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Preservative effects of composite biopreservatives on goat meat during chilled storage: Insights into meat quality, high-throughput sequencing and molecular docking

Jin Wang [a,1], Baojing Ren [b,1], Kathrine H. Bak [c], Olugbenga P. Soladoye [d], Mohammed Gagaoua [e], Jorge Ruiz-Carrascal [f], Yongfu Huang [a], Zhongquan Zhao [a], Yongju Zhao [a], Yu Fu [b,*], Wei Wu [a,**]

[a] Chongqing Key Laboratory of Herbivore Science, College of Animal Science and Technology, Southwest University, Chongqing, 400715, China
[b] College of Food Science, Southwest University, Chongqing, 400715, China
[c] Institute of Food Safety, Food Technology and Veterinary Public Health, University of Veterinary Medicine Vienna, Veterinärplatz 1, 1210, Vienna, Austria
[d] Agriculture and Agri-Food Canada, Government of Canada, Lacombe Research and Development Centre, 6000 C&E Trail, Lacombe, Alberta, T4L 1W1, Canada
[e] Physiologie, Environnement et Génétique Pour l'Animal et les Systèmes d'Élevage (PEGASE), INRAE, Institut Agro, 35590, Saint-Gilles, France
[f] Research Institute of Meat and Meat Products (IProCar), University of Extremadura, Avda. de las Ciencias s/n, 10003, Cáceres, Spain

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ABSTRACT

To investigate the preservative effect of composite biopreservatives on goat meat during chilled storage, three biopreservatives (chitosan, tea polyphenols and grape seed extract) were selected. Meat samples were soaked in composite biopreservatives at previously optimized concentrations, prior to storage at 4 °C for 12 days. Meat quality parameters including pH, TBARS, TVB-N, color, sensory index and total viable count were evaluated. 16S rDNA high-throughput sequencing combined with bioinformatics was used to assess the changes in the bacterial community, while molecular docking permitted the investigation of molecular interaction between gyrase, catechins, and anthocyanins. Treatment by composite biopreservatives contributed to the stability and maintenance of meat quality, notably by significantly reducing TBARS and maintaining meat color and sensory scores. Pseudomonadales, Bacillales and Flavobacteriales were effectively inhibited as the main spoilage bacteria in goat meat during chilled storage. Molecular docking revealed that catechins and anthocyanins could bind to DNA gyrase by hydrogen bonds and hydrophobic interactions, thus inhibiting DNA synthesis and bacterial growth. Hence, the composite biopreseratives exerted a preservative impact on the chilled goat meat.

1. Introduction

Goat meat is characterized as lean meat with favorable nutritional attributes. However, fresh goat meat is susceptible to spoilage, which may cause food safety concerns and lead to economic losses. Therefore, it is crucial to control the growth of food spoilage microorganisms as well as the chemical and biochemical changes in goat meat during storage, such as the oxidation of lipids and proteins (Domínguez et al., 2019).

Currently, some commonly used methods to preserve and extend the shelf life of meat include low-temperature preservation, modified atmosphere packaging, preservatives treatment, high-pressure treatment, and irradiation treatment among other emerging and innovative methods (Gagaoua et al., 2021; Gagaoua et al., 2022; Lamri et al., 2021; Ren et al., 2021; Wang, Chen, Bai, & Lai, 2018; Wang et al., 2019). Although three former methods are frequently used, but fresh goat meat still has a relatively short shelf-life for shipping and commercial storage. In addition, frozen meat tends to lose its tenderness, juicy taste and flavor. The physical methods require large-scale equipment and massive investment. By contrast, the application of preservatives (e.g. chemical preservatives and biopreservatives) is an effective and straightforward method (Xu, Kaur, Pillidge, & Torley, 2022). Nowadays, chemical

* Corresponding author.
** Corresponding author.
E-mail addresses: fuy987@swu.edu.cn (Y. Fu), weiwu2019@swu.edu.cn (W. Wu).
[1] These authors contributed equally to this work.

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preservatives are normally selected to extend the shelf-life of meat and meat products (Zhao et al., 2019). Nevertheless, several recent studies have revealed that chemical preservatives can exert adverse impacts on human health when used at exceeded concentration, including cell toxicity, gastrointestinal diseases, allergic reactions or even carcinogenicity (Alirezalu et al., 2021; Zhang et al., 2021). Therefore, consumers are reluctant to accept chemical preservatives. Besides, the intensive application of chemical preservatives may also lead to antimicrobial resistance (Marc Belles, Alonso, Roncales, & Beltran, 2019). On the contrary, biopreservatives are characterized by safety, nontoxicity, and extensive source, which are promising for meat preservation (Fasuan & Chukwu, 2020; Mahmoud, 2014).

Several biopreservatives have been evidenced for their potential to inhibit the growth of specific bacteria, especially those related to meat spoilage. For example, Abirami et al. (2021) demonstrated that chitosan exhibited a potent inhibitory effect on *Pseudomonas* strains. Both tea polyphenols and grape seed extract can effectively inhibit the growth of *Staphylococcus* and *Bacillus* strains, while grape seed extract specifically inhibited enteric *Bacillus* (family Enterobacteriaceae) (Gomez-Mejia et al., 2021; Mercimek Takci, Bakirhan, Ozdemir, & Yalcin, 2020; Shao, Sun, Jiang, & Yu, 2020). In addition, Zhao, Chen, Zhao, He, and Yang (2020) revealed that grape seed extract also had a significant inhibitory effect on the growth of *Pseudomonas*. Therefore, different biopreservatives have antimicrobial activities toward diverse microorganisms. However, a better understanding of the inhibitory mechanisms of different biopreservatives is a crucial step for their application to effectively delay the growth of spoilage bacteria in meat and meat products. Accordingly, a recent work has systematically reviewed different biopreservatives with the significant inhibitory effect on bacterial growth through different mechanisms, including disrupting cell membrane or cell wall, inhibiting or preventing synthesis of DNA, RNA and protein, causing cellular contents leakage, changing membrane potential and interfering with intermediate metabolism or energy production (Ren et al., 2021).

Chitosan and cardamom extract can serve as a natural coating to improve the quality of lamb during chilled storage (Sharafati-Chaleshtori and Sharafati-Chaleshtori, 2017). Zhao et al. (2019) evidenced the preservative effect of composite biopreservative containing nisin, tea polyphenols and chitosan on chilled pork, showing a reduced growth rate of lactic acid bacteria, *Pseudomonas* spp., *Staphylococcus* spp., *Brochothrix thermosphacta*, and *Enterobacteriaceae*, while tea polyphenols can exhibit a high inhibitory activity (Zhao et al., 2019). Although different biopreservatives have been employed to extend the shelf life of meat, to the best of our knowledge, research on the application of biopreservatives in the preservation of fresh goat meat has rarely been reported (Mouafo, Mbawala, Tanaji, Somashekar, & Ndjouenkeu, 2020; Ren et al., 2021). Furthermore, the changes in the bacterial community and the inhibitory mechanisms during the chilled storage of goat meat are unknown. In this regard, three biopreservatives (chitosan, tea polyphenols and grape seed extract) were specifically selected according to their inhibitory specificity and the main spoilage bacteria in meat. Thus, the hypothesis was that given the specific inhibitory activities of biopreservatives, their combined action may retard spoilage and thus extend the shelf-life of goat meat.

2. Materials and methods

2.1. Experimental design

The *longissimus lumborum* muscles of six Dazu black goats (*Capra Hircus*) (males, 10 months age and pasture-fed) were collected from the slaughterhouse in Dazu District, Chongqing, China within 24 h after slaughter and immediately transported to the laboratory in polyethylene ice packs in 30 min. The meat from each animal was trimmed to remove surrounding fat and connective tissues and then cut into 2 × 2 cm strips of 1 cm thickness. Then, meat cubes were divided into two groups (the treatment group and control group). For treatment by the composite biopreservative containing chitosan, tea polyphenols and grape seed extract (CTG), each of meat cubes was individually immersed in the composite biopreservative solution for 30 s, removed from the solution and then packed in plastic trays with one absorbent pad placed underneath the sample. Muscle cubes without any coating solution were used as the control. All trays were sealed with polyethylene (PE) cling film (oxygen transmission rate 25,000 cm^3/m^2/24 h). The headspace atmosphere in the plastic trays was air and not modified. All packed samples were stored at 4 °C. Samples were measured on Days 0, 3, 6, 9, and 12 for subsequent analysis.

2.2. Chemical reagents

Chitosan (Molecular weight, 400 kDa and deacetylation degree, ~85%), grape seed extract and tea polyphenols were purchased from Tongzhe Biotechnology Co. Ltd (Guangzhou, China). Tryptone, yeast extract, glucose and agar were purchased from Baimicrobial Technology Ltd. (Zhongshan, China). Trichloroacetic acid, gallic acid, disodium EDTA, thiobarbituric acid, magnesium oxide, sodium chloride, boric acid, hydrochloric acid, methyl red, bromocresol green and anhydrous ethanol were purchased from Kolon Chemical Co. Ltd (Chengdu, China). All other reagents were of analytical grade.

2.3. Determination of total phenolic content of tea polyphenols and grape seed extract solutions

Total phenolic content was measured according to Aybastier, Dawbaa, and Demir (2018) with slight modifications. Briefly, tea polyphenols (0.5 g) and grape seed extract (0.5 g) were dissolved in Milli-Q water (100 mL) to form a mixture of tea polyphenols and grape seed extract. After diluting the sample solution (0.2 mL) with Milli-Q water to 1 mL, 0.2 mL of Folin-Ciocalteu reagent (diluted 1:3, v/v) was added and allowed to stand for 6 min. Two milliliters of 7.50% sodium carbonate solution and 1.6 mL of Milli-Q water were then added and thoroughly mixed. The mixture was allowed to stand for 1.5 h at 25 °C and protected from light, and the absorbance was measured at 760 nm. Quantification was performed based on the standard curve generated with gallic acid.

2.4. Preparation of composite biopreservatives

The dosage and proportion of chitosan, tea polyphenols and grape seed extract in the composite biopreservatives have been optimized according to our preliminary study on each biopreservative. It was determined that chitosan (10 mg/mL) and tea polyphenols and grape seed extract (total phenolic content of 2.50 mg/mL, 1:1, w/w) had a good preservative effect in terms of maintenance of meat quality. Tea polyphenols (0.5 g) and grape seed extract (0.5 g) were dissolved in sterilized Milli-Q water of 100 mL to form a mixture with a total phenolic content of 2.5 mg/mL. Subsequently, 1 g of chitosan was dissolved in the above polyphenol solutions. The final composite biopreservative of chitosan, tea polyphenols and grape seed extract (CTG) was obtained and stored at 4 °C.

2.5. Determination of pH

Prior to measuring the pH values of goat meat samples, the pH meter (FZ-600T Ark Technology Ltd, Chengdu, China) was calibrated using three commercial standard buffers, namely 0.05 mol/L potassium hydrogen phthalate buffer (pH 4.00), 0.025 mol/L mixed phosphate buffer (pH 6.86), and 0.01 mol/L borax buffer (pH 9.18). Goat meat (5.0 g) was ground by a meat grinder (XQQ-jrjan082701 Electrical Appliance Co., Ltd, Foshan, China) and further mixed with 50 mL of Milli-Q water. The pH of the homogenate was subsequently determined in triplicate (Yu, Robyn Dorothy, & Zhongxiang, 2019).

2.6. Lipid oxidation

The lipid oxidation of samples was evaluated based on the thiobarbituric acid reactive substances (TBARS) assay described by Xiong, Chen, Warner, and Fang (2020) with slight modifications. Meat samples were minced and 10 g of meat was further weighed in a beaker. Fifty milliliters of 7.5% trichloroacetic acid (TCA) and 0.1% ethylenediaminetetraacetic acid (EDTA) were added and shaken for 30 min. Subsequently, samples were filtered twice through a double layer of filter paper. The supernatant (5 mL) was transferred to a glass tube and 5 mL of 0.02 mol/L thiobarbituric acid (TBA) was added. The tube was kept in a boiling water bath for 40 min, removed and cooled for 1 h. Thereafter, it was further centrifuged at 2000×*g* for 5 min, and the supernatant was added with 5 mL of chloroform and shaken vigorously. The upper phase was taken and the absorbance was recorded at 532 and 600 nm. The TBARS value was calculated according to the following formula.

$$\text{TBARS values (mg MDA / kg)} = \frac{(A_{532} - A_{600}) \times 72.06 \times 100}{155m}$$

Where A_{532} is the absorbance of the solution measured at 532 nm, A_{600} is the absorbance of the solution measured at 532 nm, m is the muscle weight (g), 72.06 is molecular weight of MDA, and the constants originate from the dilution factor and the molar extinction coefficient of the thiobarbituric acid (TBA) reaction product.

2.7. Determination of total volatile basic nitrogen (TVB-N)

TVB-N was measured according to Baehaki, Herpandi, and Rosalina (2019) with slight modifications. Samples were first minced, and then 10 g of meat was added to 75 mL of Milli-Q water, followed by shaking for 30 min. Then, the mixture stood for 10 min and was further filtered. The mixture was distilled using an automatic K9860 Kjeldahl nitrogen analyzer (Jingcheng Instrumentation Co Ltd, Qingdao, China). TVB-N values were calculated according to the consumption of hydrochloric acid (0.1 mol/L) and expressed as mg N/100 g meat.

2.8. Determination of color

Color was measured according to Holman et al. (2021). Goat meat cubes were cut so that muscle fiber orientation was perpendicular to the exposed surface; they were placed onto individual black trays. The samples were allowed to bloom at the chilled temperature (4 °C) within 1 min. L^* (Lightness), a^* (redness) and b^* (yellowness) were measured using a colorimeter (CS-420 Vipshop Precision Instruments Ltd, Shenzhen, China). Illuminant was set to 'D65' and standard observer at 10° with a 2.5 cm aperture. Chroma (C*) and hue angle (h*) were further calculated using the following equations.

$$C^* = \left[\left(a^{*2} + b^{*2}\right)^{1/2}\right]$$

$$h^* = \left[(b^*/a^*)tan^{-1}\right]$$

2.9. Sensory meat evaluation and sensory index

A panel containing 10 panelists (6 females and 4 males) was subjected to professional training with fresh goat meat, refrigerated goat meat (2 days), and spoiled goat meat. The trained panelists, at a practice phase, evaluated the color (C), odor (O), and texture (T) of samples given the storage periods. Thereafter, the panelists evaluated the samples daily without knowing the storage period. After completing this practice with good performance (1 month), assessors participated in the sensory evaluation. Sensory evaluation was conducted in individual booths under controlled conditions of light, temperature and humidity. A set of six samples (three from the CTG treatment group and three from the control group) were randomly coded with 3-digit numbers and then presented to panelists. In total, 5 sensory sessions were conducted on each storage time point (Days 0, 3, 6, 9 and 12). Each panelist tested all meat samples by scoring color (C), odor (O) and texture (T) via watching, sniffing and touching each sample using a scale from 1(worst) to 5 (best). The final sensory index (SI) was calculated as follows: SI = (2 × C + 2 × O + T)/5. When SI ≤ 2.5, the meat quality was considered unacceptable (Khazandi et al., 2017).

2.10. Total viable bacterial count

Total viable count (TVC) was determined by plate count agar to evaluate the microbial growth in meat samples according to Li et al. (2022). Five grams of meat cubes were transferred into a sterile homogenizing bag containing 45 mL of 0.85% saline, followed by tapping for 1 min at medium speed with a tap homogenizer (ZQ-08X Scramble Scientific Instruments Ltd, Shanghai) to produce a 1:10 (w/w) sample homogenate. After homogenization, 1 mL was pipetted into 9 mL sterile saline solution and further diluted to produce 1:100, 1:1000, and 1:10, 000 dilutions. Subsequently, 100 μL of each concentration was pipetted into Petri dishes, which were further incubated at 37 °C for 48 h in triplicate. All the operations were conducted under aseptic conditions. The results were expressed as log CFU/g meat.

2.11. NovaSeq high-throughput sequencing

Five grams of meat was removed on day 0, 3, 6, 9, and 12, followed by addition of 45 mL PBS for homogenization. The mixture was then centrifuged at 200×*g* for 5 min and the pellets were discarded. The supernatant was further centrifuged at 10000×*g* for 10 min, and the bacterial pellet was collected. Bacterial DNA was extracted using the Bacterial DNA Isolation Kit following the manufacturer's guidelines (Ark Biosafety Technology Ltd., Beijing, China) (Zhao et al., 2021). The extracted DNA was purified, and the V3–V4 region of the 16s rDNA of the extracted samples was amplified by PCR with two primers 341F (CCTACGGGAGGCAGCAG) and 806R (GGACTACHVGGGTWTCTAAT). The PCR was performed three times with 25 μL of 2 × Premix Taq, 1 μL of Primer-F (10 μM), 1 μL of Primer-R (10 μM), 50 ng DNA, and nuclease-free water was added to 50 μL. The procedure was as follows. The samples were held at 94 °C for 5 min, 30 × 30s cycles at 94, 52 and 72 °C, respectively, followed by 72 °C for 10 min. The PCR products were detected by 1% agarose gel electrophoresis for fragmentation. The average length and concentration of the PCR products were checked by 1% agarose gel electrophoresis. After comparing the concentrations of the PCR products using GeneTools Analysis Software (Version 4.03.05.0, SynGene), the volume required for each sample was calculated according to the equal mass principle, and each PCR product was mixed. The PCR product mix was recovered using the E.Z.N.A.® Gel Extraction Kit (Omega, USA), and the target DNA fragments were recovered by elution in TE buffer and sequenced on the NovaSeq platform.

2.12. Molecular docking analysis

Molecular structures of catechins and anthocyanins were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov). *Pseudomonas putida (Arthrobacter siderocapsulatus)* is the main dominant spoilage bacteria in meat, so protein sequences of DNA gyrase subunit A (Uniprot ID: A0A126S1Q8) and DNA gyrase subunit B (UniProt ID: P13364) from *Pseudomonas putida (Arthrobacter siderocapsulatus)* were submitted to Swiss Model to construct homologous protein models (https://swissmodel.expasy.org). The structures of polyphenols and enzymes were imported into SYBYL, and then the Surflex-Dock (SFIC) standard mode was used in the SYBYL-X 2.1.1 software (Luo et al., 2022). The original ligand molecule was separated from the receptor pocket, and hydrogenated and water molecules and other ligands were removed. The processed protein was saved in PDB format as receptor

protein.

Catechins and anthocyanins were molecularly docked with the processed protein using the Surflex-Dock program in SYBYL-X 2.1.1 software. The affinity between polyphenols and receptor protein was analyzed by scoring function, and the interaction force was visualized by LigPlus to reveal the interaction mechanism. The molecular docking technology was used to analyze the affinity of polyphenols and receptor proteins. The Total-Score function T-Score is a function-derived SYBYL-X 2.1.1 software and consists mainly of Crash and Polar. C-Score is a function to evaluate the reliability of docking results by combining conformational prediction function, binding free energy function, common evaluation function, etc., which is based on the calculation of four values, D-SCORE, PMF-SCORE, G-SCORE, and CHEM-SCORE.

2.13. Statistical analysis

The data were expressed as mean ± standard error (SE). The results were analyzed by two-way analysis of variance (ANOVA) with storage time and biopreservative treatment as factors. Three replicates (muscle cube samples) in two groups (the CTG treatment and the control groups) were prepared for each day. Unless otherwise stated, each sample was measured in triplicate (a total of 6 data was collected for each test). In the case of sensory evaluation, the data were averaged over the 10 panelists to minimize the effects of pallenlist differences. Tukey's test was employed to determine the discrepancy between different storage times and between different biopreservative treatments. Pearson correlation analysis was applied to analyze the relationship between meat indices. The significance level was established at $P < 0.05$. All statistical analyses were performed by SPSS 22.0 and SigmaPlot 14.0 software.

3. Results and discussion

3.1. Physicochemical indicators of goat meat

3.1.1. pH

pH is an important indicator of raw meat quality related to protein degradation and microbial growth (Pabast, Shariatifar, Beikzadeh, & Jahed, 2018). As shown in Table 1, the pH values of the two treatments differed on day 0, possibly due to the preservative effect of composite biopreservatives on the pH of meat itself. However, according to Xiong et al. (2020), grape seed extract did not affect the pH of pork during chilled storage. The difference in the initial pH may be due to the presence of chitosan or tea polyphenols. With the extension of storage time, the pH values in both the control and the CTG treatment showed a significantly increasing trend. According to Karabagias, Badeka, and Kontominas (2011), this trend was partially due to the degradation of proteins and release of amino acids, causing the formation of alkaline reactive compounds such as NH_3 and amines, thus leading to a slight pH increase. Furthermore, there was no significant difference between the CTG treatment and the control during the other storage times ($P > 0.05$). On day 12, the pH values for the control and CTG treatment were 8.50 and 8.33, respectively, which means the control had a slightly higher pH than the CTG treatment. As chitosan exhibits an effective antibacterial activity against several microorganisms, including fungi, yeast, and bacteria, it can reduce pH changes and inhibit the spoilage of meat (Xiong et al., 2020). Therefore, it was ultimately shown that the final goat meat quality of the CTG treatment was slightly better than the control in terms of pH.

Table 1
The changes in pH values of the control and the CTG-treated goat meat during chilled storage at 4 °C.

Group	Storage time (days)				
	0	3	6	9	12
Control	4.61 ± 0.09^{ey}	5.64 ± 0.13^{dx}	6.39 ± 0.05^{cx}	7.50 ± 0.08^{bx}	8.50 ± 0.07^{ax}
CTG	5.41 ± 0.19^{ex}	5.78 ± 0.03^{dx}	6.42 ± 0.01^{cx}	7.20 ± 0.07^{bx}	8.33 ± 0.09^{ax}

Note: Data are mean values ± S.E. Mean values with different letters within the same column indicate significant differences. $^{a\text{-}e}$ Means within a row with no common superscript differ significantly ($P < 0.05$). ^{x,y} Means within a column with no common superscript differ significantly ($P < 0.05$).

3.1.2. TBARS

TBARS is a common index to measure the concentration of secondary products produced during lipid oxidation, and a higher TBARS value indicates a higher degree of lipid oxidation in meat (Domínguez et al., 2019; Ebadi, Khodanazary, Hosseini, & Zanguee, 2019). The difference in TBARS values between the CTG treatment and the control during chilled storage was more pronounced, probably due to the antioxidant activity of tea polyphenols and grape seed extract. The formation of free radicals at the early phase can be inhibited, or free radical chain reactions by acting as an electron donor can be interrupted, which resulted in slight changes in TBARS values in the CTG treatment and a significant difference in the control (Rababah et al., 2011). As depicted in Fig. 1, the TBARS values for the CTG treatment and the control were 0.08 and 0.09 mg MDA/kg on Day 0, respectively. However, the control group exhibited a significant rise in TBARS levels after Day 3, indicating a substantial degree of lipid oxidation. In the study by Hussain et al. (2021) on the impacts of storage time on ground lamb meat, the initial concentration of TBARS values in all samples ranged from 0.42 to 0.49 mg MDA/kg. It was much higher than the initial results in this study, indicating that goat meat was less susceptible to oxidation compared to ground lamb meat. On Day 12, the CTG treatment has an average value of 0.21 mg MDA/kg, while the control reached 2.3 mg MDA/kg, approximately 11 times higher than the CTG treatment samples. Overall, the composite biopreservatives containing tea polyphenols and grape seed extract displayed an efficient inhibitory activity against lipid oxidation.

3.1.3. TVB-N value

TVB-N is normally used to assess the freshness of meat due to protein degradation into nitrogenous compounds by enzymes and bacteria during the storage process (Xiong et al., 2020). Holman et al. (2021) found that the TVB-N concentration of beef was related to its color, microbial count, tenderness, and moisture content. As low TVB-N

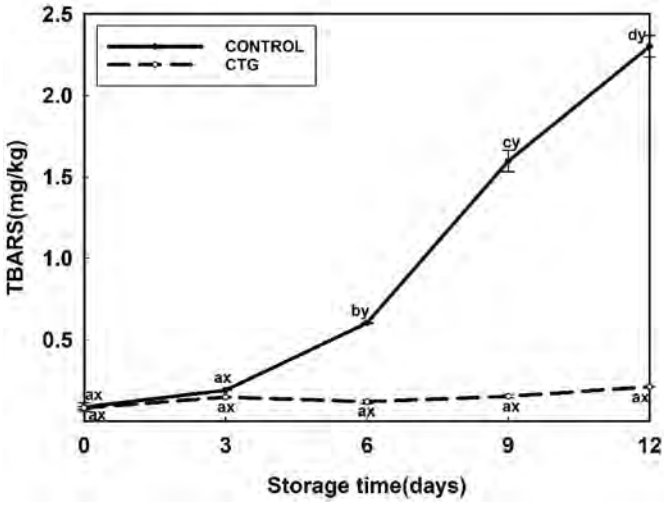

Fig. 1. The changes in TBARS values (means ± S.E.) of the control and the CTG-treated goat meat during storage at 4 °C for 0–12 days. Different lowercase superscripts (a–e) mean significant difference ($P < 0.05$) between different storage days. Lowercase superscripts (x and y) denote significant difference ($P < 0.05$) within different treatment on the same day.

concentrations indicate fresh meat, it tends to exhibit higher redness values and water retention, and lower microbial counts and protein hydrolysis levels (Xiong et al., 2020). The changes in TVB-N during the chilled storage of goat meat are shown in Fig. 2. On Day 3, 9, and 12, the TVB-N values of the CTG treatment group were significantly lower than the control group ($P < 0.05$). In addition, the TVB-N values started to show a more remarkable increasing trend after 9-day chilled storage in both groups. In this study, the contents of TVB-N were elevated with the extended storage time, which was consistent with the results of Diao, Huan, and Chitrakar (2020) on the application of garlic aqueous extracts-carboxymethyl chitosan coating solution for the preservation of chicken meat. Based on above results, it can be concluded that the samples treated with CTG exhibited improved performance in reducing TVB-N contents as compared to the control group to a certain extent.

3.2. Sensory indicators of goat meat

3.2.1. Color

Table 2 lists the color changes between the control and CTG treatment during 12-day storage, as reflected by the different values of L^*, a^*, and b^*. The L* values exhibited a decreasing tendency in both groups with the extension of storage. However, the CTG treatment showed more potency in maintaining the stability of L^* values, especially in the first six days ($P > 0.05$). Besides, as the pattern of L^* values, the CTG treatment also led to a slightly higher a^* values than the control. A previous study by Weber et al. (2007) revealed that grape seed extract was abundant in phenolic compounds and proanthocyanidins that caused discoloration, which might explain the lower a^* values in the CTG treatment than in the control on Day 3. The tendency of a^* values was similar to the study of Belles, Alonso, Roncales, and Beltran (2017) that a declined a^* value in lamb meat was observed during storage. By contrast, the b^* values were not significantly different in the present study ($P > 0.05$). Taken together, the chroma values and hue angle confirm that the discoloration was more pronounced in the control group compared to the CTG treatment group. Although the composite biopreservatives slightly affected the color of goat meat, they still contributed to the mitigation of meat discoloration during chilled storage.

3.2.2. Sensory index

The changes in sensory indices of goat meat for 12-day chilled storage are shown in Fig. 3. With an extended storage time, both groups showed a gradual decrease in sensory indices. However, the control samples had an unsatisfactory sensory index of 2.4 on Day 9 (SI $\leq$ 2.5), whereas the CTG treatment exhibited a drop to 2.4 until Day 12. The sensory index of CTG treatment was lower than the control on Day 0, since the color and odor of tea polyphenol and grape seed extract solution in the composite biopreservative itself can affect goat meat. Hence, it resulted in lower scores for odor and color compared to the control. According to Belles et al. (2017), no taste and flavor differences were found between the control and green tea catechin-treated samples.

Table 2
The changes in indices of the control and the CTG-treated goat meat during chilled storage at 4 °C.

Color index	Group	Storage time (days)				
		0	3	6	9	12
L^*	Control	46.90 ± 0.09^{ay}	44.43 ± 0.13^{by}	42.54 ± 0.16^{cy}	40.07 ± 0.61^{dy}	37.72 ± 0.23^{ey}
	CTG	45.69 ± 0.34^{ax}	45.23 ± 0.48^{ax}	45.18 ± 0.25^{ax}	40.34 ± 0.34^{bx}	39.67 ± 0.15^{bx}
a^*	Control	15.88 ± 0.74^{by}	17.87 ± 0.64^{ay}	13.59 ± 0.21^{cy}	11.79 ± 0.16^{dx}	9.48 ± 0.17^{ey}
	CTG	16.83 ± 0.15^{ax}	13.37 ± 0.30^{bx}	13.91 ± 0.91^{bx}	11.70 ± 0.21^{cx}	10.77 ± 0.15^{dx}
b^*	Control	15.80 ± 0.30^{by}	15.54 ± 0.21^{by}	16.58 ± 0.34^{ay}	15.72 ± 0.08^{by}	15.66 ± 0.39^{by}
	CTG	14.61 ± 0.37^{ax}	14.75 ± 0.08^{ax}	14.34 ± 0.27^{ax}	14.06 ± 0.55^{ax}	14.28 ± 0.28^{ax}
C^*	Control	22.40 ± 0.59^{bx}	23.69 ± 0.32^{ay}	21.44 ± 0.17^{cy}	19.65 ± 0.04^{dy}	18.31 ± 0.26^{ey}
	CTG	22.29 ± 0.19^{ax}	19.91 ± 0.11^{bx}	19.98 ± 0.67^{bx}	18.29 ± 0.41^{cx}	17.89 ± 0.19^{dx}
h°	Control	44.87 ± 0.66^{dy}	41.03 ± 1.08^{ey}	50.66 ± 0.75^{cy}	53.12 ± 0.41^{by}	58.82 ± 0.70^{ay}
	CTG	40.95 ± 0.68^{ex}	47.82 ± 0.64^{cx}	45.92 ± 1.11^{dx}	50.21 ± 0.78^{bx}	52.99 ± 0.27^{ax}

Note: Data are mean values ± S.E. Mean values with different letters within the same column indicate significant differences. [a-e] Means within a row with no common superscript differ significantly ($P < 0.05$). [x,y] Means within a column with no common superscript differ significantly ($P < 0.05$).

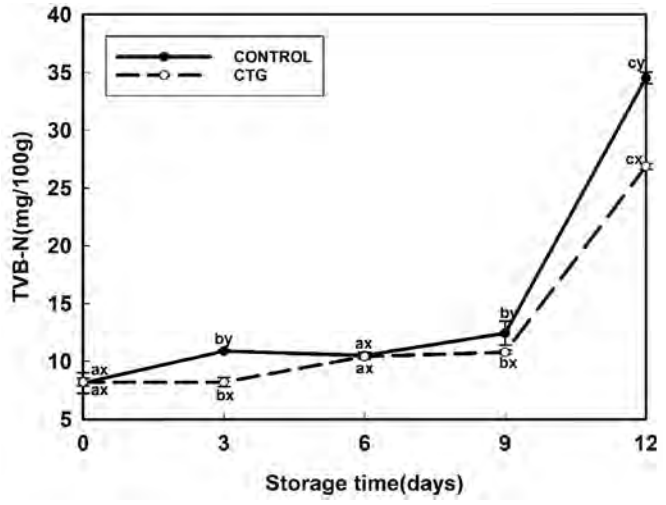

Fig. 2. The changes in TVB-N values (means ± S.E.) of the control and the CTG-treated goat meat during storage at 4 °C for 0–12 days. Different lowercase superscripts (a–e) mean significant difference ($P < 0.05$) between different storage days. Lowercase superscripts (x and y) denote significant difference ($P < 0.05$) within different treatment on the same day.

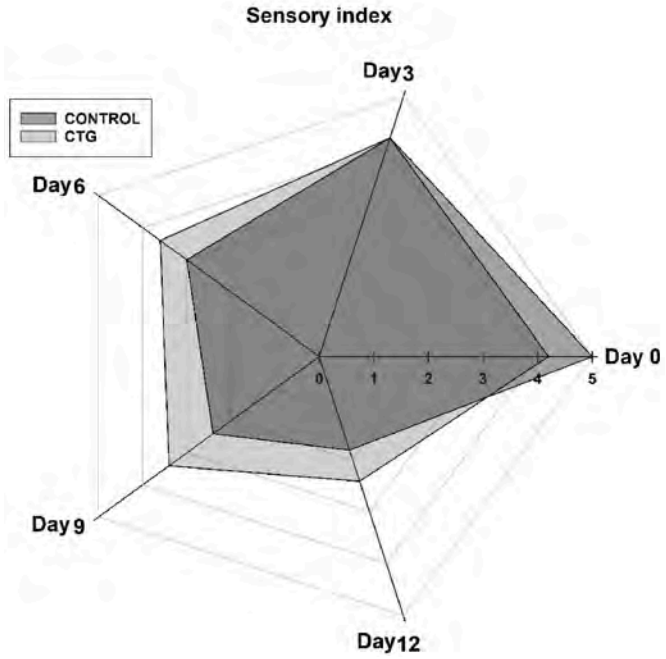

Fig. 3. The changes in the sensory index of the control and the CTG-treated goat meat during storage at 4 °C for 0–12 days.

The more significant effect on odor might be due to grape seed extract. After 3-day chilled storage, all the sensory indices of the control were lower than the CTG treatment (Fig. 3), which suggested a better ability of the CTG treatment to maintain the sensory quality of goat meat during long-term chilled storage.

3.3. Total viable count (TVC)

TVC is a commonly used method that estimates the total number of microorganisms in meat (Li et al., 2019). The changes in TVC of the CTG treatment and the control are shown in Fig. 4. On Day 0, the TVC values of the CTG treatment and the control groups were 2.9 and 3.1 log CFU/g, respectively. Compared to the control, the TVC value of the CTG treatment decreased by approximately ten-fold, confirming that the addition of biopreservative had a significant inhibitory effect on the growth of bacteria in meat ($P < 0.05$). The difference in TVC between the CTG treatment group and the control group increased gradually as the storage time extended. Nevertheless, the control group exhibited a slightly higher growth rate compared to the CTG treatment, which is consistent with the findings reported by Hussain et al. (2020). The control group reached 6.4 log CFU/g on Day 9. However, the CTG treatment group reached 6.1 log CFU/g until Day 12, which indicated that it could extend the shelf-life of fresh goat meat by 3 days in terms of microbial safety.

The main reason for this phenomenon is the broad-spectrum inhibitory effect of chitosan against a wide range of microorganisms. It can result in the hydrolysis of peptidoglycan through charged treatments in the polymeric backbone and their ionic interactions with bacterial wall components, leading to leakage of intracellular electrolytes and consequently microbial death (Goy, Morais, & Assis, 2016). Chitosan has a good inhibitory effect on Gram-negative bacteria (Gagaoua et al., 2021). Tea polyphenols can interfere with intracellular intermediate metabolism or energy by scavenging free radicals directly by reacting with them to produce more stable phenol-oxygen radicals or inhibiting metal ions that catalyze oxidation by complexation (Yin, Cheng, Zhang, & Wu, 2020). It can also easily transfect inside the cells, which inhibits nucleic acid synthesis by binding to DNA gyrase and disturbing energetic systems (Shao et al., 2020). Shao et al. (2020) found that the potent antibacterial activities of corn distarch phosphate/carboxymethyl cellulose composite films containing tea polyphenol might be due to the interaction between phenolic hydroxyl treatment in tea polyphenol and the amino or carboxyl treatment in cell protein, which inhibited the growth of bacteria. Grape seed extract contains a series of polyphenols, so the partial hydrophobic nature of phenolic compounds can enhance their accumulation and attachment to the bacterial cytoplasmic membrane. Therefore, polyphenolic compounds are expected to exhibit antibacterial activity through interactions with the outer cell membrane of bacteria. The antibacterial efficiency of these polyphenols is closely correlated to the presence of reactive groups such as hydroxyls, galloyl moieties and conjugated double bonds (Amankwaah, Li, Lee, & Pascall, 2020).

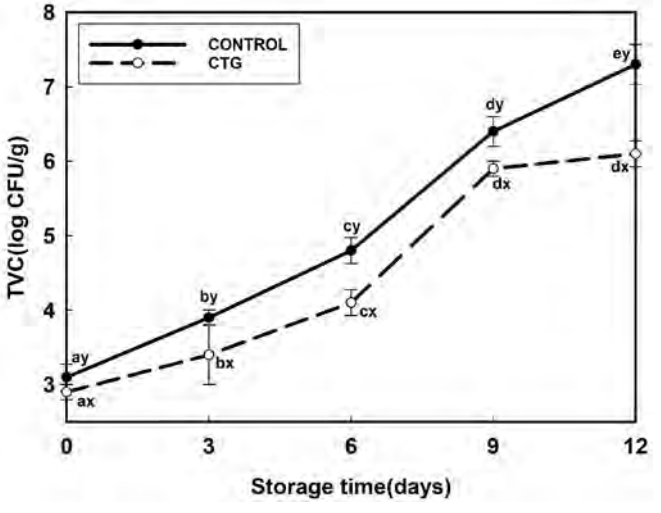

Fig. 4. The changes in total viable count (means ± S.E.) of the control and the CTG-treated goat meat during storage at 4 °C for 0–12 days. Different lowercase superscripts (a–e) mean significant difference ($P < 0.05$) between different storage days. Lowercase superscripts (x and y) denote significant difference ($P < 0.05$) within different treatment on the same day.

3.4. Correlation analysis between meat indices

In order to reveal the potential relationship between L^*, a^*, b^*, pH, TBARS, TVB-N and TVC indices, Pearson correlation analysis was carried out for control and CTG treatment group, respectively. As listed in Tables S1 and S2, the correlation coefficients (r values) show quite similar patterns in the control and CTG treatment group. Both b^* and TVB-N values showed insignificant interactions with other indices ($P > 0.05$). However, L^*, a^*, pH, TBARS and TVC values demonstrated significant correlations with $r > 0.85$ or $r < -0.85$. In addition, it is noteworthy that all correlation coefficients with significant changes were lower in CTG treatment, which suggested the meat indices were affected by biopreservatives treatment to some degree.

3.5. High-throughput sequencing

To further elucidate the variation of microbial diversity in goat meat during storage, high-throughput sequencing technology was applied to sequence and analyze the changes in the microbial community. By combining and filtering the DNA sequences from each sample, 1107589 high-quality valid sequences were obtained with an efficiency rate of 93.04–95.62%. Valid sequences were clustered into 2255 operational taxonomic units (OTUs) using a similarity threshold of 97% and coverage of at least 99% for all samples, indicating that almost all bacteria in the goat meat samples could be detected. Sequencing analysis indicated a total of 15 orders, including *Pseudomonadales*, *Bacillales*, *Flavobacteriales*, *Lactobacillales*, *Aeromonadales, Enterobacteriales, Micrococcales, Rhizobiales, Corynebacteriales, Betaproteobacteriales, Bacteroidales, Clostridiales, Alteromonadales* and *Xanthomonadales*.

The rank abundance curve can provide a visual representation of the richness and evenness of microbial species contained in the goat meat samples. According to Fig. 5a, the curve for Day 0 was the flattest, indicating that the microbial colonies in goat meat were more complex

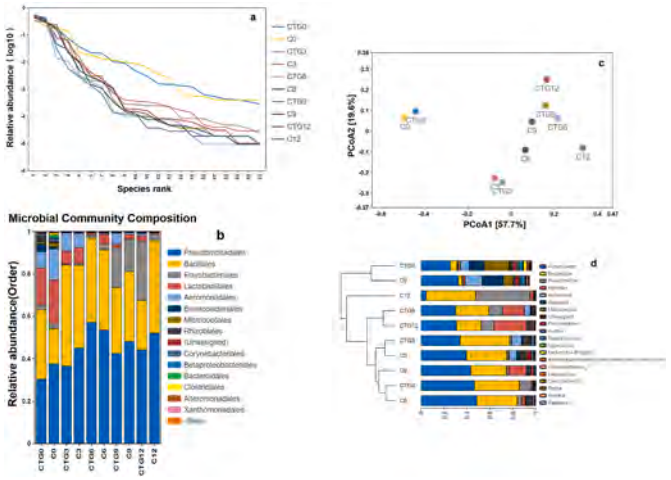

Fig. 5. High-throughput sequencing results of the control and the CTG-treated goat meat during storage at 4 °C for 0–12 days. (a) The rank-abundance curve; (b) The relative abundance (%) of bacterial taxa at the order-level; (c) PCoA plot; (d) The cluster analysis.

and diverse at the beginning (Day 0). As the storage time increased until Day 12, the curve dropped rapidly and steeply, indicating a gradual decrease in microbial diversity. It is important to note that the curve slope was greater in the CTG treatment than in the control on the same storage day, showing that the addition of composite biopreservatives had a potent inhibitory effect on the growth of microorganisms. According to Fig. 5b, *Pseudomonadales* (37.49%), *Bacillales* (16.81%), *Lactobacillales* (20.66%) and *Aeromonadales* (14.60%) were the main groups of bacteria present in the goat meat investigated in this study. Combined with the rank abundance curve (Fig. 5a), the results showed that only a small number of microorganisms species caused the spoilage of goat meat. Except for Day 6, *Pseudomonadales* were less abundant in the CTG treatment group with the addition of composite biopreservatives than in the control group. Our findings are in agreement with those of Abirami et al. (2021) who demonstrated that chitosan could form chitosan films to exhibit an inhibitory effect on the growth of *Pseudomonadales*. Thus, it can be inferred that the inhibited growth of *Pseudomonas* is key for the effective preservation of goat meat during chilled storage.

Among the spoilage bacteria in chilled goat meat, *Pseudomonas* accounted for about 30% as the dominant flora, followed by *Enterobacter*, *Lactobacillus*, and *Staphylococcus* spoilage bacteria. However, the percentages of each bacteria group in meat during storage are slightly different between studies (Wen et al., 2022). *Bacillales* were the second most predominant bacteria causing spoilage of goat meat during chilled storage, reaching 47.74% on Day 3 in the CTG treatment, followed by a decrease to 23.40%. In contrast, *Bacillales* in the control showed an increasing trend, eventually reaching 43.72% (Fig. 5a), which demonstrated that chitosan, tea polyphenols and grape seed extract had an excellent inhibitory effect on the growth of *Bacillales* in goat meat. At the same time, the percentage of *Lactobacillales* decreased with the extended storage time, from 20.66% to 0.59% in the control and from 17.63% to 2.29% in the CTG treatment. Overall, the combined application of chitosan, tea polyphenols and grape seed extract exhibited the most significant inhibitory effect on *Lactobacillales*.

The percentage of *Flavobacteriales* increased with extended storage time, so it can be predicted that the inhibition of *Flavobacteriales* growth can also be beneficial for the preservation of goat meat. It was strain ANORD5^{T}, a mesophilic, chemoheterotrophic aerobic bacteria with an optimal growth pH of 7.0–8.5, while *Flavobacterium* (*phylum Bacteroidetes*) contains four validly described families, two of which (*Icariidae* and *Flavobacterium*) were predominantly distributed in the marine environment (Wiese et al., 2018). Fig. 5b shows that the abundance of this species was significantly higher on Day 9 when pH was 7.5 and 7.2 for the control and the CTG treatment, respectively, which is the optimal pH for the growth of these bacteria, thus confirming the reason for the increased abundance of *Flavobacteriales*.

As illustrated in Fig. 5c and d, it is possible to visually analyze and compare the differences and similarities of the microbial composition between different samples. These two groups of samples had similar colony composition on Day 0 and are not showing significant differences until Day 6. Afterwards, the differences between the CTG and the control started to differ significantly, probably due to the exponential growth pattern of the microorganisms beginning to be evident. It is worth noting that the microbial composition of the control group was significantly different from that of the CTG treatment on Day 12. The present study suggested that the control without the addition of composite biopreservatives was severely spoiled on Day 12. Furthermore, a similar conclusion can be reached according to Fig. 5c, which showed that the combined usage of chitosan, tea polyphenols and grape seed extract had a potent antibacterial effect. Taken together, more targeted inhibition of various bacteria, namely *Pseudomonadales*, *Bacillales*, and *Flavobacteriales*, could better preserve goat meat by ascertaining the suitable biopreservatives in subsequent studies.

3.6. Molecular docking analysis

Phenolic substances (e.g. anthocyanins and catechins) in tea polyphenols and grape seed extract can inhibit or prevent the synthesis of DNA, RNA and protein of microorganisms (Shao et al., 2020). Catechins and anthocyanins are the main bioactive compounds in tea polyphenols and grape seed extract, respectively. It has been shown that GyrA and GyrB, two important subunits of DNA gyrase, have been identified as suitable targets for designing antimicrobial agents (Dighe & Collet, 2020). The interactions of catechins and anthocyanins with GyrA and GyrB from Pseudomonas were analyzed using molecular docking techniques to further understand the mechanism of their antimicrobial activity. The higher value of Total Score and C Score indicates the higher affinity between phenolic substances and subunits. The C scores of anthocyanins with GyrA and GyrB were both 4 and the T scores were −1 and 2.93, while the C scores of catechins with GyrA and GyrB were 3 and 4 and the T scores were 3.93 and 7.09. This might suggest that catechin exhibited a higher affinity with DNA gyrase and thus can exhibit a better inhibitor effect on DNA synthesis.

The 2D diagrams of the interaction of catechins and anthocyanins with GyrA and GyrB are shown in Fig. 6. Hydrogen bonds and hydrophobic interactions were responsible for their binding. The binding sites of catechin for GyrA included Ser53 and Tyr50, while the binding sites for GyrB were Arg318, Glu266, Asp315, Arg518, and Glu522, respectively. The hydrogen bond sites of anthocyanins with GyrA were Asp137 and Lys140, while the hydrogen bonding sites with GyrB were Asn265, Glu266, Arg318, Asp315, Phe264 and Gln519. In addition, the hydrophobic interaction also contributed to the binding of polyphenols and DNA gyrase. Overall, both anthocyanins and catechins can bind to DNA gyrase, while catechins displayed a better affinity. Hence, tea polyphenols may be more effective to inhibit the DNA gyrase of *Pseudomonas*.

4. Conclusion

The composite biopreservative containing chitosan, tea polyphenols and grape seed extract had a remarkable preservative effect on goat meat during chilled storage at 4 °C. The application of composite biopreservatives can extend the shelf life of goat meat by about 3 days. Especially, the composite biopreservative treatment resulted in a significant reduction in lipid oxidation. High-throughput sequencing revealed that *Pseudomonadales*, *Bacillales*, and *Flavobacteriale* were the main spoilage bacteria in chilled goat meat, and the composite biopreservatives were extremely effective in inhibiting the growth of *Pseudomonas*. Both catechin and anthocyanin can interact with DNA gyrase through hydrogen bonds and hydrophobic interaction to inhibit DNA synthesis and bacterial growth. The present study contributes to the application of composite biopreservatives in the preservation of goat meat by a specific selection of biopreservatives to inhibit meat spoilage during chilled storage.

CRediT authorship contribution statement

Jin Wang: Writing – original draft, Methodology. **Baojing Ren:** Writing – original draft, Methodology. **Kathrine H. Bak:** Writing – review & editing. **Olugbenga P. Soladoye:** Supervision, Writing – review & editing. **Mohammed Gagaoua:** Writing – review & editing. **Jorge Ruiz-Carrascal:** Writing – review & editing. **Yongfu Huang:** Writing – review & editing, Resources. **Zhongquan Zhao:** Writing – review & editing, Resources. **Yongju Zhao:** Writing – review & editing, Resources. **Yu Fu:** Conceptualization, Writing – review & editing, Funding acquisition, Supervision. **Wei Wu:** Conceptualization, Writing – review & editing, Funding acquisition, Supervision, Resources.

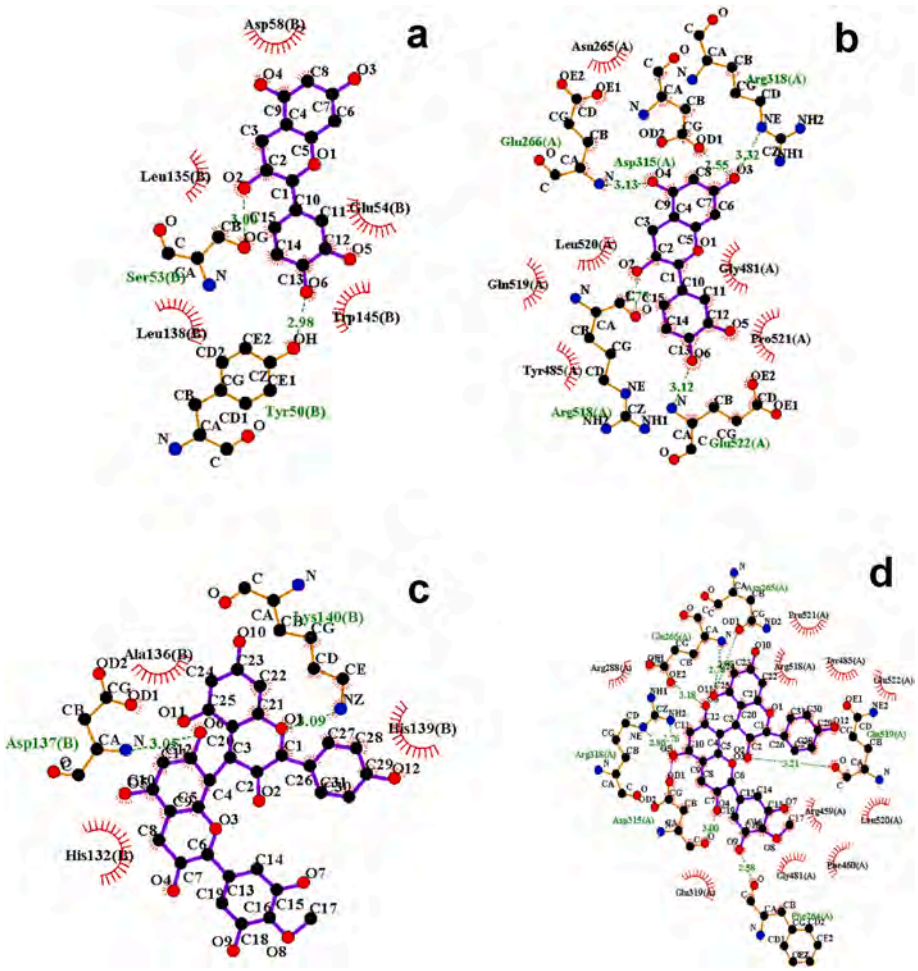

Fig. 6. Molecular docking of catechin and anthocyanin with GyrA and GyrB. (a) catechin with GyrA; (b) catechins with GyrB; (c) anthocyanin with GyrA; (d) anthocyanin with GyrB. Note: The green dashed line (green font) indicated the hydrogen bond interaction between ligand (anthocyanin or catechin) and receptor (GyrA or GyrB) residue. Brick red and black amino acids indicated hydrophobic interaction between ligand and receptor residues, and the number of marks reflects the interaction distance. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Declaration of competing interest

The authors declare no conflicts of interest.

Data availability

Data will be made available on request.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2023.115033.

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