – review & editing, Methodology. **Sung Gu Han:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101807.

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