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The effects of *Trametes versicolor* fermented *Rosa roxburghii* tratt and coix seed quild on the nutrition, sensory characteristics and physical and chemical parameters of yogurt

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ARTICLE INFO

Keywords: Trametes versicolor Rosa roxburghii tratt and coix seed yogurt vitamin C γ-aminobutyric acid Polysaccharides Plackett–Burman design Response surface

ABSTRACT

Trametes versicolor can produce aromatic flavor in growth and development, widely used in food fermentation. This study used a One-Factor-at-a-Time (OFAT) test and response surface analysis to study the optimum fermentation parameters of *Rosa roxburghii* tratt and coix seed yogurt by *Trametes versicolor*. The best fermentation process is as follows: skim milk powder 17 %, sucrose content 4 %, *Rosa roxburghii* tratt and coix seed liquid 36 %, fermentation temperature 39 °C, inoculum 2 %, strain ratio 2:1:1(LB12: BLH1: Q-1), fermentation time 9.5 h. Under this fermentation process, the sensory score was 82.11, the contents of vitamin C, GABA, and total live bacteria in this yogurt were 13.89, 2.58, and 1.02 times higher than in common yogurt. Correlation analyses showed a significant contribution of the leavening agent to the GABA content of yogurt. This study provides a foundation for producing Rosa roxburghii tratt and coix seed yogurt with high sensory and nutritional value.

1. Introduction

Yogurt is a widely consumed and popular food worldwide. Edible fungi, which are large fungi that can be utilized as resources, have high protein, low sugar, low cholesterol, and high added value (Y. Zhang et al., 2021). Edible fungi is a traditional fermentation strain in the food processing industry, attracting attention for its ability to produce aromatic flavors during growth and development, similar to plant-like fragrances. Z. Wang, Gao, He, Zeng, Qin, and Chen (2022) found Four kinds of edible fungi that were used to ferment soybean dregs, which reduced the bean flavor and obtained the new aromatic flavor of soybean dregs. *Trametes versicolor* edible fungi belong to basidiomycetes, polyporaceae, *Thrombus* genus, which is rich in essential vitamins, amino acids, and polysaccharides. *Trametes versicolor* mainly depends on its own, containing a variety of enzymes, such as laccase, cellulase, protease, and so on, to degrade biomass into simple nutrients for its use. The structures of polysaccharides in fermentation broth, mycelium and fruiting body of *Trametes versicolor* are different, but they all have obvious antiviral, anti-tumor, anti-inflammatory and other pharmacological activities.

Rosa roxburghii Tratt is rich in nutrients and functional ingredients, especially vitamin C (VC) and superoxide dismutase (SOD). It is a well-known antioxidant that can quickly inactivate free radicals and oxidants and enhance the body's antioxidant defense mechanism (J. W. Xu, S. K. Vidyarthi, W. B. Bai, & Z. L. Pan, 2019). Coix seed rice contains phenols, flavonoids, polysaccharides, protein, fiber, and vitamins. The components of coix seed rice have strong antioxidant, anti-inflammatory, and anti-obesity activities (Erkaya-Kotan, 2020).

The microorganism plays a crucial role in shaping the flavor of yogurt flavor. The quality of fermented products can be improved by

https://doi.org/10.1016/j.fochx.2023.100969

Received 9 August 2023; Received in revised form 24 October 2023; Accepted 25 October 2023 Available online 28 October 2023 2590-1575/© 2023 The Authors. Published by Elsevier Ltd. This is an open access article under the C0



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inoculating suitable strains to control fermentation (Gu et al., 2021). In addition to *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which are commonly used in yogurt, more and more probiotics are used as yogurt starter, such as *Lactiplantibacillus paraplantarum*, *Bifidobacterium*, *Lactobacillus* acidophilus (T. Zhang et al., 2019), *Lactobacillus rhamnosus* (Kamal, Alnakip, Abd, & Bayoumi, 2018), etc.

In this research, L. plantarum subsp. plantarum LB12, B. animalis subsp. lactis BLH1, S. thermophilus Q-1 were used as fermentation strains of Rosa roxburghii tratt and coix seed yogurt. The effects of different processing parameters on sensory, nutrition (VC, polysaccharides), and microorganisms of Rosa roxburghii tratt and coix seed yogurt were studied by Plackett-Burman (PB) design and Box-Behnken (BBD) design. A new nutritious and healthy Rosa roxburghii tratt and coix seed yogurt with edible fungus flavor was developed. Based on obtaining the fermentation technology of Rosa roxburghii tratt and coix seed yogurt, to further understand the effect of Trametes versicolor fermentation on the quality of yogurt, we studied the quality and nutrition of two kinds of vogurt in the fermentation process. The Rosa roxburghii tratt and coix seed liquid fermented without Trametes versicolor (CY) and the Rosa roxburghii tratt and coix seed liquid fermented for 2 days (YCY) were selected to make vogurt according to the previously optimized fermentation process to explore the changes of pH, soluble solids, color, reducing sugar, polysaccharides, VC and γ -aminobutyric acid (GABA) and the number of living bacteria in the two kinds of yoghurt.

2. Materials and methods

2.1. Materials and strains

Skim milk powder, sucrose (local supermarket); *Rosa roxburghii* tratt (Guizhou Qiang Biotechnology Co., Ltd., China); coix seed (Guizhou Renxin Agricultural Development Co., Ltd., China). *Trametes versicolor*: GDMCC 5.178, purchased in Guangdong Microbial Strain Preservation Center. Lactic acid bacteria: *L. plantarum* subsp. *plantarum* LB12 (China Center for Type Culture Collection (CCTCC) NO: M2022948) was isolated from sour soup and preserved in a typical Chinese culture preservation center; laboratory-screened strain *B. animalis* subsp. *lactis* BLH1 (China Center for Type Culture Collection (CCTCC) NO: M20221979) was isolated from red sour soup and preserved in China General microbial strains Preservation and Management Center; *S. thermophilus* Q-1 was screened from Shanhua yogurt, China.

2.2. Mediums

Potato medium: PDB medium 25 g, potassium dihydrogen phosphate 2 g, peptone 2 g, magnesium sulfate 1 g, vitamin B1 8 mg, distilled water 1000 mL. MRS Medium (Shanghai Bio-way Technology Co., Ltd. China). PTYG Medium (Aleshan (Guangzhou) Biotechnology Co., Ltd., China). MC medium (Shanghai Bio-way Technology Co., Ltd, China). MC field MRS: After sterilization of MRS broth, add 500 μ g/mL L-Cysteine hydrochloride (Shanghai Bo Biotechnology Co., Ltd., China) and 100 μ g/mL Lin-mupirocin (Shanghai Bo Biotechnology Co., Ltd., China). An additional 2 % agar was required when the liquid medium was changed into the solid medium. All medium was sterilized at 121 °C for 20 min. LB12 was cultured in MRS broth medium for 24 h at 37 °C, BLH1 in PTYG liquid medium at 37 °C, 48 h. Q-1 in MRS broth medium aerobic culture at 37 °C for 48 h.

2.3. Liquid fermentation process of Trametes versicolor

Edible fungal liquid fermentation was carried out using the method of Gao (2021) with slight modifications. The tip mycelium of *Trametes versicolor* slant medium was dug up and inoculated into potato medium (100 mL) at 27 $^{\circ}$ C, 170 r/min for 4 days, and the strain was activated. The culture was homogenized under aseptic conditions to obtain seed suspension at 4 $^{\circ}$ C and set aside.

2.4. Preparation of Rosa roxburghii tratt and coix seed liquid fermented by Trametes versicolor

Wash the harmless coix seed rice with clean water three times, soak at 25 °C for 12 h, then beat according to the ratio of material to water at 1:15 (w / v), gelatinize 20 min in a water bath at 90 °C, add hightemperature α -amylase (200 U/g), hydrolyze 45 min in a water bath at 90 °C, finally add glucoamylase (300 U/g), adjust the water bath temperature to 65 °C, saccharify 80 min, refrigerate and set aside. Take out the Rosa roxburghii tratt fruit preserved at -20 °C, thaw at room temperature, remove the leaves and stalks, squeeze the juice with the original juicer, then pass 200 mesh, place it in a brown bottle in refrigerate. Coix seed solution (121 °C, 20 min) and Rosa roxburghii tratt juice (90 °C, 20 min) were sterilized, respectively. After cooling, the mixture of coix seed liquid: Rosa roxburghii tratt juice = 7:3 by volume was added to the suspension of 4 % Trametes versicolor seed bacteria suspension and fermented for 2 days (27 °C, 170 r/min). Then, the fermentation liquid was homogenized (6000 rpm, 1 min), ultrasonic (80 w, 1 h), over 200 mesh, sterilized in a brown bottle (65 °C, 30 min), refrigerated and set aside.

2.5. Preparation of Rosa roxburghii tratt and coix seed yogurt

Water, sucrose, and skimmed milk powder were mixed in a particular proportion, homogenized (6000 rpm, 5 min), heated 10 min at 90 °C, cooled to room temperature, and mixed with the fermentation broth of *Rosa roxburghii* tratt and coix seed fermented before. The activated lactic acid bacteria seed liquid (2×10^8 CFU/mL) was added and fermented at a specific temperature until the pH reached 4.6 \pm 0.1. The fermentation was terminated and ripened at 4 °C for 24 h.

2.6. Optimization of fermentation process conditions

The effects of different processing parameters on *Rosa roxburghii* tratt and coix seed yogurt were investigated using sensory organs, polysaccharides, and VC as indexes. The above single factors were optimized by PB experimental design, and four significant high-level factors were screened out. With the sensory score, the content of VC and polysaccharides as indicators, and according to the results of the One-Factorat-a-Time (OFAT) experiment, 1 level and-1 level were selected for each factor, as shown in Table S1. According to the significant factors obtained by the PB experiment, taking the contents of senses, VC, and polysaccharides as response indexes, the response surface method (RSM) was designed for four factors, such as *Rosa roxburghii* tratt and coix seed liquid, skim milk powder, sucrose and inoculation amount, by using Design-Expert 8.0 software, as shown in Table S2.

2.7. Sensory evaluation

The sensory characteristics of yogurt were evaluated by Baba, Jan, Punoo, Wani, Dar, and Masoodi (2018), and some improvements were made. Twenty teachers and graduate students who were familiar with the evaluation characteristics of *Rosa roxburghii* tratt and had experience in sensory evaluation of food-related courses were selected to evaluate the yogurt samples. The sensory evaluation scale is shown in Table S3. The yogurt sensory assessment is conducted in a food laboratory with good ventilation, light, and space. The four sensory aspects of tissue state, color, flavor, and taste were evaluated. The four sensory attributes were independently assessed using a score of 1 to 5. Finally, each sensory attribute score is multiplied by five times to get an index score. The higher the index score, the higher the quality of yogurt (Yoshikawa, Chen, Zhang, Scaboo, & Orazaly, 2014).

2.8. Determination of pH, soluble solids, and color

The pH value of the yogurt sample was recorded by a digital pH

meter (Shanghai Lichen Bangxi Instrument Technology Co., Ltd., China) and calibrated with standard pH 4 and 7 buffers before use. The soluble solids of the samples were detected by a digital display sugar meter. Calibrate the instrument with deionized water before use. Put the sample into a measuring cup and use a previously calibrated portable colorimeter to determine color properties, including L* (luminance), a* (red-green intensity), and b* (yellow-blue intensity).

2.9. Determination of polysaccharides and reducing sugars

The supernatant of the yogurt sample was centrifuged (6790 \times g/min, 20 min), and the supernatant of 5 mL was absorbed into the centrifuge tube. After adding 20 mL anhydrous ethanol, the supernatant was precipitated for 16 h and then centrifuged (6790 \times g/min 20 min). The supernatant was discarded, and the residue was re-dissolved and diluted 100 \times with water. Absorbance A was determined at 490 nm using a spectrophotometer with phenol–sulfuric acid (Chen & Huang, 2019).

The yogurt sample was centrifuged, and the supernatant of 1 mL was diluted with distilled water. Reducing sugar concentration was determined by the method with dinitro salicylic acid reagent (Luo, Guo, Wu, & Wan, 2018).

2.10. Determination of vitamin C and γ -aminobutyric acid

2.10.1. Determination of vitamin C

The method for determining VC in yogurt was modified according to GB 5009.86–2016 (China, 2016b). The chromatographic column was Agilent C18 column (internal diameter 4.6 mm, particle size 5 μ m), the detector was UV detector, the mobile phase was 0.1 % phosphoric acid solution-250 mm methanol (98:2), passed through 0.45 μ m filter membrane, ultrasonic 30 min, the flow rate was 0.7 mL/min, the detection wavelength was 245 nm, the column temperature was 25 °C, 20 μ L injection. The 0.5 g yogurt sample was weighed in a beaker, dissolved, and transferred to 50 mL with metaphosphoric acid solution (20 g/L). After ultrasonic 10 min, the sample was centrifuged (1697 \times g, 5 min). The supernatant passed through a 0.22 μ m filter membrane and was detected on the machine.

2.10.2. Determination of γ -aminobutyric acid

Absorb the sample of 10 mL yoghurt, centrifuge for 10 min at 15300 \times g, absorb the supernatant of 5 mL through 0.45 µm filter membrane, then take the supernatant of yogurt 0.2 mL, and then add 0.5 mol/L sodium bicarbonate solution of 0.2 mL and 1 % FDNB solution of 0.2 mL (dissolved with acetonitrile and placed in a brown bottle). The mixture was placed in a water bath at 60 °C to avoid light, and the derivatization reaction was carried out for 1 h. The filtrate is to be tested through the 0.22 µm aqueous phase filter membrane. The chromatographic column was the AcclaimTM120 C18 (4.6 \times 250 mm, 5 µm) with a UV detector. The mobile phase was 0.12 % phosphoric acid solution-100 % acetonitrile (50:50), the filter membrane was 0.22 µm, the ultrasonic 30 min, the flow rate was 1 mL/min, the detection wavelength was 370 nm, the column temperature was 35 °C, and the injection volume was 10 µL.

2.11. Microbial count

According to the procedure of GB 4789.35–2016 (China, 2016a), the samples were analyzed by microbiology. According to the estimated contents of BLH1, LB12, and Q-1 in the samples to be tested, 2 to 3 suitable diluents were selected continuously, and 0.1 mL was absorbed for plate counting. Q-1 was cultured on an MC Agar plate at 37 °C for 48 h, BLH1 on a modified MRS agar plate at 37 °C for 72 h, and LB12 on an MRS agar plate at 37 °C for 48 h.

2.12. Statistical analysis

All the experimental samples are in triplicate, and the experimental results are expressed as mean \pm standard deviation. The statistical analysis of all data is done using the software SPSS 21.0 (International Business Machine Inc., USA). The difference was analyzed by the Tukey test, and the means with different letters were significant. Design Expert 8.0 software (Stat-Ease Inc., USA) was used for multiple regression analysis of PB and RSM experimental design.

3. Results and discussions

3.1. OFAT test

3.1.1. Effect of skim milk powder on Rosa roxburghii tratt and coix seed yogurt

Skimmed milk powder retains most of the nutrients of milk and has good solubility, emulsifying, and foaming properties, which can increase the solid content in raw materials (Marafon, Sumi, Granato, Alcantara, Tamime, & Nogueira de Oliveira, 2011). Therefore, it is widely used in baking and dairy products (Phosanam, Chandrapala, Huppertz, Adhikari, & Zisu, 2020). As shown in Fig. 1A, when skim milk powder was 16 %, the sensory score of vogurt was the highest, with 80.48. However, with the increase in skim milk powder content, VC and polysaccharides show an upward trend, reaching a peak at 18 %, VC content is 1.15 mg/ g, and polysaccharides content is 4.17 mg/mL (P < 0.05). In Fig. 2A, skim milk powder had no significant effect on BLH1. With the increase of skim milk powder addition, the number of Q-1 first increased and then decreased, highest when added at less than 16 %. But LB12 showed a dynamic process. LB12 and Q-1 contribute highly to the number of living bacteria in yogurt, so the total number of living bacteria in yogurt increases first and then decreases with the increase of skimmed milk powder, reaching a peak at 16 %. The total number of living bacteria is 9.31 log CFU/mL. According to the above analysis, 16 % skim milk powder was selected to add yogurt.

3.1.2. Effect of sucrose on Rosa roxburghii tratt and coix seed yogurt

As shown in Fig. 1B, the higher the sucrose addition, the greater the overall sensory score of yogurt. The sensory score was the highest when sucrose was added at a 5 % concentration. Sucrose's expansion property enhances yogurt's total solids, texture, main body, viscosity, and humidity (Torrico, Tam, Fuentes, Gonzalez Viejo, & Dunshea, 2019). Our findings indicate that sucrose can increase the content of polysaccharides in yogurt (Fig. 1B), but it does not have a significant effect on the content of VC content (P > 0.05). When the sucrose amount ranges from 4 % to 5%, the polysaccharides content in yogurt reaches 3.30 mg/mL-3.49 mg/mL. With the increase of sucrose concentration, the number of BLH1 and Q-1 in yogurt initially rises and then declines (Fig. 2B). BLH1 had the highest viable count at a 2 % sucrose addition, while Q-1 reaches its peak level of live bacteria at 4 %. The change in the count of LB12 was not significantly affected by the shift in sucrose concentration. The total number of living bacteria in yogurt peaked when the sucrose concentration was 4 %. Adding 4 % sucrose is more appropriate for combining sensory, nutrition, and viable bacteria.

3.1.3. Effect of adding amount of Rosa roxburghii tratt and coix seed liquid on Rosa roxburghii tratt and coix seed yogurt

As shown in Fig. 1C, with the addition of *Rosa roxburghii* tratt and coxi seed fermentation broth, the sensory score of yogurt increased initially and then decreased. When the additional amount was 35 %, the sensory score of yogurt was the highest. *Rosa roxburghii* tratt juice is rich in VC and polysaccharides (H. Li, Fang, Wang, & Chen, 2022). *Trametes versicolor* can also produce ample polysaccharides during the fermentation of *Rosa roxburghii* tratt and coix seed liquid (K. Wang, 2017). Polysaccharides are among the primary active constituents of coix seed, possessing anti-inflammatory and immunological activities (Y. Li et al.,



Fig. 1. Effect of skim milk powder, sucrose, Rosa roxburghii tratt and coix seed liquid inoculation amount, and strain ratio on the sensory, VC and polysaccharide of Rosa roxburghii tratt and coix seed yogurt.

2019). Adding more *Rosa roxburghii* tratt and coix seed liquid resulted in a gradual increase in the VC content of yogurt, while the content of polysaccharides increased at first and then decreased, reaching its peak at 35 %. Furthermore, *Rosa roxburghii* tratt and coix seed solution at 40 % concentration could promote the growth of BLH1 and inhibit the growth of Q-1 (P < 0.05), while the number of LB12 showed a dynamic process (Fig. 2C). In summary, 35 % of the fermentation broth of *Rosa roxburghii* tratt and coix seed was selected as the best proportion.

3.1.4. Effect of inoculation amount on Rosa roxburghii tratt and coix seed yogurt

The impact of inoculation amount on the quality of Rosa roxburghii tratt and coix seed yogurt is shown in Fig. 1D. When the inoculation amount is below 2 % (v/w), the starter produces insufficient acid, resulting in subpar curd formation and a low sensory score. Conversely, when the inoculation amount exceeds 2% (v/w), the lactic acid bacteria produce excessive acid, leading to increased acidity and a decrease in sensory score (Fig. 1D). Li et al. (M. D. Li et al., 2022) study also found that with the rise in inoculation amount, the acid production rate of starter rose, the pH of yogurt significantly decreased, and the sensory score declined. Fig. 2D shows that the amounts of bacteria had no significant effect on the VC content in yogurt, which was maintained at 1.06 mg/g. Meanwhile, an increase in the inoculation amount resulted in a significant decrease in polysaccharide content (P < 0.05) due to the fermentation strains' utilization of polysaccharides and other nutrients. When the inoculation amount is 2 %, the content of polysaccharides in yogurt is the highest, reaching 4.69 mg/mL. Fig. 2D reveals that the effect of 1 % - 5 % inoculation amount on the total bacteria in yogurt follows a pattern of initial increase, subsequent decrease, and eventual growth. When the inoculation amount is 2 %, there is the highest number of living bacteria.

3.1.5. Effect of strains ratio on Rosa roxburghii tratt and coix seed yogurt

The acid production rate varies among different strains, and there is an interaction between strains (Atwaa, Shahein, El-Sattar, Hijazy, Albrakati, & Elmahallawy, 2022). Strains interact to create their unique growth model, and the fermentation process can be better controlled by selecting the appropriate proportion of strains (Zheng et al., 2020). When the strain ratio (LB12: BLH1: Q-1) was 1:2:1 or 1:1:2, the sensory score of yogurt was higher (Fig. 1E). When the strain ratio (LB12: BLH1: Q-1) was 2:2:1 or 1:2:1, the yogurt had higher contents of polysaccharides and VC (Fig. 1E). This shows that the effect of strains on polysaccharides and VC is related to not only due to specific strains, but also the combined effect of multiple strains. As shown in Fig. 2E, when the strain ratio (LB12: BLH1: Q-1) is 1:2:1, the number of BLH1 in yogurt is the highest, and when the inoculation ratio is 2:1:1, the number of total bacteria, LB12 and Q-1 in yogurt was higher. Therefore, the suitable inoculation proportion of yogurt is 2:1:1.

3.1.6. Effect of fermentation time on Rosa roxburghii tratt and coix seed yogurt

Lactic acid bacteria ferment lactose, producing lactic acid that interacts with milk protein to produce the unique texture and sour taste of yogurt. The production of lactic acid reduces the pH value of milk. When the pH reaches approximately 4–5, casein gel forms, causing the milk protein to solidify and giving yogurt its unique gel-like appearance



Fig. 2. Effect of skim milk powder, sucrose, Rosa roxburghii tratt and coix seed liquid inoculation amount, and strain ratio on the number of living bacteria of Rosa roxburghii tratt and coix seed yogurt.

(Ahmad, Hao, Li, Zhang, Ding, & Lyu, 2022). The fermentation time is too short, the yogurt curd is not good, too long, the yogurt taste is too sour, and the whey precipitates too much (Zheng et al., 2020). The optimal fermentation time, denoted as 9.5 h, yields the best taste and curd consistency (Fig. 1F). At the same time, the contents of VC in yogurt with a fermentation time of 8.5–9.5 h were significantly higher than those with fermentation time 10–10.5 h (P < 0.05). In addition, the number of total bacteria, LB12 and BLH1 in yogurt increased significantly during 8.5 h-9.5 h, So the best fermentation time was 9.5 h (Fig. 2F).

3.1.7. Effect of fermentation temperature on Rosa roxburghii tratt and coix seed yogurt

Fig. 1G shows that the highest overall sensory score of yogurt is achieved when fermented at 37 °C. It is found that decreasing the temperature from 43 to 45 °C to 32–39 °C, results in a thicker, smoother, and richer yogurt due to a denser protein matrix obtained under these

conditions (Guénard-Lampron, Villeneuve, St-Gelais, & Turgeon, 2020; Yang et al., 2021). The changes in VC and polysaccharide contents in yogurt are insignificant at 33–37 °C. However, at 37–39 °C, the content of polysaccharides decreased significantly (P < 0.05). While the content of VC increased significantly (P < 0.05). As the fermentation temperature rises, the number of BLH1 gradually increases, while the number of Q-1 and LB12 decreases gradually, the total number of bacteria initially increases and then decreases (Fig. 2G). At 41 °C, a large amount of whey precipitates, inhibiting bacterial growth and slowing down the rate of acid production, affecting yogurt's taste. According to the above results, 37 °C is more suitable for fermenting *Rosa roxburghii* tratt and coix seed yogurt.

3.2. PB Optimization experiment

Through the analysis of the OFAT experiment, the key variables affecting the quality of yogurt were determined by PB design. Table 1

Table 1 PB experiment results.

| Test number | X_1 | X_2 | X_3 | X4 | X5 | X ₆ | X7 | X ₈ | X9 | X ₁₀ | X ₁₁ | sensory | polysaccharides (mg/mL) | VC (mg/g) |
|-------------|---------|---------|---------|---------|---------|----------------|---------|----------------|---------|-----------------|-----------------|-----------------------------------|-----------------------------------|---------------|
| 1 | $^{-1}$ | $^{-1}$ | $^{-1}$ | $^{-1}$ | $^{-1}$ | $^{-1}$ | 1 | 1 | $^{-1}$ | 1 | 1 | 3.62 ± 0.64 | 2.92 ± 0.20 | 1.28 ± 0.02 |
| 2 | 1 | 1 | $^{-1}$ | 1 | $^{-1}$ | $^{-1}$ | 1 | 1 | 1 | $^{-1}$ | $^{-1}$ | 3.50 ± 0.40 | 3.46 ± 0.05 | 1.88 ± 0.07 |
| 3 | 1 | 1 | 1 | $^{-1}$ | $^{-1}$ | 1 | 1 | $^{-1}$ | $^{-1}$ | $^{-1}$ | 1 | 3.72 ± 0.81 | 4.42 ± 0.51 | 1.36 ± 0.07 |
| 4 | 1 | 1 | 1 | 1 | 1 | $^{-1}$ | $^{-1}$ | 1 | $^{-1}$ | 1 | 1 | 3.90 ± 5.81 | 3.65 ± 0.34 | 1.90 ± 0.02 |
| 5 | 1 | $^{-1}$ | $^{-1}$ | $^{-1}$ | 1 | $^{-1}$ | $^{-1}$ | $^{-1}$ | $^{-1}$ | $^{-1}$ | $^{-1}$ | 4.17 ± 1.67 | 3.89 ± 0.14 | 1.38 ± 0.03 |
| 6 | 1 | $^{-1}$ | $^{-1}$ | 1 | 1 | 1 | 1 | $^{-1}$ | 1 | 1 | 1 | 3.84 ± 4.00 | 3.16 ± 0.25 | 1.85 ± 0.03 |
| 7 | $^{-1}$ | 1 | $^{-1}$ | $^{-1}$ | 1 | 1 | $^{-1}$ | 1 | 1 | $^{-1}$ | 1 | 3.92 ± 3.04 | 3.29 ± 0.16 | 1.45 ± 0.02 |
| 8 | 1 | $^{-1}$ | 1 | $^{-1}$ | $^{-1}$ | 1 | $^{-1}$ | 1 | 1 | 1 | $^{-1}$ | 4.00 ± 1.39 | 3.81 ± 0.08 | 1.61 ± 0.00 |
| 9 | $^{-1}$ | 1 | $^{-1}$ | 1 | $^{-1}$ | 1 | $^{-1}$ | $^{-1}$ | $^{-1}$ | 1 | $^{-1}$ | 4.00 ± 1.83 | 3.11 ± 0.20 | 1.83 ± 0.03 |
| 10 | $^{-1}$ | $^{-1}$ | 1 | 1 | $^{-1}$ | $^{-1}$ | $^{-1}$ | $^{-1}$ | 1 | $^{-1}$ | 1 | 3.84 ± 2.40 | $\textbf{2.84} \pm \textbf{0.11}$ | 1.89 ± 0.02 |
| 11 | $^{-1}$ | 1 | 1 | $^{-1}$ | 1 | $^{-1}$ | 1 | $^{-1}$ | 1 | 1 | $^{-1}$ | 3.68 ± 4.72 | 3.48 ± 0.20 | 1.38 ± 0.03 |
| 12 | $^{-1}$ | $^{-1}$ | 1 | 1 | 1 | 1 | 1 | 1 | $^{-1}$ | $^{-1}$ | $^{-1}$ | $\textbf{3.82} \pm \textbf{3.41}$ | 2.70 ± 0.20 | 1.85 ± 0.02 |
| | | | | | | | | | | | | | | |

X: Skinosnilkoholuder (%), X Rosa roxburghii trRenpundtationisteinihistionisteinisteiniksisteinihistionisteinihisteinihisteinihi

shows the design matrix of 12 series consisting of 7 variables and the corresponding response values to sensory, polysaccharides, and VC content. Based on the results of the PB experiment (Table 2), the effects of various factors on sensory perception, polysaccharides, and vitamin C were analyzed. By conducting variance analysis, the linear regression equation with sensory (Y_1), polysaccharides (Y_2), and vitamin C (Y_3) as response values were obtained as follows:

 $\begin{array}{l} Y_1 \!=\! +76.29 \!-\! 1.48 X_1 + 2.05 X_2 + 0.26 X_4 + 0.39 X_5 \!-\! 0.35 X_7 + 2.23 X_8 + \\ 0.084 X_{10}. \end{array}$

 $Y_{3}{=}{+}1.63 + 0.03X_{1} + 0.22X_{4} + 1.667X_{5}{-}0.033X_{7} + 0.03X_{8} + 8.333X_{10}.$

The sensory score variance response analysis is shown in Table S4, and the regression model is obtained according to the analysis of variance P = 0.0482 < 0.05, indicating significance and a good fit. Skim milk powder, sucrose, and fermentation temperature significantly affect the sensory of yogurt. The variance response analysis of polysaccharides is shown in Table S5, revealing a *P*-value of 0.0224 < 0.05. The results showed that the model was significant and the fitting degree was good. Skim milk powder, *Rosa roxburghii* tratt and sucrose exerted notable effects on the polysaccharide content in the system. The VC variance response analysis is shown in Table S6. According to the analysis of variance, the regression model is obtained, in which P = 0.0157 < 0.05, indicating significance and a satisfactory fit. Only *Rosa roxburghii* tratt and coix seed liquid significantly affected VC in yogurt (P = 0.0011 < 0.01).

Based on the PB variance analysis of sensory organs, polysaccharides, and VC, skim milk powder, sucrose, *Rosa roxburghii* tratt and coix seed liquid, and fermentation temperature were selected to optimize the response surface with 4 factors and 3 levels.

3.3. Response surface optimization experiment

The response surface method can more intuitively analyze the interaction between multiple variables and find the optimal combination of experimental parameters, which has been widely used in food processing (C. Wang, Yin, Zhao, Zheng, Xu, & Yue, 2021). The results of the response surface experimental design are presented in Table 2. Tables S7-S9 show the analysis of variance for the sensory score (Y₃), polysaccharides (Y₄), and VC (Y₅). The regression equations for these variables with respect to the independent variables skim milk powder (A), sucrose (B), *Rosa roxburghii* tratt seed rice liquid (C), and fermentation temperature (D) are as follows:

Table S7 showed that the model was highly significant, with a misfit of 0.2524 > 0.05, indicating that the model's fitting degree was satisfactory. The corresponding P < 0.05 of A, D and A² showed that these two factors significantly impacted the sensory score of yogurt. The effect was as follows: skim milk powder > fermentation temperature. Fig. S1a shows that the interaction between factors BD 0.0099 < 0.01, and had a significant effect on sensory scores.

In Table S8, the model is 0.0078 < 0.01, indicating high significance and suggesting that it accurately reflects the experiment's actual situation. A, B, C, and A², P < 0.05, significantly impacting polysaccharides' content. The order of influence is as follows: *Rosa roxburghii* tratt seed rice liquid > sucrose > skim milk powder. The interaction of Table S8 and Fig. S1b demonstrates that the interaction of BC significantly affected the polysaccharide content.

Table S9 shows that the model had a P < 0.0001, demonstrating its high significance. A, C, and B² were found to have noticeable impacts on VC content, and the order of effect on VC content in one item was as follows: *Rosa roxburghii* tratt seed rice liquid > skim milk powder > sucrose. By considering both Table S9 and Fig. S1c, it can be observed that the *p*-value of AB was higher than that of AD, and both were below 0.05. Therefore, the interaction of AD had a more significant effect on the content of VC in *Rosa roxburghii* tratt and Coix seed yogurt.

3.4. Verification test

The optimum results were obtained from the response surface analysis: skim milk powder 17.16 %, sucrose 4.01 %, *Rosa roxburghii* tratt and coix seed liquid 36.19 %, fermentation temperature 39 °C. Under these optimal conditions, the predicted value of sensory score was 84.61, the polysaccharide value was 3.78 mg/mL, and the predicted value of VC was 1.66 mg/g. In order to facilitate actual experimental operation, the optimized conditions were revised: skim milk powder 17 %, sucrose 4 %, *Rosa roxburghii* tratt and coix seed liquid 36 %, fermentation temperature 39 °C. The sensory score, polysaccharides content, and VC content were 82.11, 3.74 mg/mL, and 1.53 mg/g, respectively, which closely matched the predicted results of the response surface analysis. That indicates that the industrial fermentation conditions of *Rosa roxburghii* tratt and coix seed yogurt obtained in this experiment were reliable.

3.5. Physicochemical changes of yogurt during fermentation

The mixed liquid of Rosa roxburghii tratt and coix seed was selected without adding Trametes versicolor (CY) and Trametes versicolor Response surface experimental design results.

| 1 | 1 | 0 | | | | | |
|----------------|-------------------------|----------------|---|----------------------------------|--|-----------------------------------|---|
| Test number | Skim milk powder (%) | Sucrose (%) | <i>Rosa roxburghii</i> tratt and coix seed liquid (%) | Fermentation temperature (°C) | Sensory score | polysaccharides (mg/ mL) | VC (mg/ g) |
| 1 | 0 | 0 | 0 | 0 | $\begin{array}{c} 83.60 \pm \\ 1.19 \end{array}$ | 3.26 ± 0.13 | $\begin{array}{c} 1.46 \pm \\ 0.04 \end{array}$ |
| 2 | -1 | 0 | 1 | 0 | $\begin{array}{c} 71.60 \pm \\ 0.75 \end{array}$ | 3.33 ± 0.07 | $\frac{1.83}{0.00}\pm$ |
| 3 | 1 | 0 | -1 | 0 | $\begin{array}{c} 81.00 \pm \\ 2.54 \end{array}$ | $\textbf{3.60} \pm \textbf{0.18}$ | $\begin{array}{c} 1.32 \pm \\ 0.03 \end{array}$ |
| 4 | -1 | 1 | 0 | 0 | $\begin{array}{c} \textbf{76.00} \pm \\ \textbf{1.25} \end{array}$ | $\textbf{3.84} \pm \textbf{0.18}$ | $\begin{array}{c} 1.59 \pm \\ 0.01 \end{array}$ |
| 5 | 0 | 1 | 0 | -1 | $\begin{array}{c} 80.00 \pm \\ 2.78 \end{array}$ | $\textbf{3.45} \pm \textbf{0.13}$ | $\begin{array}{c} 1.48 \pm \\ 0.01 \end{array}$ |
| 6 | 0 | 0 | 0 | 0 | $\begin{array}{c} \textbf{78.00} \pm \\ \textbf{2.37} \end{array}$ | $\textbf{3.86} \pm \textbf{0.06}$ | $\begin{array}{c} 1.56 \pm \\ 0.05 \end{array}$ |
| 7 | -1 | 0 | -1 | 0 | $\begin{array}{c} \textbf{72.60} \pm \\ \textbf{2.76} \end{array}$ | $\textbf{3.29} \pm \textbf{0.02}$ | $\begin{array}{c} 1.31 \pm \\ 0.01 \end{array}$ |
| 8 | -1 | 0 | 0 | -1 | $\begin{array}{c} \textbf{77.00} \pm \\ \textbf{2.11} \end{array}$ | $\textbf{3.29}\pm\textbf{0.05}$ | $\begin{array}{c} 1.62 \pm \\ 0.00 \end{array}$ |
| 9 | 0 | 0 | 1 | -1 | $\begin{array}{c} \textbf{79.00} \pm \\ \textbf{2.01} \end{array}$ | $\textbf{3.40}\pm\textbf{0.03}$ | $\frac{1.83}{0.00}\pm$ |
| 10 | 0 | 1 | 0 | 1 | $\begin{array}{c} 88.00 \pm \\ 3.30 \end{array}$ | $\textbf{3.43} \pm \textbf{0.15}$ | 1.59 ± 0.00 |
| 11 | 0 | 1 | -1 | 0 | 78.00 ± 0.13 | $\textbf{4.60} \pm \textbf{0.11}$ | 1.37 ± 0.04 |
| 12 | 1 | 0 | 1 | 0 | $\begin{array}{c} \textbf{72.00} \pm \\ \textbf{0.79} \end{array}$ | $\textbf{3.34}\pm\textbf{0.04}$ | $1.78~\pm$ 0.01 |
| 13 | -1 | 0 | 0 | 1 | 78.00 ± 1.26 | $\textbf{4.30} \pm \textbf{0.22}$ | 1.42 ± 0.00 |
| 14 | 0 | 0 | 0 | 0 | 75.00 ± | $\textbf{2.88} \pm \textbf{0.17}$ | 1.60 ± |
| 15 | 0 | -1 | 0 | 1 | 80.60 ± | $\textbf{3.66} \pm \textbf{0.24}$ | 1.52 ± 0.04 |
| 16 | 0 | 0 | 0 | 0 | 80.00 ± | $\textbf{3.09} \pm \textbf{0.07}$ | 1.56 ± |
| 17 | 0 | -1 | 0 | -1 | 80.00 ± | $\textbf{3.89}\pm\textbf{0.13}$ | 1.48 ± |
| 18 | 1 | 0 | 0 | 1 | 79.00 ± | $\textbf{4.28}\pm0.05$ | 1.59 ± |
| 19 | 0 | 0 | 0 | 0 | 88.00 ± | $\textbf{3.74} \pm \textbf{0.17}$ | 1.51 ± |
| 20 | 0 | 0 | 1 | 1 | 72.00 ± | $\textbf{3.93} \pm \textbf{0.14}$ | 1.86 ± 0.01 |
| 21 | 1 | 0 | 0 | -1 | 84.00 ± | $\textbf{4.04} \pm \textbf{0.08}$ | 1.44 ± 0.04 |
| 22 | 0 | 1 | 1 | 0 | 73.60 ± | $\textbf{3.78} \pm \textbf{0.28}$ | 1.67 ± 0.01 |
| 23 | 1 | 1 | 0 | 0 | 84.20 ± 3.9 | $\textbf{3.58} \pm \textbf{0.06}$ | 1.33 ± 0.05 |
| 24 | 0 | 0 | -1 | -1 | 75.80 ± | $\textbf{3.22}\pm\textbf{0.09}$ | 1.20 ± |
| 25 | 0 | 0 | -1 | 1 | 79.00 ± | $\textbf{4.23}\pm\textbf{0.04}$ | 1.27 ± 0.00 |
| 26 | 0 | -1 | -1 | 0 | 74.00 ± | $\textbf{3.25}\pm\textbf{0.08}$ | 1.21 ± 0.04 |
| 27 | -1 | -1 | 0 | 0 | 2.55 76.00 ± 2.56 | $\textbf{3.94} \pm \textbf{0.08}$ | 1.42 ± 0.04 |
| 28 | 1 | -1 | 0 | 0 | 81.00 ± | $\textbf{3.68} \pm \textbf{0.06}$ | 1.46 ± |
| 29 | 0 | -1 | 1 | 0 | 75.20 ± 3.72 | 3.41 ± 0.08 | 1.74 ± 0.01 |

fermented for 2 days (YCY). Subsequently, *Rosa roxburghii tratt* and coix seed yogurt was prepared using optimized conditions.

3.5.1. Changes in pH, soluble solids, and color

The pH value, soluble solids, and color changes during yogurt fermentation are shown in Table 3. The pH trend of the two kinds of yogurt samples was similar. The decrease in pH is attributed to the consumption of lactose and the production of lactic acid and other metabolites by *Lactiplantibacillus paraplantarum, Streptococcus thermophilus* and *Bifidobacterium* (Huang, Yu, Zhou, Sun, Liu, & Liang, 2020). Lowering the pH promotes the solidification of yogurt by facilitating casein binding in milk micelles (Fox, 2001). The initial pH of the two

kinds of yogurt samples was about 5.6, during this time (2 h), microorganisms adapted to the substrate. Between 2 and 6 h, the pH decreased as the bacteria metabolized lactose more rapidly in preparation for the next stage (Bezerra, Souza, & P Correia, 2012). Due to intense microbial activity, tthe pH rapidly reduced from 6 to 8 h. After 8 h of fermentation, the pH decreased to 4.97 (CY) and 4.89 (YCY), respectively. The pH value of 5.0 is essential in the acidification process of yogurt, as it marks the beginning of gel formation (Tribst, Ribeiro, Leite Junior, de Oliveira, & Cristianini, 2018). After 9.5 h fermentation, the pH decreased, approaching the isoelectric point of casein (pH = 4.6), which is sufficient for yogurt production. Similar pH changes during milk fermentation for yogurt production have also been observed in other studies

Table 3

Physicochemical changes of yogurt during fermentation.

| Indicator | Samples | Fermentation time (h) | | | | | | | |
|---------------------|---------|-------------------------|---------------------------------------|---------------------------------------|---------------------------------------|------------------------|------------------------|--|--|
| | - | 0 | 2 | 4 | 6 | 8 | 9.5 | | |
| pН | CY | 5.59 ± 0.01^a | 5.59 ± 0.01^{ab} | 5.57 ± 0.01^{ab} | 5.53 ± 0.02^{b} | 4.97 ± 0.03^{b} | 4.50 ± 0.01^{c} | | |
| | YCY | $5.68\pm0.02^{\rm a}$ | $5.67\pm0.02^{\rm a}$ | $5.64\pm0.01^{\rm a}$ | 5.59 ± 0.01^a | $4.89\pm0.00^{\rm b}$ | $4.42\pm0.02^{\rm b}$ | | |
| SSD% | CY | 14.43 ± 0.06^{ab} | 14.63 ± 0.06^{a} | 14.47 ± 0.06^{ab} | $14.23\pm0.12^{\rm b}$ | 11.70 ± 0.25^{c} | $10.93\pm0.12^{\rm d}$ | | |
| | YCY | $14.33\pm0.06^{\rm a}$ | $14.33\pm0.06^{\rm a}$ | 14.30 ± 0.00^a | $14.13\pm0.06^{\rm b}$ | $10.83\pm0.06^{\rm c}$ | $10.80\pm0.00^{\rm d}$ | | |
| L* | CY | $58.80\pm0.36^{\rm c}$ | $59.27\pm0.64^{\rm c}$ | $59.97 \pm 1.15^{\mathrm{bc}}$ | $61.70\pm0.10^{\rm b}$ | $64.23\pm0.42^{\rm a}$ | $65.40\pm0.70^{\rm a}$ | | |
| | YCY | $59.00\pm0.70^{\rm c}$ | $59.47\pm0.90^{\rm c}$ | $56.50 \pm 0.17^{\rm d}$ | $61.70\pm0.10^{\rm b}$ | $65.20\pm1.04^{\rm a}$ | $65.40\pm0.70^{\rm a}$ | | |
| a* | CY | -1.40 ± 0.62^{ab} | -0.93 ± 0.21^{ab} | $-1.53\pm0.40^{\rm b}$ | $-0.07\pm0.15^{\rm b}$ | $0.03\pm0.06^{\rm a}$ | $0.40\pm0.10^{\rm a}$ | | |
| | YCY | -1.10 ± 0.53^{ab} | -1.00 ± 0.36^{ab} | $-1.73\pm0.06^{\rm b}$ | $-2.27\pm0.93^{\rm b}$ | -0.07 ± 0.32^{a} | $0.00\pm0.50^{\rm a}$ | | |
| b* | CY | 3.83 ± 0.61^{ab} | $3.67\pm0.64^{\rm b}$ | $5.83 \pm 1.47^{\rm a}$ | $3.73\pm0.59^{\rm b}$ | 3.87 ± 0.15^{ab} | $3.67\pm0.21^{\rm b}$ | | |
| | YCY | 0.97 ± 0.47^{bc} | 2.23 ± 1.86^{ab} | -0.10 ± 0.36^{c} | 1.57 ± 0.49^{abc} | 3.57 ± 0.40^{a} | $3.50\pm0.17^{\rm a}$ | | |
| LB12 viable counts | CY | $6.47\pm0.26^{\rm d}$ | $6.81\pm0.03^{\rm d}$ | $7.15\pm0.11^{\rm c}$ | $7.85\pm0.08^{\rm b}$ | $8.11\pm0.00^{\rm b}$ | 8.54 ± 0.09^{a} | | |
| | YCY | $6.60\pm0.02^{\rm c}$ | $6.76\pm0.01^{\rm c}$ | $7.17\pm0.00^{\rm b}$ | $7.33\pm0.23^{\rm b}$ | $7.40\pm0.00^{\rm b}$ | 8.40 ± 0.09^{a} | | |
| Q-1 viable counts | CY | $6.37\pm0.30^{\rm e}$ | $6.77\pm0.02^{\rm d}$ | $7.05\pm0.05^{\rm d}$ | $7.43\pm0.08^{\rm c}$ | $8.20\pm0.02^{\rm b}$ | $9.21\pm0.03^{\rm a}$ | | |
| | YCY | $6.50\pm0.13^{\rm e}$ | $6.73\pm0.03^{\rm d}$ | $7.32\pm0.04^{\rm c}$ | $7.32\pm0.04^{\rm c}$ | $7.77\pm0.01^{\rm b}$ | $8.95\pm0.01^{\rm a}$ | | |
| BLH1 viable counts | CY | $6.26\pm0.05^{\rm c}$ | $6.38\pm0.09^{\rm c}$ | 6.40 ± 0.06^{c} | $7.19\pm0.11^{\rm b}$ | $7.32\pm0.16^{\rm b}$ | 7.82 ± 0.04^{a} | | |
| | YCY | $6.31\pm0.06^{\rm c}$ | $6.38\pm0.11^{\rm c}$ | $6.40\pm0.11^{\rm c}$ | $7.17\pm0.01^{\rm b}$ | 7.69 ± 0.11^{a} | $7.97\pm0.29^{\rm a}$ | | |
| Total viable counts | CY | $6.86\pm0.21^{\rm f}$ | $7.17\pm0.02^{\rm e}$ | $\textbf{7.45} \pm \textbf{0.05}^{d}$ | 8.06 ± 0.08^{c} | $8.49\pm0.02^{\rm b}$ | 9.31 ± 0.04^{a} | | |
| | YCY | $6.97\pm0.04^{\rm f}$ | $\textbf{7.13} \pm \textbf{0.02}^{e}$ | $\textbf{7.58} \pm \textbf{0.02}^{d}$ | $\textbf{7.77} \pm \textbf{0.08}^{c}$ | 8.13 ± 0.04^{b} | 9.12 ± 0.04^{a} | | |

Significant differences between letters in the same row of the table (p < 0.05); CY: Rosa r^{oxburghii} tratt and coix seed yoghurt, YCY fermented Rosa roxburghii tratt and coix seed yogurt with Trametes versicolor. SSD: soluble solids.L*: brightness; +a*: redness, -a*: greenness; +b*: yellowness, -b*: blueness.

(Medeiros, Souza, & Correia, 2015).

In the process of fermentation, the pH value of the YCY group decreased rapidly, possibly due to the high activity of the culture and the existence of fermentation components. The change in soluble solids in the two types of yogurt followed a similar trend as the pH value. Kristo, Biliaderis, and Tzanetakis (2003) suggest that low total solid content decreases buffering capacity, leading to a faster decline in pH when the same amount of acid is produced. Therefore, one of the reasons for the rapid decrease in pH value in the YCY group at 8 h was the lower content of soluble solids compared to CY.

Table 3 shows the L* (luminance), a* (redness) and b* (yellowness) values of the samples during yogurt fermentation. After 9.5 h of fermentation, the L* values and brightness of the two samples increased. the a* values of CY and YCY groups increased from -1.40 ± 0.62 and -1.10 ± 0.53 to 0.40 ± 0.10 and 0.00 ± 0.50 , respectively. *Rosa roxburghii* tratt is a yellow fruit, and the coix seed is beige. After fermentation, the brightness and redness of the two kinds of yogurt increased while the YCY group increased. That is due to the fermentation of *Rosa roxburghii* tratt and coix seed liquid by *Trametes versicolor*, which causes a variation in the b* value of the raw material. Consequently, there is a difference in the b* value between the CY and YCY samples.

3.5.2. Changes in reducing sugar and polysaccharides

The changes in the contents of polysaccharides and reducing sugars in the fermentation process of the two kinds of yogurt are shown in Fig. 3. In Fig. 3a, it can be observed that during the early and middle stages of fermentation (0 h-6 h), the polysaccharides content of CY was higher than that of YCY group, but in the later stage of fermentation (8 h-9.5 h), both groups experienced a significant decrease in polysaccharide content (P < 0.05). This decrease can be attributed to the slow growth and limited number of microorganisms in the initial stages of fermentation, resulting in low utilization of polysaccharides. Conversely, lactic acid bacteria adapted to the environment and grew rapidly in the later stage, leading to a rapid decrease in polysaccharide content. The higher polysaccharide content in YCY compared to CY at the end of fermentation (9.5 h) may be due to a slightly lower number of microorganisms in the former group (Table 3). Rosa roxburghii tratt coix seed liquid fermented by Trametes versicolor can produce polysaccharides, which are considered as one of the main bioactive components. It has biological activities such as enhancing immunity, anti-tumor, anti-radiation, antiaging, anti-oxidation and so on (Xie et al., 2015). As shown in Fig. 3b, the content of reducing sugar decreased from 62.27 \pm 1.54 to 56.79 \pm

1.42 (CY) and 66.34 ± 2.18 to 55.43 ± 2.71 (YCY) from 0 h to 9.5 h, respectively. Lactic acid bacteria significantly reduced the content of reduced sugar in yogurt, but the decrease in polysaccharides was even more significant. That can be attributed to the higher total amount of reducing sugar compared to polysaccharides, resulting in a slower decline. After 6 h of fermentation, CY exhibited a slightly higher rate of reducing sugar dissolution than YCY, which may be related to the glucose consumption rate by microorganisms (Hoang, Ferng, Ting, Huang, & Hsu, 2016).

3.5.3. Changes of vitamin C and γ -aminobutyric acid

VC in yogurt mainly comes from Rosa roxburghii (J. Xu, S. K. Vidyarthi, W. Bai, & Z. Pan, 2019). Fig. 3c shows that VC in the two kinds of yogurt changed dynamically with increased fermentation time. There was no significant change in VC content before fermentation (0 h) and after fermentation (9.5 h). That indicates that VC remains relatively stable during the yogurt fermentation, resisting decomposition and utilization. It is worth noting that the VC content of CY was only slightly lower than that of YCY group at 8 h. That may be due to the shaker culture fermentation condition of Trametes versicolor, which caused more loss of VC content of Rosa roxburghii tratt and coix seed fermented by adding Trametes versicolor, resulting in lower VC content in YCY fermentation compared to the CY group. GABA is a natural amino acid produced by the L-glutamic acid α decarboxylation catalyzed by glutamate decarboxylase (GAD). It is a bioactive compound that acts as a major inhibitory neurotransmitter in the central nervous system. It plays many metabolic roles in the citric acid cycle of microorganisms and plants (Garavand, Dalv, & G. Gómez-Mascaraque, 2022). As shown in Fig. 3d, the GABA content in YCY was significantly higher than that in the CY group for 0 h, which indicated that the fermentation of Rosa roxburghii tratt and coix seed liquid could increase the GABA content in yogurt. The content of GABA in the two kinds of yogurt increased with fermentation time (2–9.5 h), from 1.51 \pm 0.05 to 1.80 \pm 0.00 (CY) and 1.33 ± 0.00 to 2.27 \pm 0.03 (YCY), respectively. That demonstrates that lactic acid bacteria fermentation can increase the content of GABA in two kinds of yogurt. Similar results have been obtained by Fan et al., (2023).

3.5.4. Changes in the number of viable bacteria

Microorganisms play an important role in yogurt fermentation. The changing trend of the number of living bacteria in the fermentation process of the two kinds of yogurt is shown in Table 3. The number of living bacteria in the two kinds of yogurt significantly increased because



Fig. 3. Polysaccharides (a), sensory score (b), vc (c) and gaba(d) changes of two kinds of yogurt (cy and YCY) during fermentation, (e) is correlation analysis of *Trametes versicolor* fermented *Rosa roxburghii* tratt and coix seed yogurt (YCY).

the raw materials' nutrients such as amino acids and polysaccharides provided suitable growth conditions for bacterial growth (Ghosh, Chattoraj, & Chattopadhyay, 2013). After 2 h of fermentation, the live bacterial count in LB12 for both types of yogurt showed a significant increase. at 9.5 h, LB12 reached 8.54 ± 0.09 (CY) and 8.40 ± 0.09 (YCY). However, the growth of LB12 was inhibited in YCY, possibly due to the lower pH value compared to CY. The acidic environment of YCY affected the growth. From 0 to 9.5 h of fermentation, the number of living bacteria of Q-1 increased by 2.84 log CFU/mL in CY and 2.45 log CFU/mL in YCY, indicating a better growth rate of Q-1 in the two kinds of yogurt. There was no significant difference in the live bacterial count of BLH1 between the two types of yogurt. The growth rates of the three strains were Q-1 > LB12 > BLH1. After 9.5 h of fermentation, the total number of living bacteria in CY was higher than that in the YCY group.

3.6. Correlation analysis

Fig. 3e shows the Pearson correlation coefficients among the variables studied in YCY yogurt. Positive correlation indicates mutual promotion, while negative correlation suggests reciprocal inhibition. First of all, it can be seen that the VC content remains unaffected by other factors. Polysaccharides, reducing sugars, soluble solids, and pH correlated negatively with the number of living bacteria. That is because the strain utilizes sugars during yogurt fermentation, and increased lactic acid bacteria leads to stronger acid production and a lower pH. However, the number of living bacteria can promote GABA content, suggesting that BLH1, Q-1 and LB12 can metabolically produce GABA content. Previous studies have also shown that lactic acid bacteria can produce GABA (D. Wang, Wang, Lan, Wang, Zhao, & Hu, 2021). It can also be seen that L*, a*, and b* are mutually inhibited by polysaccharides, reducing sugars, soluble solids, and pH, but mutually promoted by the number of viable bacteria. The correlation analysis revealed the intrinsic relationship between different indicators of yogurt, which can help the subsequent improvement and control of yogurt quality.

4. Conclusion

Our study used the response surface model to optimize the processing parameters of *Rosa roxburghii* tratt and coix seed yogurt. There was no significant difference in sensory, VC, and polysaccharide contents between the predicted and predicted values. At the same time, the effects of *Trametes versicolor* fermentation on the physical and chemical indexes and functional components of yogurt were studied. We found that the *Trametes versicolor* fermentation of the *Rosa roxburghii* tratt and coix seed liquid increased the content of polysaccharides and GABA in yogurt, and slightly decreased the content of VC and the level of microorganisms. In this study, a new type of high-quality yogurt was obtained and the deep processing of *Rosa roxburghii* tratt and coix seed was realized at the same time.

CRediT authorship contribution statement

Yue Tian: Data curation, Writing – original draft. Shasha Zheng: Data curation, Software. Laping He: Resources, Writing – review & editing, Supervision. Cuiqin Li: Software, Validation, Supervision. Shunbin Qiao: Funding acquisition. Han Tao: Funding acquisition. Xiao Wang: Software, Supervision. Xuefeng Zeng: Resources.

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.

Acknowledgements

This work was financially supported by the High-level innovative talents training project of Guizhou Province (QKHPTRC-GCC[2022] 026-1), the Science and Technology Project of Guizhou Province (QKHCG[2021]YB031), Key Agricultural Project of Guizhou Province (QKHZC-[2021]YB184, QKHZC-[2021]YB278, QKHZC-[2021]YB142, QKHZC[2019]2382 and QKHZC[2016]2580), National Natural Science Foundation of China (31660010 and 31870002), Guizhou Science and Technology Program (QKHPTRC-[2020]5004), Qiankehe talents project ([2018]5781 and [2017]5788-11), and Guizhou University special position ((2021)C13).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100969.

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