Italian Journal of Food Science, 2025; 37 (1): 319–332

Effects of *Carya cathayensis* Sarg. and *Torreya grandis* nuts on the physicochemical properties and lactic acid bacteria of yogurt

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Academic Editor: Prof. Giuseppe Zeppa - Università di Torino, Italy

Received: 6 July 2024; Accepted: 10 November 2024; Published: 1 January 2025

ORIGINAL ARTICLE

Abstract

Yogurts containing nuts were prepared by adding 2% or 5% *Carya cathayensis Sarg.* and *Torreya grandis* nuts and inoculating with 20 kinds of probiotics. Both nuts blend well with the yogurts, and they enrich the sensory quality of the yogurt, giving it the unique flavor of *C. cathayensis* Sarg. and *T. grandis* nuts, which significantly increase the nutritional value of yogurt and improve the viscosity and water-holding capacity of yogurt. *Both nuts* are beneficial to the growth and reproduction of *Streptococcus thermophilus* and *L. delbrueckii* subsp. *bulgaricus*.

Keywords: *Carya cathayensis* Sarg.; *Torreya grandis;* Yogurt; physicochemical properties; lactic acid bacteria

Introduction

Nuts are a beneficial source of bioactive substances such as high-quality plant proteins, fiber, minerals, tocopherols, phytosterols, and phenolic compounds (Ros *et al.*, 2010). Thus, nuts may reduce the incidence of several chronic diseases, including cardiovascular diseases (Rusu *et al.*, 2019), reduce the risk of weight gain and obesity, and have cholesterol-lowering effects (Martínez-González and Bes-Rastrollo, 2011). Among the various bioactive compounds found in nuts, folic acid, selenium, n-3 and n-6 fatty acids, and vitamin E hold significant importance due to their reported health benefits, while dairy products do not serve as a good source of these components (Ros *et al.*, 2010). Therefore, the combination of nuts and fermented dairy can provide nutrient-rich dairy products (Yilmaz-Ersan *et al.*, 2022).

Yogurt is a typical fermented dairy product and usually contains active probiotics (Jiao *et al.*, 2023). Chestnuts enhance probiotic activity, antioxidant capacity, and gamma-aminobutyric acid in yogurt (Yilmaz-Ersan *et al.*, 2022). Tiger nut significantly improves yogurt's physical and chemical properties; its protein lowers the pH of the base system, increases microbial lag time, and reduces the acidification rate (Kizzie-Hayford *et al.*, 2016). Cashew increased lactic acid bacteria (LAB) counts, and *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Lactobacillus plantarum* were more suitable to use as starter cultures in cashew milk–based yogurt production. Total phenolic and flavonoid content of cashew was beneficial to LAB, especially the *lactobacillus* spp. strains closely related to antioxidant capacity content (Shori *et al.*, 2022). Hazelnut, almond, and pistachio have some effect on the physicochemical and instrumental textural

characteristics of yogurt, and they also increased the counts of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. Another benefit of using nuts as a fortifier in yogurt is their rich dietary fiber and protein content (Bertolino *et al*. 2015). Supplementation with hazelnuts can be effective in increasing the total solid matter content in yogurt, which is important for gel formation, firmness, and viscosity (Udayarajan *et al.*, 2022). Similarly, nuts contain a prebiotic source of indigestible carbohydrates (Ozturkoglu-Budak *et al.*, 2016). The addition of nuts increases the nutritional value of yogurt (Ozturkoglu-Budak *et al.*, 2016; Udayarajan *et al.,* 2022).

Both *C. cathayensis* Sarg. and *T. grandis* nuts are abundant in Xuancheng, Anhui Province. They are rich in beneficial lipids, proteins, micronutrients, and bioactive compounds (He *et al*., 2016; Hu *et al*., 2018). *C. cathayensis* Sarg. is rich in phenolic compounds, vitamin E, phytosterols, squalene, carotenoids, and so on (Alasalvar and Bolling, 2015). The main objective of this study was to evaluate the effects of *C. cathayensis* Sarg. and *T. grandis* nuts on yogurt fermentation, physicochemical properties, and lactobacilli counts. We hypothesized that the composition of certain bioactive components detected in nuts would contribute to the production of new functional yogurt.

Materials and Methods

Materials and reagents

Lactobacillus strains were purchased from Qingdao Kemetsen Food Science and Technology Co., Ltd. and contained 20 strains, including *L. plantarum* Lrld-22, which was isolated from Qinghai yak fermented milk. The *L. plantarum* Lrld-22 was kept in the microbiology laboratory of Anqing Vocational and Technical College. Milk was purchased from Yili Dairy Co., Ltd., and *C. cathayensis* Sarg. and *T. grandis* nuts were supplied by Anhui Zhanshi Food Corporation.

Preparation of *C. cathayensis* Sarg. and *T. grandis* nuts yogurt

The modified method of Liu (2018) was employed to manufacture nut yogurt. *C. cathayensis* Sarg. and *T. grandis* nuts were crushed and weighed at a level of 2 g and 5 g/100 mL, respectively, and sugar was added at a ratio of 6 g/100 mL to formulate a mixture of 500 mL, which was sterilized at 90°C for 20 min. It was cooled to 42°C, then inoculated with 4% (v/v) of yogurt fermenter and incubated at 42°C for 6 h. *C. cathayensis* Sarg yogurts ($CCSY_{2\%}$ and $CCSY_{5\%}$) and *T. grandis* yogurts ($TGY_{2\%}$ and $TGY_{5\%}$) with different concentrations of 2 g and 5 g/100 mL were prepared, and yogurts without nuts were used as a control, and the yogurt samples were refrigerated at 4°C.

Nutrient composition analysis of yogurt

The Kjeldahl method (Kirk, 1950) determined the total protein of yogurt and nuts. The fat content of yogurt and nuts was determined using a Soxhlet extraction system (Velp Scientific, Usmate, Italy) according to AOAC International (2019, methods 2000.18). Carbohydrates in yogurt and nuts, as well as dietary fiber, were determined according to AOAC International (2019). The total solids content of yogurt was determined according to AOAC International (2019, method 925.23). The physicochemical parameters of the ingredients were determined using the dairy rapid analysis system to complete the determination (Zhejiang University Youchuang Technology Co., LTD., Hangzhou, China). All experiments were repeated thrice.

Determination of the physicochemical properties of yogurt

The syneresis and water-holding capacity (WHC) were determined according to the method of Isanga and Zhang (2009). The syneresis was calculated as: syneresis (%) = (V1/V2) × 100 (1), where V1 is the volume of whey collected after discharge and V2 is the volume of yogurt sample. WHC of yogurt was determined using the centrifugation (Sigma 3-18K, Sartorius AG, Göttingen, Germany) method of Isanga and Zhang (2009), with some modifications. The WHC was calculated by centrifuging 25 g of yogurt at 4500 × g for 15 min at 4°C as: WHC (%) = (1-M1/M2) × 100 (2), where M1 is the weight of the whey after centrifugation and M2 is the weight of the yogurt sample. The viscosity values (Pa.s) of the yogurt samples were measured at a temperature of 25°C using a programmable viscometer (Haake, Karlsruhe, Germany) with a RV-5 spindles (Gocer and Koptagel, 2023).

Determination of LAB counts

The total number of LAB was detected by the plate counting method. One milliliter of yogurt sample was taken and diluted tenfold with sterile saline (mass fraction 0.9%). About 0.1 mL of the dilution was aspirated with a pipette gun into a sterile petri dish, and MRS medium at 42°C was poured and mixed (Liu, 2018). The plates were placed in a MCO-18AC carbon dioxide incubator (Sanyo, Panasonic, Japan) at 37°C for 72 h. The number of colonies was expressed as log CFU/mL, and the experiments were repeated 10 times.

16Sr RNA gene amplification and MiSeq sequencing

Total genomic DNA was extracted using the DNA Extraction Kit (Thermo Fisher, Germany) according to the manufacturer's instructions, and PCR was performed with Takara Ex Taq (Takara). Sequencing data was pre-processed by Trimmomatic software and achieved using QIIME software (version 1.8.0). Then, VSEARCH software was used to remove primer sequences and cluster valid tags to generate operational taxonomic units (OTUs) with 97% similarity. α diversity (Shannon and Chao1 indices) and Bray–Curtis distance were calculated by R package Vegan (v2.5.7), and the Kruskal–Wallis test of GraphPad Prism 8 software was used to analyze the statistical differences between Shannon and Chao1 indices and the abundance of bacterial groups (Pichler *et al*., 2018). Principal component analysis (PCoA) and KEGG metabolic pathway differences were calculated using R package AP (v5.6.2) and PICRUST2 software (Pichler *et al*., 2018).

Sensory evaluation of yogurt

Sensory evaluation of yogurt was carried out on the first day of storage at 4°C. Referring to Shori *et al.* (2022), a 40-person, untrained female panel with members ranging in age from 20 to 45 years (mean age of 23) comprising teachers and students at the Department of Food Quality and Safety at Anqing Vocational and Technical College conducted the evaluation. The evaluation assessed six components, namely, taste, color, aroma, flavor, texture, and overall preference. Each component was evaluated based on a 10-point scale (Shori *et al*., 2022).

Statistical analysis

All the experiments were repeated more than thrice. Data were expressed as mean ± standard deviation. Statistical significance was analyzed using one-way analysis of variance (ANOVA) by GraphPad Prism 8.0. The threshold for statistical significance was established at $p < 0.05$.

Results and Discussion

The pH changes and the total number of LAB in yogurt during fermentation

During the fermentation process, as LAB metabolized acid production using lactose, the pH of all kinds of yogurts decreased continuously (Figure 1A), ultimately reaching 4.75–4.84. The pH of different yogurts did not

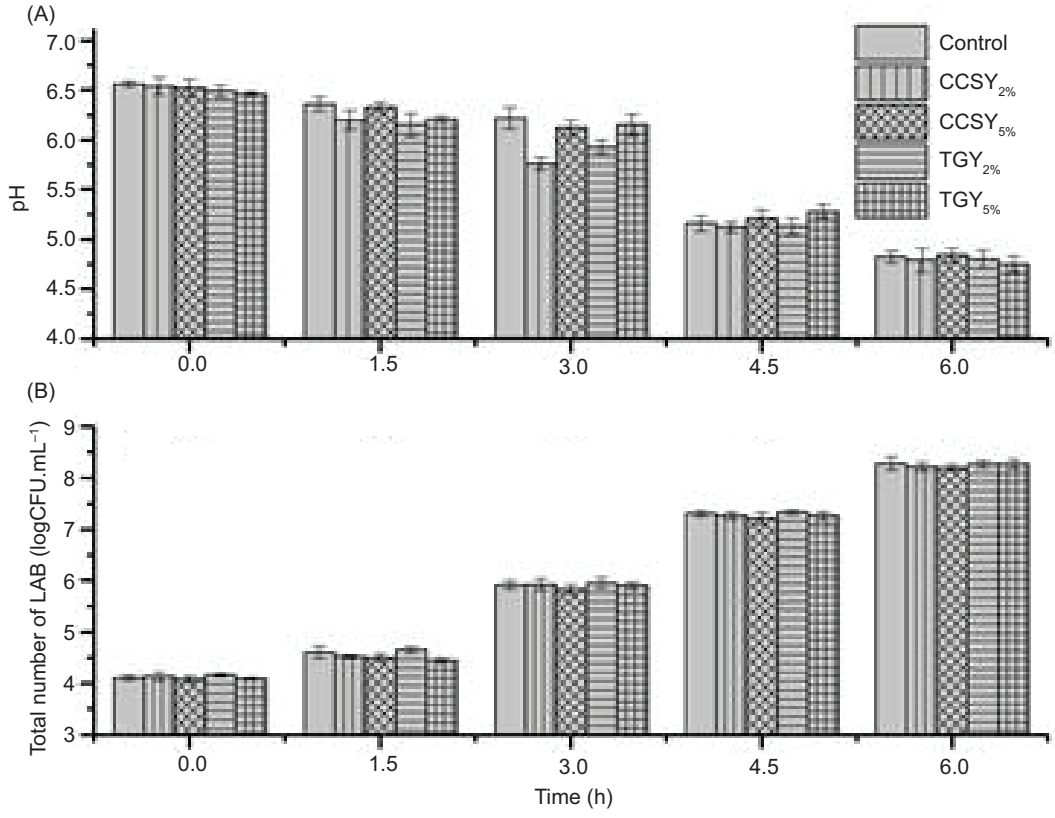

Figure 1. The change of pH and *Lactobacillus* counts during the fermentation.

change significantly during the fermentation phase, suggesting that the incorporation of *Carya cathayensis* Sarg. and *Torreya grandis* nuts within this concentration range did not impede the metabolic synthesis of LAB (Isanga and Zhang 2009; Udayarajan *et al.*, 2022).

In both control and nut-contained yogurt, LAB went through adaptation, and logarithmic and stabilization periods (Figure 1B). The LAB were in the adaptation period for 0–1.5h, and the total colony number was about 4.10 CFU/mL. The addition of 2% and 5% *C. cathayensis* Sarg. and *T. grandis* resulted in a slightly lower pH than the control, possibly due to the nuts' active components assisting the LAB in adapting to the environment (Piekarska-Radzik and Klewicka, 2021). During the 1.5–4.5h when LAB were in the logarithmic phase (Huang *et al*., 2023), *lactobacilli* counts rose rapidly, which means vigorous metabolism led to acceleration of pH decline. At 6 h, all kinds of yogurt reached the stabilization period with the LAB count of 8.20–8.28 CFU/mL. Throughout the fermentation period, there was no significant difference in the LAB count among five different yogurts.

Effect of two nuts on LAB

To further understand if *C. cathayensis* Sarg. and *T. grandis* nuts influence the growth of specific strains of LAB, we performed 16Sr RNA sequencing on yogurt samples. Because the two kinds of nuts at 2% and 5% concentrations had no significant effect on the total number of LAB and fermentation acid production, control and yogurt with a 2% addition of nuts were selected considering the reduction in sequencing costs. At the genus level, the addition of *T. grandis* nuts increased the number of *Streptococcus* spp. of the yogurt, while *Lactobacillus* spp. decreased (Figure S1). At the species level, *S. thermophilus* and *L. delbrueckii* subsp. *Bulgaricus* accounted for more than 95% of the total abundance of *lactobacilli* in $TGY_{2\%}$, with *S. thermophilus* having a 9.36% increase in abundance compared to the control. The abundance of *Lactococcus lactis* in $TGY_{2\%}$ increased, while the abundance of *L. plantarum*, *Lactobacillus crispatus*, *Lactobacillus fermentum*, and *Bifidobacterium animalis* showed no significant changes. On the contrary, the abundance of *L. delbrueckii* subsp. *Bulgaricus* decreased in abundance by 10.37% (Figure 2A). The difference was that the abundance of *Lactobacillus* spp. in $CCSY_{2\%}$ fermented milk was significantly higher than that in control and $TGY_{2\%}$ groups, and the abundance of *S. thermophilus* was significantly lower than that in control and $TGY_{2\%}$ at the species level, with an abundance of about 50% of the abundance in the control and only 44% of the abundance in $TGY_{2\%}$. In addition, the abundance of *L. Johnsonii* and *B. animalis* was slightly lower than that of the control and $TGY_{2\%}$ groups, respectively. The abundance of *L. delbrueckii* subsp. *Bulgaricus* in the $CCSY_{2\%}$ group was 2.7 and 1.8 times higher than that of the $TGY_{2\%}$ and control abundance, respectively, and the abundance of *L. lactis*, *L. crispatus*, *L. fermentum,* and *Brettanomyces claussenii* were slightly more abundant than in the other two groups. Mandalari *et al.* (2008) also reported that almond lipids have a role in the growth of probiotic bacteria, such as *Bifidobacteria* and *Eubacterium rectale*. This may

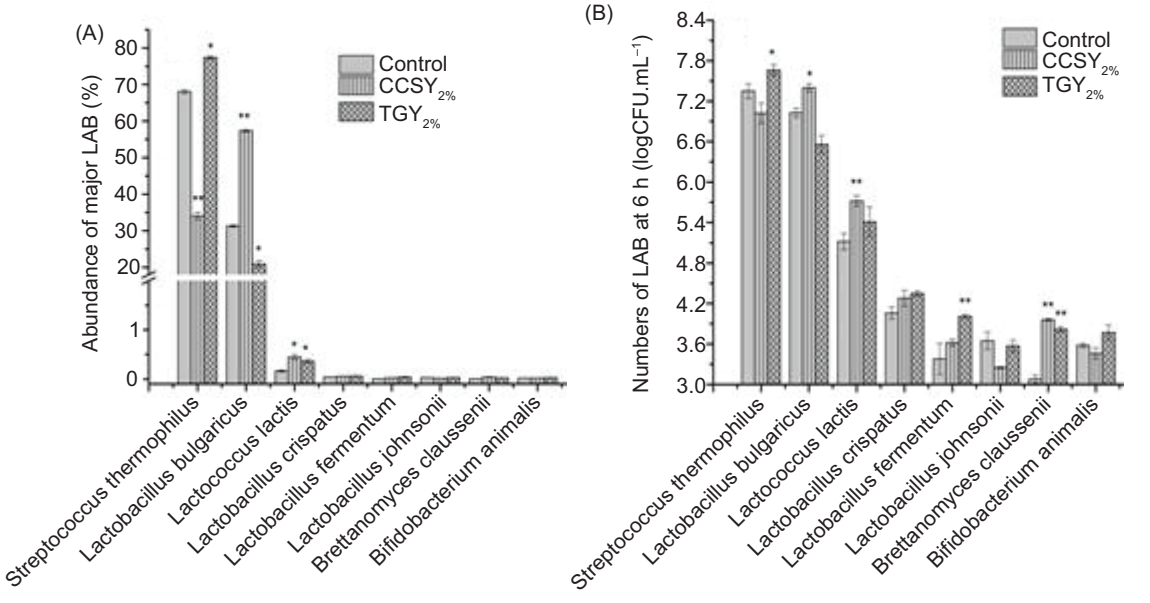

Figure 2. Abundance and numbers of major LAB in yogurt samples at 6 h. * and ** denote a significant difference at P<0.05 and P<0.01, respectively, compared with control.

be ascribed to the intrinsic variations in nut contents, including oligopeptides, polyphenols, and dietary fiber (Gupta and Abu-Ghannam, 2012).

The viable culture counting approach was used to further investigate the effects of *C. cathayensis* Sarg. and *T. grandis* nuts on the populations of the primary LAB. The results are compatible with the sequencing data (Figure 2B). While *C. cathayensis* Sarg. enhanced the growth and reproduction of *L. delbrueckii* subsp. *bulgaricus*, the addition of *T. grandis* nuts benefited the growth and reproduction of *S. thermophilus*. Furthermore, adding both nuts had a growth-promoting effect on four probiotic species, namely, *L. lactis*, *L. crispatus*, *L. fermentum*, and *B. claussenii*, compared to the control (Figure 2B), with abundance of results and counts in agreement. According to reports, cashew nuts are a rich source of vegetable protein (23%), carbohydrates (29.30%), essential fatty acids (44%), and unsaturated fats (82%). These nutrients can serve as a rich growth medium, enhancing the growth and metabolic activity of LAB (Bruno *et al*., 2019).

Effects of nuts on LAB's KEGG metabolic pathways

The addition of 2% *C. cathayensis* Sarg. promotes terpenoids and polyketides metabolism, amino acid metabolism, membrane transport, replication and repair, signal transduction, and translation (Figure 3). The metabolic abundance of glycan biosynthesis and metabolism, nucleotide metabolism, endocrine system, and xenobiotic biodegradation and metabolism were significantly ($P<0.05$) upregulated, indicating upregulation of the metabolic abundance of *L. delbrueckii* subsp., *L. lactis*, *L. crispatus*, *L. fermentum*, *L. lactis*, *L. cepaci*, and *L. cepa. crispatus*. *L. fermentum* and *B. claussenii* were slightly more abundant than the other two groups (control and $TGY_{2\%}$).

L. bulgaricus, *L. crispatus*, *L. fermentum*, and *B. claussenii* colonies, which were differentially higher than the control, support the hypothesis that enhanced carbohydrate metabolism, amino acid metabolism, and nucleotide metabolism may promote the growth and multiplication of LAB in $CCSY_{2\%}$ samples. Immune system, cell motility, transcription and energy metabolism, environmental adaptation, metabolism of cofactors and vitamins, lipid metabolism, folding, sorting, and degradation were downregulated compared with the control, and polyphenol flavonoids in *C. cathayensis* Sarg. may be involved in oxygen consumption and interfere with the energy of *S. thermophilus*. It's possible that the polyphenol flavonoids in *C. cathayensis* Sarg. interfere with the energy metabolism of *S. thermophilus*, lipid metabolism, and gene transcription, leading to a decrease in abundance and viable counts compared to the control. The addition of *C. cathayensis* Sarg. altered the physicochemical properties and composition of the liquid milk system, possibly challenging the environmental adaptation of *B. animalis* and *L. Johnsonii*. Environmental adaptation of *B. animalis* and *L. Johnsonii* was challenged and may have further affected some of their functional protein folding and degradation, immune system, and cellular activity (Table S1); this also resulted in lower abundance and colony counts than the control group to varying degrees. Two percent of *T. grandis* was added to promote xenobiotic

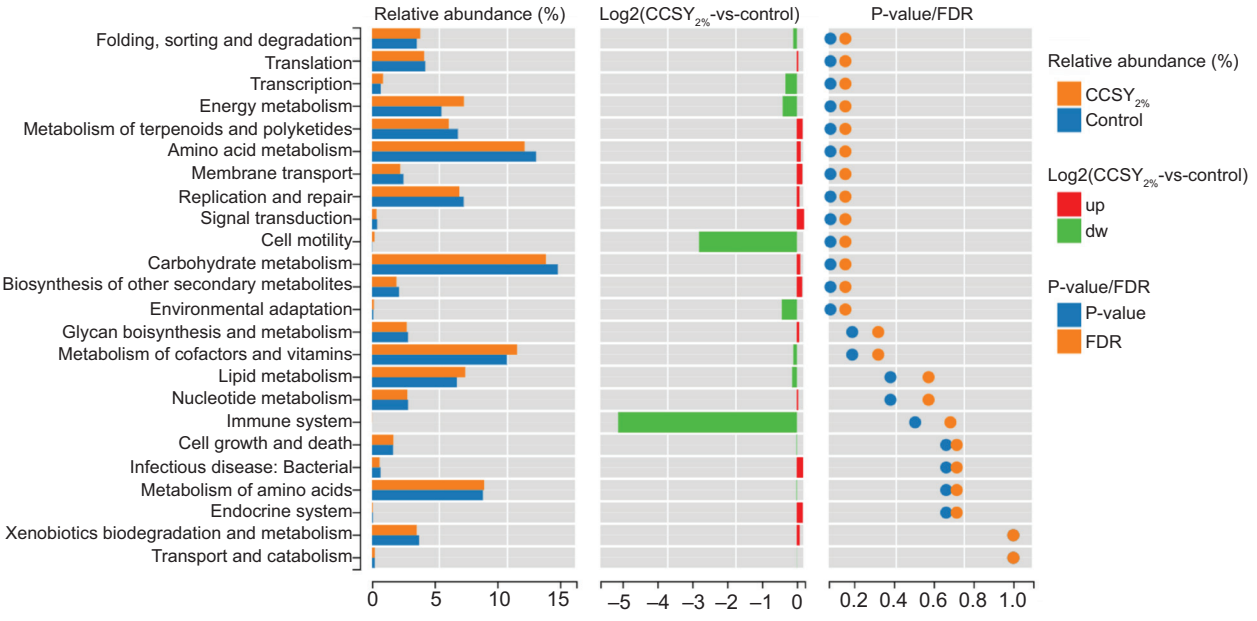

Figure 3. Effect of *C. cathayensis* Sarg on KEGG pathways in LAB.

biodegradation and energy metabolism. Energy metabolism, environmental adaptation, cell growth and death, metabolism of other amino acids, lipid metabolism, nucleotide metabolism, and translation were significantly upregulated, which may better explain the possible upregulation of metabolic abundance in *T. grandis* of *Streptococcus* spp. as well as *L. lactis*, *L. crispatus*, *L. crispatus*, *L. fermentum*, and *B. animalis* abundances (Figure 4). In particular, *Streptococcus* spp. requires an acclimatized environment, adequate energy, and amino acid supplementation for growth during the pre-fermentation period, and the amino acids and lipids in *T. grandis* fortify the nutrients required for their cellular growth, affixing a significant upregulation of their primary bile acid biosynthesis and fatty acid synthesis in particular (Table S2). Conversely, *T. grandis* also enhanced cell motility, the endocrine system, and the metabolism of terpenoids and polyketides in *L. delbrueckii* subsp. *bulgaricus* and *L. crispus* (Figure 4). Carbohydrate metabolism and amino acid metabolism pathways are adversely affected to some extent, especially starch and sucrose metabolism, lipopolysaccharide biosynthesis, flagellar assembly, and bacterial chemotaxis abundance, which were significantly downregulated, resulting in lower numbers of *L. delbrueckii* subsp. *bulgaricus* and *L. crispus* compared to controls (Figure 4).

How the two nuts affect the metabolism, growth, and reproduction of *L. delbrueckii* subsp. *bulgaricus* is still unclear, and in-depth transcriptomics and metabolomics are needed to explore the possible mechanisms. Metabolomics technology was used to reveal significant changes in the metabolites, including antioxidant and anti-inflammatory properties, in walnut milk fermented by *L. plantarum*. The researchers identified key pathways, including lipid and amino acid metabolism, steroid hormone biosynthesis, bile secretion, protein digestion, oxidative phosphorylation, and the tricarboxylic acid cycle. Walnut and other nuts often affect the primary metabolism of LAB, including carbohydrate metabolism, fat metabolism, and amino acid metabolism (Li *et al.*, 2023; Udayarajan *et al.*, 2022), which is consistent with the addition of *C. cathayensis* Sarg and *T. grandis*.

Effect of two kinds of nuts on the nutritional composition of yogurt

C. cathayensis Sarg. and *T. grandis* nuts are rich in protein, fat, carbohydrates, and dietary fiber (Table S3), and the addition of 2% and 5% nuts significantly enriched the nutritional composition of yogurt. The protein, fat, and dietary fiber contents of CCSY and TGY were significantly higher than those of the control yogurt. The two nut yogurts had a protein increase of 0.2–0.7%, and the fat in $CCSY_{2\%}$, $CCSY_{5\%}$, $TGY_{2\%}$, and $TGY_{5\%}$ was higher than the control by 0.5%, 1.7%, 0.7%, and 1.9%, respectively. Yogurt dietary fiber content and carbohydrate increase were in the range of 0.09–0.34% and 0.2–0.8%, respectively. Furthermore, the total solids and nonfat milk solids content also increased with the addition of 5% *C. cathayensis* Sarg. The addition of 5% *T. grandis* resulted in a significant increase in total solids and nonfat milk solids content of 4.5% and 2.1%, respectively.

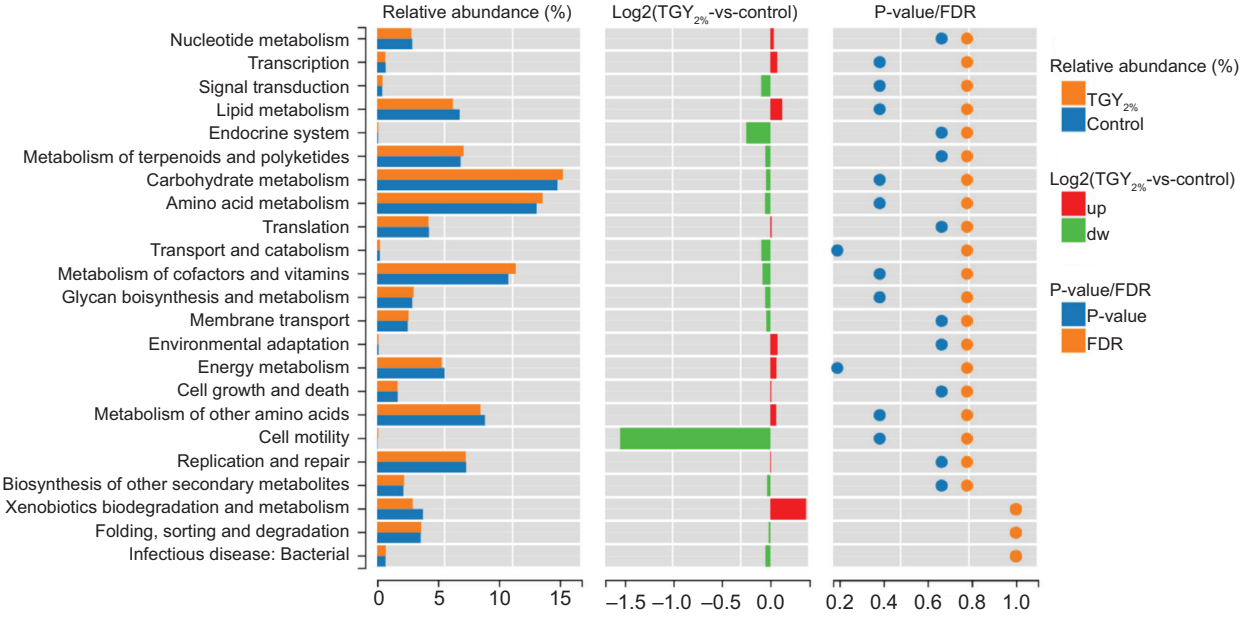

Figure 4. Effect of *T. grandis* on KEGG in LAB.

Cashew is known to contain alkaloids, tannins, flavonoids, phenolics, and other compounds, as well as high levels of nutrients such as proteins, carbohydrates, essential fatty acids, unsaturated fats, vitamins, and minerals (Shori *et al*., 2022), which also enhance yogurt's nutrient richness.

Effect of two kinds of nuts on pH and viscosity of yogurts during refrigeration

During the 28-day storage period, the LAB continued to metabolize and create organic acids, resulting in an increase in acidity in all yogurt samples and a gradual drop in pH (Figure 5).

On days 14 and 21, adding 5% *C. cathayensis* Sarg. and *T. grandis* slowed down post-acidification, but other than that, significant changes in pH did not occur during the refrigeration period compared to the control. On the 28th day, both the control and nut yogurts had a pH of approximately 4.25. During the refrigeration period, *L. rhamnosus*, *L. casei*, and *L. plantarum* fermented the lactose into lactic and other organic acids, leading to a notable decline in the pH of the nut yogurt. The viscosities of the control yogurts were in the range of 2.25–2.6 Pa.s. The viscosities of yogurts containing nuts were higher than those of the control, primarily due to the increase in non-fat milk solids in the CCSY and TGY. In addition, the viscosity of the five yogurts increased during storage for 28 days at 4°C.

Effect of the nuts on syneresis and WHC of yogurts during refrigeration

There was no significant difference in the syneresis among five kinds of yogurt during 28 days of refrigeration (Figure 6). The syneresis of the control yogurt was in the range of 22.0–24.1%, whereas the syneresis of the CCSY and TGY varied in the range of 21.2–24.0%. Over the same storage period, the WHC of both nut yogurts increased to varying degrees compared to the control yogurt, and there was an upward trend with increasing nut addition. The addition of 5% *C. cathayensis Sarg.* and *T. grandis* significantly increased the WHC of the yogurts by a maximum of 6.4 and 8.1%, respectively. The increased WHC of the nut yogurt may be due to the dietary fiber and protein

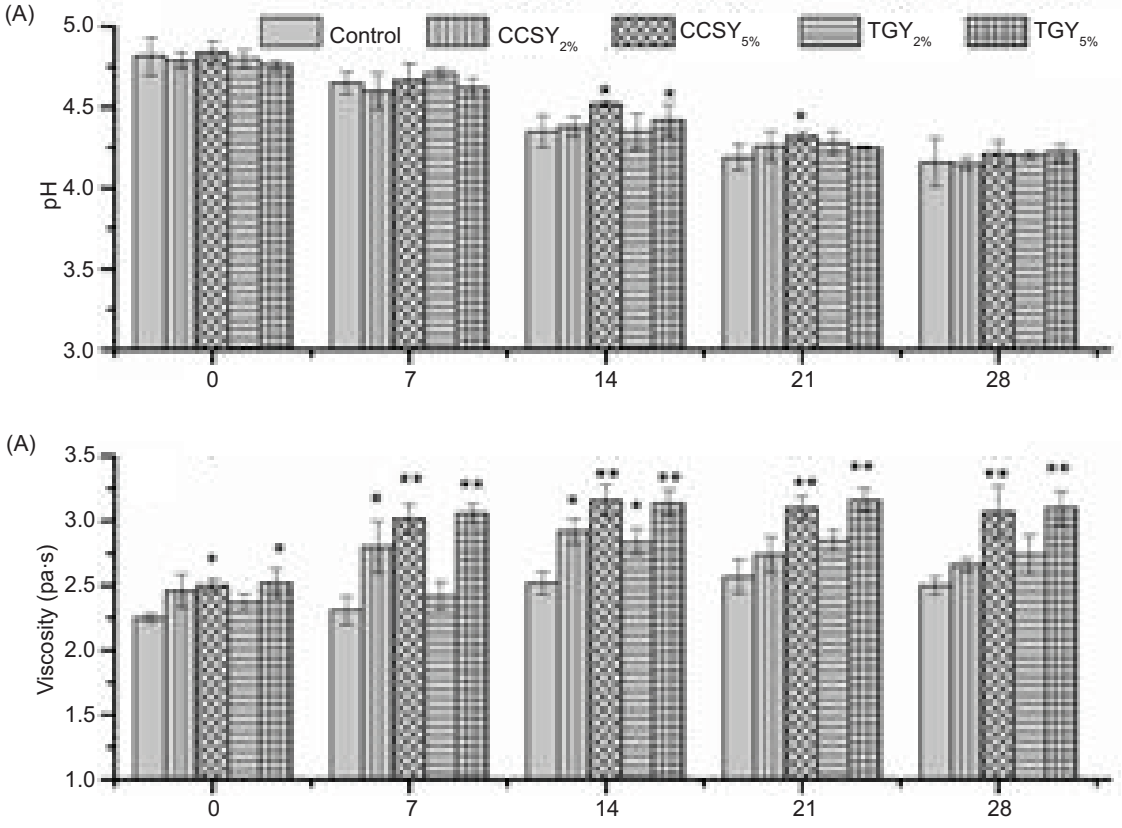

Figure 5. Changes in pH and viscosity of yogurts during refrigeration. * and ** denote a significant difference at P<0.05 and P<0.01, respectively, compared with control.

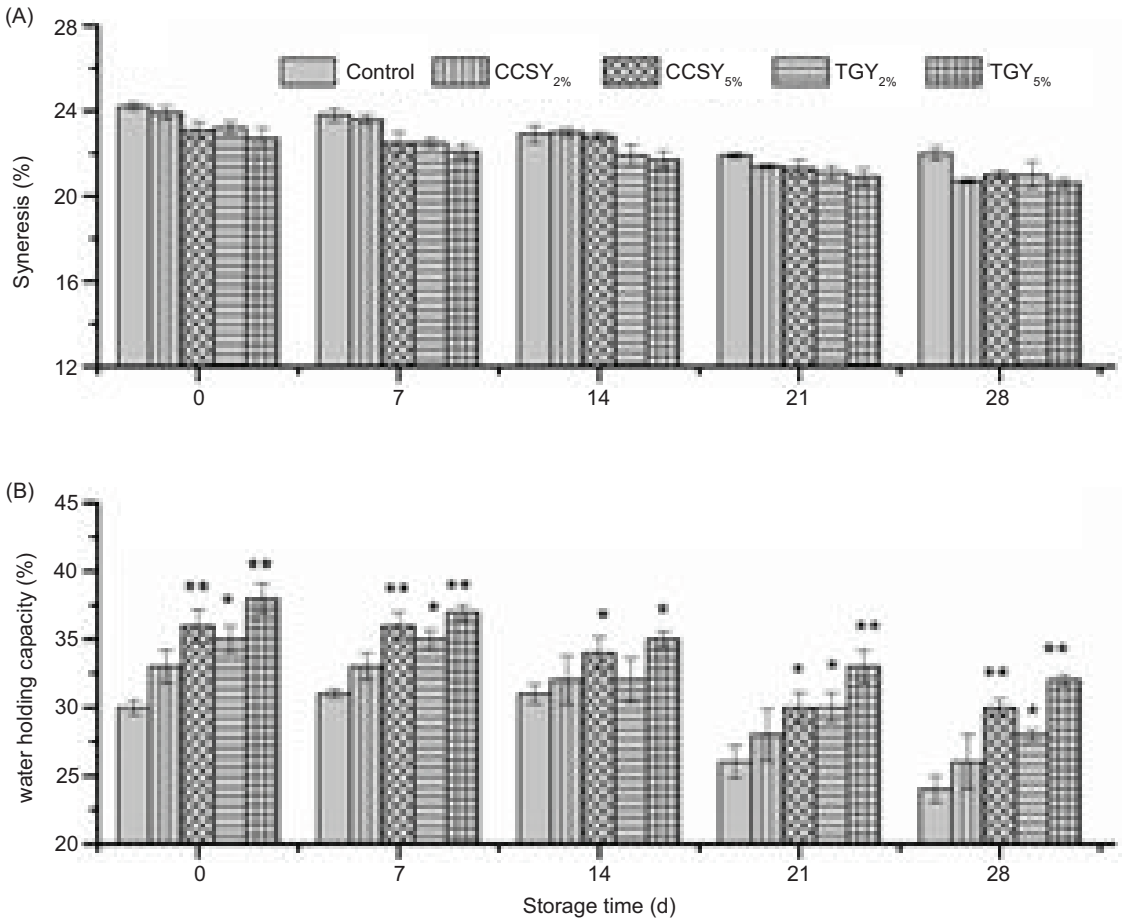

Figure 6. Changes of syneresis and water-holding capacity of yogurts during refrigeration. * and ** denote a significant difference at P<0.05 and P<0.01, respectively, compared with control.

from two kinds of nuts, as well as both generating a cohesive network structure (Yu *et al.*, 2022).

The number of LAB during refrigeration

The total number of LAB and the main LAB, *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, showed a slow decrease in all samples during 28 days of storage (Table 1). There was no significant difference in the total LAB counts of control, CCSY, and TGY throughout the cold storage period, with the total LAB counts of the three being in the ranges of 7.93–8.32 log CFU/mL, 7.86–8.30 log CFU/mL, and 7.90–8.28 log CFU/mL, respectively. During the same refrigeration period, the number of viable *S. thermophilus* in the CCSY decreased compared to the control, while the number of viable bacteria in the TGY increased to varying degrees compared to the control. In addition, the *S. thermophilus* viable counts of TGY were significantly higher than those of CCSY during the same refrigeration period, up to 0.77 logCFU/mL. The *L. delbrueckii* subsp. *bulgaricus* counts of TGY were differently increased compared with the control, with a maximum increase of 0.38 logCFU/mL. The viable count of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* decreased slowly in all control, CCSY, and TGY during the whole period of refrigeration, with a significant decrease in the control on the 29th day compared to the 1st day. In the CCSY, the numbers of *S. thermophilus* decreased significantly on day 29, whereas *L. delbrueckii* subsp. *bulgaricus* viable counts did not change significantly. Throughout the storage process, the total colony counts of the control, CCSY, and TGY lactic acid bacteria were greater than 10^7 CFU/mL.

Effect of nuts on the sensory quality of yogurt

C. cathayensis Sarg. and *T. grandis* had significant ($p < 0.05$, $p < 0.01$) effects on the aroma, taste, texture, and overall acceptability of the yogurt. Compared to the control, adding 2% or 5% *C. cathayensis* Sarg. and *T. grandis*

Table 1. Numbers of LAB in yogurt samples during refrigerated storage.

LAB (log CFU·mL^{-1})	Day	Control	$CCSY_{2\%}$	$CCSY_{5\%}$	$TGY_{2\%}$	$TGY_{5\%}$
	1	8.32 ± 0.14^{A}	8.30 ± 0.07^{A}	8.19 ± 0.09^{A}	8.28 ± 0.12^{A}	8.20 ± 0.18^{A}
	7	8.19 ± 0.10^{A}	8.16 ± 0.05^{A}	8.08 ± 0.04^{A}	8.18 ± 0.03^{A}	8.15 ± 0.22^{A}
Total number of LAB	14	8.14 ± 0.05^{A}	8.14 ± 0.11^{A}	8.03 ± 0.15^{A}	8.11 ± 0.14^{A}	8.10 ± 0.12^{A}
	21	8.06 ± 0.08^{A}	8.03 ± 0.16^{A}	7.95 ± 0.13^{A}	8.04 ± 0.07^{A}	8.01 ± 0.07^{A}
	28	7.93 ± 0.04^{B}	7.90 ± 0.12^{B}	7.86 ± 0.21^{A}	7.92 ± 0.19^{A}	7.90 ± 0.13^{A}
	1	7.41 ± 0.17^{aA}	7.09 ± 0.15^{bA}	7.06 ± 0.08^{bA}	7.86 ± 0.22^{bcA}	7.56 ± 0.09^{acA}
	7	7.28 ± 0.13^{aA}	6.96 ± 0.21^{aA}	6.93 ± 0.05^{aA}	7.80 ± 0.21^{bA}	7.45 ± 0.27^{aA}
S. thermophilus	14	7.12 ± 0.10^{aA}	6.85 ± 0.13^{aA}	6.78 ± 0.16^{abA}	7.72 ± 0.18^{cA}	7.27 ± 0.11^{adA}
	21	7.01 ± 0.09^{aB}	6.72 ± 0.16^{aB}	6.70 ± 0.11^{aA}	7.67 ± 0.25^{bA}	7.18 ± 0.15^{cA}
	28	6.88 ± 0.10^{aB}	6.62 ± 0.11^{aB}	6.62 ± 0.07^{aB}	7.39 ± 0.23^{bB}	7.16 ± 0.12^{bB}
	1	7.06 ± 0.07^{aA}	7.44 ± 0.16^{bA}	7.20 ± 0.19^{abA}	6.76 ± 0.14^{acA}	6.69 ± 0.08^{acA}
	7	7.00 ± 0.23^{aA}	7.32 ± 0.15^{aA}	7.11 ± 0.12^{aA}	6.67 ± 0.25^{bA}	6.59 ± 0.15^{bA}
L. bulgaricus	14	6.98 ± 0.08^{aA}	7.22 ± 0.19^{aA}	7.03 ± 0.07^{aA}	6.59 ± 0.16^{bA}	6.51 ± 0.09^{bA}
	21	6.85 ± 0.06^{aA}	7.15 ± 0.26^{aA}	6.92 ± 0.06^{aA}	6.56 ± 0.27^{abA}	6.49 ± 0.10^{acA}
	28	6.76 ± 0.12^{aB}	7.12 ± 0.07^{bA}	6.77 ± 0.18^{aB}	6.50 ± 0.21^{aA}	6.45 ± 0.21^{aA}

Different lowercase letters in the same row indicate a difference in *lactobacilli* counts at $p<0.05$ compared to the control group; different uppercase letters in the same column indicate a difference in *lactobacilli* counts at $p<0.05$ compared to the start of cold storage.

nuts had different levels of effect on the taste of yogurt (Figure 7). The addition of 5% *C. cathayensis* Sarg. and *T. grandis* changed the color significantly ($p<0.05$) from creamy white to light yellow. In terms of aroma, *C. cathayensis* Sarg. and *T. grandis* nuts enriched the yogurt with a nutty aroma that was pleasing to the judges, with a significant ($p < 0.05$) increase in aroma sensory scores compared to the control. Similarly, 2% and 5% of *C. cathayensis* Sarg. and *T. grandis* gave the yogurt an attractive nutty flavor, which became more intense with increasing nut concentration. Two kinds of yogurt curd were completely free of whey precipitation and had a more viscous, homogeneous tissue with a smooth, crack-free surface and a good state of organization, with a score of over 8.0 and significantly ($p < 0.05$) higher than that of the control. The two yogurts that contained nuts had overall sensory scores of about 8.0 or higher with distinctive aromas and flavors from yogurt, *C. cathayensis* Sarg., and *T. grandis* nuts. Their overall acceptability was higher than that of the control. Researchers have also enriched the fermented dairy with other nuts and showed differences in color, flavor, consistency, and general acceptability (Bensmira and Jiang, 2015).

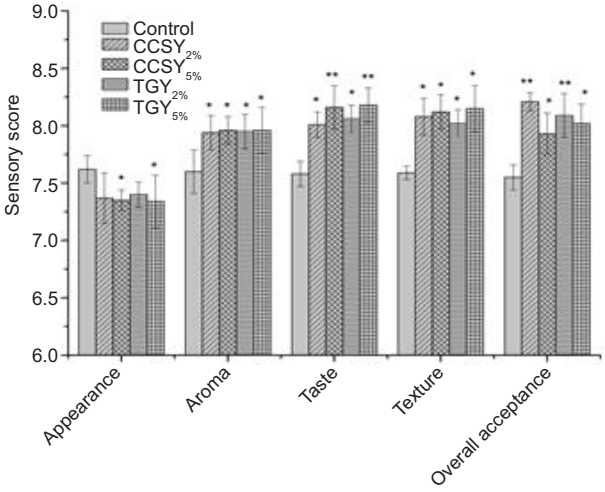

Figure 7. Sensory evaluation of yogurt. * and ** denote a significant difference at P<0.05 and P<0.01, respectively, compared with control.

Conclusion

Both *C. cathayensis Sarg.* and *T. grandis* nuts blended well with the yogurt, significantly increasing the nutritional value of the yogurt and improving its viscosities and WHCs. *They* both could promote the growth and reproduction of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*. *C. cathayensis* Sarg. could promote terpenoids and polyketides metabolism, amino acid metabolism, membrane transport, replication and repair, signal transduction, and translation of LAB. *T. grandis* was able to improve xenobiotic biodegradation, environmental adaptation, and cell growth of LAB. The precise mechanism by which the two nuts influence the metabolism of *lactobacilli* remains unclear; however, integrating

transcriptome and metabolomic investigations may elucidate this relationship.

Acknowledgments

Thanks to Dr. Ziyang Ye and Xinxia LV for the help with the revision and improvement of the paper. Thanks to Yaqi Qin for helping to prepare the yogurts.

Authors Contribution

Dong Liu was involved in conceptualization, methodology, formal analysis, investigation, software, data curation, writing of the original draft, and reviewing and editing. Huayin Zhao and Gang Liu were concerned with methodology and supervision. Ming Ye and Xinhua Liu were responsible for conceptualization, formal analysis, project administration, resources, supervision, funding acquisition, original draft preparation, and reviewing and editing.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

Funding for this work was provided by the Anqing Science and Technology Project (Grant Nos. 2022Z0003 and 2021Z0004), the Major Natural Science Research Project of colleges and universities of Anhui Province (Grant Nos. 2024AH040182 and KJ2021zd0164), and modern agricultural industry practice centers on integrated production and instruction (2023cj2x011). This is a company-commissioned project (W2021JSKF0706).

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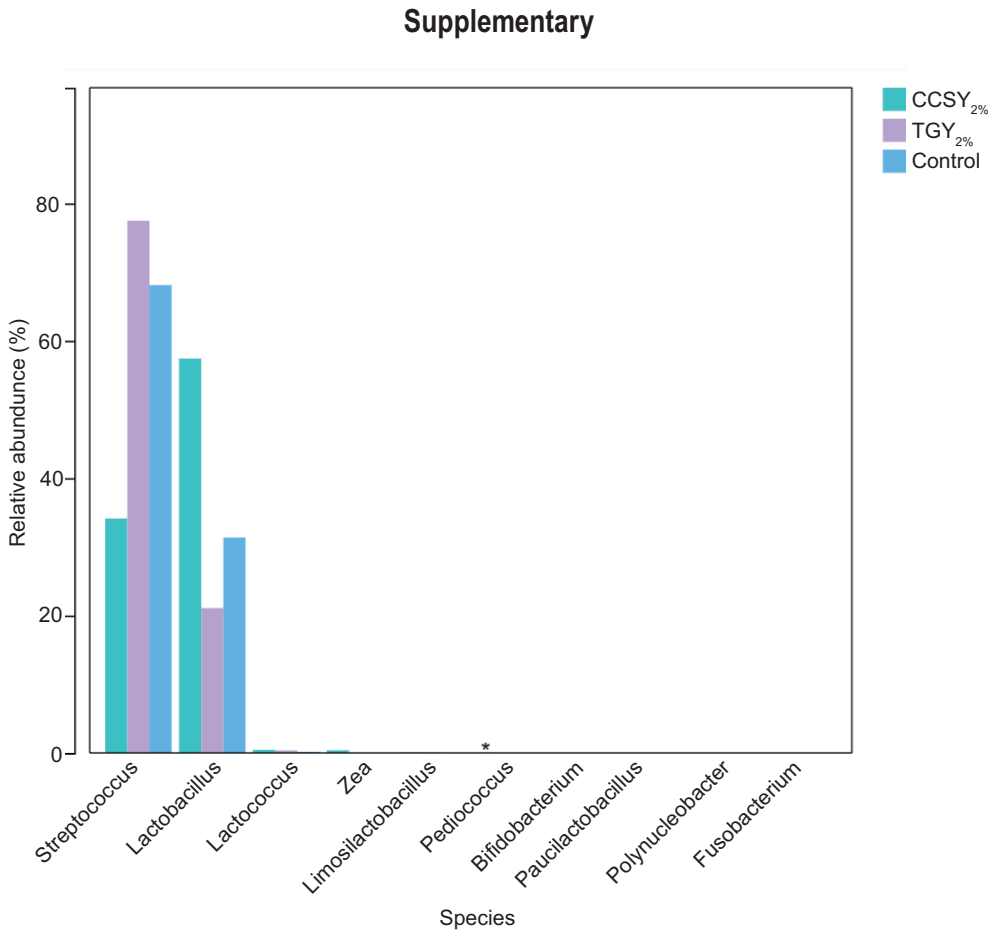

Figure S1. Effect of two nuts on differential lactic acid bacteria at the genus level.

Table S1. Effects of *C. cathayensis* Sarg. on LAB's KEGG metabolic pathways.

Function	$CCSY_{2\%}$ (%)	Control (%)	P-value	FDR
Xenobiotics biodegradation and metabolism	3.571264	3.770435	1.0	1.0
Immune system	0.01444	4.14E-4	0.506555	0.683849
Folding, sorting, and degradation	3.853562	3.58106	0.080856	0.155937
Translation	4.156352	4.257459	0.080856	0.155937
Transcription	0.87778	0.700677	0.080856	0.155937
Energy metabolism	7.329696	5.536275	0.080856	0.155937
Metabolism of terpenoids and polyketides	6.129259	6.857248	0.080856	0.155937
Amino acid metabolism	12.170475	13.09058	0.080856	0.155937
Membrane transport	2.253288	2.510754	0.080856	0.155937
Transport and catabolism	0.222874	0.222333	1.0	1.0
Cell growth and death	1.711137	1.692641	0.662521	0.715523
Infectious diseases: bacterial	0.6067	0.685586	0.662521	0.715523
Glycan biosynthesis and metabolism	2.767334	2.884518	0.19043	0.321351
Metabolism of other amino acids	8.947698	8.848862	0.662521	0.715523
Lipid metabolism	7.436758	6.777946	0.382733	0.574099
Replication and repair	6.963311	7.315841	0.080856	0.155937
Metabolism of cofactors and vitamins	11.562529	10.765565	0.19043	0.321351
Signal transduction	0.35921	0.413857	0.080856	0.155937
Cell motility	0.200044	0.028644	0.080856	0.155937
Nucleotide metabolism	2.822295	2.886893	0.382733	0.574099
Infectious diseases: parasitic	0.001198	6.83E-4	0.662521	0.715523
Immune diseases	0.0	3.0E-6	0.504985	0.683849
Carbohydrate metabolism	13.86324	14.81423	0.080856	0.155937
Digestive system	6.93E-4	2.07E-4	0.080856	0.155937
Endocrine system	0.070922	0.079596	0.662521	0.715523
Biosynthesis of other secondary metabolites	1.955794	2.164923	0.080856	0.155937
Environmental adaptation	0.152147	0.11277	0.080856	0.155937

Table S2. Effects of *T. grandis* on LAB's KEGG metabolic pathways.

Function	$TGY_{2\%}$ (%)	Control (%)	P-value	FDR
Nucleotide metabolism	2.816308	2.887273	0.662521	0.777742
Xenobiotics biodegradation and metabolism	2.921124	3.770366	1.0	1.0
Transcription	0.666976	0.700927	0.382733	0.777742
Signal transduction	0.441692	0.413874	0.382733	0.777742
Lipid metabolism	6.219459	6.776341	0.382733	0.777742
Immune diseases	1.0E-5	3.0E-6	1.0	1.0
Endocrine system	0.094373	0.079535	0.662521	0.777742
Metabolism of terpenoids and polyketides	7.10208	6.860136	0.662521	0.777742
Folding, sorting, and degradation	3.620325	3.581747	1.0	1.0
Infectious diseases: parasitic	0.002589	6.86E-4	0.19043	0.777742
Carbohydrate metabolism	15.256471	14.808861	0.382733	0.777742
Amino acid metabolism	13.588652	13.090956	0.382733	0.777742
Digestive system	9.17E-4	2.06E-4	0.19043	0.777742
Immune system	0.001661	4.19E-4	0.662521	0.777742
Translation	4.220297	4.258287	0.662521	0.777742

(*continues*)

Table S2. Continued.

Function	$TGY_{2\%}$ (%)	Control (%)	P-value	FDR
Transport and catabolism	0.237079	0.222374	0.19043	0.777742
Metabolism of cofactors and vitamins	11.375	10.769657	0.382733	0.777742
Glycan biosynthesis and metabolism	2.990777	2.884451	0.382733	0.777742
Infectious diseases: bacterial	0.709565	0.685774	1.0	1.0
Membrane transport	2.578168	2.507088	0.662521	0.777742
Environmental adaptation	0.10708	0.112738	0.662521	0.777742
Energy metabolism	5.301363	5.535653	0.19043	0.777742
Cell growth and death	1.680468	1.692682	0.662521	0.777742
Metabolism of other amino acids	8.490649	8.85057	0.382733	0.777742
Cell motility	0.085677	0.029283	0.382733	0.777742
Replication and repair	7.277208	7.315593	0.662521	0.777742
Biosynthesis of other secondary metabolites	2.214031	2.164517	0.662521	0.777742

Table S3. Composition of milk, *Carya cathayensis Sarg.*, *Torreya grandis*, and yogurts.

Composition	*Carya cathayensis Sarg.*	*Torreya grandis*	Milk	Control	$CCSY_{2\%}$	$CCSY_{5\%}$	$TGY_{2\%}$	$TGY_{5\%}$
Crude protein (g/100 g)	18.1±0.5	13.2±0.2	3.2±0.1	3.2±0.4	3.5±0.3*	3.9 ±0.2**	3.4±0.3*	3.6±0.5*
Fat (g/100 g)	50.6±0.3	56.8±0.3	3.8±0.3	3.8±0.2	4.3±0.4**	5.5±0.3**	4.5±0.5**	5.7±0.4**
Carbohydrates (g/100 g)	25.1±0.2	26.2±0.1	4.8±0.2	5.8±0.3	6.0±0.1	6.5±0.4*	6.2±0.5	6.6±0.2*
Dietary fiber (g/100 g)	7.4±0.3	8.1±0.2	/	0.03±0.01	0.12±0.02**	0.37±0.06**	0.15±0.03**	0.36±0.05**
Total solids (g/100 g)	/	/	12.3±0.2	18.0±0.4	19.4±0.2	22.2±0.3**	19.3±0.5	22.5±0.6**
Solid nonfat (g/100 g)	/	/	8.5±0.2	14.7±0.5	15.1±0.3	16.7±0.2*	14.8±0.3	16.8±0.1*