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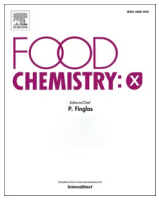

Physicochemical, structural and metabolic products of yogurt as affected by *Coriolus versicolor* fermented sweet potato pulp water

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ABSTRACT

Sweet potato pulp water (SPPW) is a kind of sweet potato starch processing by-product with rich nutrition but low utilization. The impacts of different proportions of *Coriolus versicolor* (*C. versicolor*, *CV*) fermented sweet potato pulp water (CV-SPPW) on the physicochemical, structural and metabolic properties of yogurt were investigated. Compared with 0% group, the hardness index, elasticity index and cohesion of the 10% sample group increased by 1.9-fold, 55.7% and 1.39-fold, respectively. When CV-SPPW was added at an amount of 10%, the microstructure and sensory scores of yogurts were considered as the optimal. Metabolic pathway analysis indicated that the changes of yogurts were mainly involved in sugar metabolism and amino acid metabolism, and that the carbohydrate metabolites produced mainly included cellobiose, maltitol, d-trehalose and d-maltose. The CV-SPPW improved the structural characteristics of yogurts to varying degrees and the fermented yogurts exhibited better viscosity properties.

1. Introduction

Sweet potato pulp water (SPPW) is a by-product of the sweet potato starch processing industry, and its annual output is one million tons. SPPW is rich in nutrients including proteins, carbohydrates, and nitrogen (Hu, Wu, et al., 2019). However, currently, there are still huge problems associated with the processing and utilization of SPPW, and its processing utilization rate is low, which not only brings huge economic losses to the enterprises, but also causes huge environmental pollution in treated unreasonably. (Cheng et al., 2015a; Yang et al., 2022). At present, the commonly used methods for SPPW treatment include magnetic flocculation (Mohamed Noor et al., 2022), and the modified chitosan method (Karchiyappan & Pachaiappan, 2022). However, the cost of such treatment is extremely high, and the expense of pollution control is even higher than that of the product. Therefore, with regard to seeking technical methods to promote the effective and value-added utilization of SPPW, it is a vital technical difficulty to advance the resourceful utilization of starch wastewater in the current starch slurry water industry, particularly in food products.

To overcome the disadvantages of unstable quality and insufficient gelation of yogurts, additives including guar gum and hydroxypropyl distarch phosphate are added as thickeners and stabilizers (Cui et al., 2014; Rafiq et al., 2020). However, the excessive intake of these chemical additives can cause allergic reactions in the body, disrupt intestinal homeostasis, and result in health risks including obesity (Sambu et al., 2022). Considering the mentioned disadvantages of chemical additives, it is vital to find new and safe methods to enhance the characteristics of yogurts. *Coriolus versicolor* (*C. versicolor*, *CV*) mushroom, also known as *Yunzhi* (China), is a kind of edible and medicinal fungus, and the active substances including polysaccharides produced by metabolism exert the antioxidant and antitumor effects (Saleh et al., 2017). Previous studies have indicated that the combination of *C. versicolor* fermentation broth and yogurt can improve the fermentation matrix and storage stability of yogurt, which may be caused by the significant effects of proteins and polysaccharides produced by *C. versicolor* fermentation on the structure and quality of yogurt, including improving the texture, taste, stability and nutritional value of yogurt (Zheng, 2023a). In addition, similar studies include *Ganoderma*

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lucidum fermentation broth that not only promotes the growth of lactic acid bacteria, but also enhances the taste and tissue state of yogurt by affecting the ability of yogurt gel to bind water (Li, 2012; Li & Xia, 2003). The addition of *C. versicolor* fermented sweet potato pulp water (CV-SPPW) improves the fermentation environment of lactic acid bacteria (*LAB*) (Fan et al., 2023a). Moreover, it has been previously indicated that polysaccharide has electrostatic and hydrophobic interactions with proteins in the presence of hydrogen bonds, therefore enhancing the network structure of the yogurts (Sun et al., 2023a). The presence of glycosidic bonds in polysaccharide can form more stable polysaccharide-protein complexes with proteins, improving the structural quality of food (Peng et al., 2022a). In summary, the *C. versicolor* fermentation broth was added to the yogurt to obtain a yogurt product with more diversified nutrition and more stable quality.

The addition of CV-SPPW as a substrate can change the metabolic environment of *LAB* in yogurts, affects the *LAB* metabolic pathway, and generates sugars to enhance the structural properties of yogurts. Carbohydrates including D-fructose and β-D-glucose can significantly improve the dehydration shrinkage, apparent viscosity, and elastic behavior of yogurts (Fan et al., 2023b). However, few studies have reported the use of SPPW as a liquid fermentation medium for *C. versicolor* and the effect of fermentation products on the physicochemical, structural, and metabolic profiles of yogurts. In this study, the effects of adding different proportions of CV-SPPW on the physicochemical, structural, and metabolic aspects of yogurts were evaluated, hoping to provide novel ideas for the industrial production of new yogurts with more stable quality.

2. Materials and methods

2.1. Materials

The *C. versicolor* strain was purchased from Minyuan fungi industry (Chongqing, China), while *lactobacillus* was obtained from Chuan Xiu International Trade Co. (Tongzhou, Beijing). Skim milk powder was purchased from Yili Co., Ltd. Sweet potatoes, cane sugar, and cornmeal were purchased from Walmart Supermarkets (Guiyang, Guizhou, China). Analytical grade chemicals and solvents included sulfuric acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium carbonate, 3,5-dinitrosalicylic acid, anhydrous ether, phenol, anhydrous ether, ether, magnesium acetate, sodium hydroxide, ferrous sulfate ($FeSO_4$), copper sulfate, absolute ethanol, magnesium acetate, and acetone. Food-grade glucose, peptone, magnesium sulfate, and potassium dihydrogen phosphate were purchased from Sinopharm Group Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Production of SPPW

The fresh sweet potato was cut into 1 cm × 1 cm × 1 cm pieces, then ground into pulp with a juicer based on the material-liquid ratio of 1: 2.5, passed through a 100-mesh sieve, and prepared for subsequent use. The SPPW obtained was homogenized with a high-speed disperser (3000 r/min, 1 min).

2.3. Preparation and principal component analysis of CV-SPPW

C. versicolor was cultured in potato glucose agar (PDA), stored at 4 °C, and subcultured once a month. Subsequently, the tubes were seeded in PDA enrichment medium (PDA + KH_2PO_4 + $MgSO_4$) at 26 °C for 8 days. The medium made of SPPW was autoclaved at 121 °C for 20 min and cooled to room temperature. Then, the seeds were tilted to select the 1 cm × 1 cm × 1 cm culture with a sterile inoculation loop under sterile conditions. The obtained SPPW was used as the culture medium of *C. versicolor*, and the optimized culture conditions were as follows: 24 h standing at 27 °C without shaking, followed by incubation at 170 rpm for 5 days. The *C. versicolor* fermented SPPW medium was consisted of glucose 25 g/L, peptone 3 g/L, KH_2PO_4 2 g/L, $MgSO_4$ 1 g/L, and cornstarch 1 g/L.

The main components of CV-SPPW were tested as follows. Total polysaccharides, and reducing sugars were examined with reference to the method of Yang Zhengbin et al. (Yang et al., 2023). The soluble protein concentration was determined with the Coomassie brilliant blue method (SN/T 3926–2014).

2.3.1. Monosaccharide composition of CV-SPPW polysaccharides

The Dionex ICS-5000 high performance anion exchange chromatography (HPAEC) with a CarboPacTMPA20 (3 mm × 50 mm) column was used to determine monosaccharide composition. Briefly, the sample was dissolved with 2 mol/L trichloroacetic acid, hydrolyzed to the final volume of 50 mL, and later filtered with a 45 μm microporous membrane.

2.3.2. Fourier transform infrared (FTIR) spectroscopy analysis

CV-SPPW polysaccharides were freeze-dried, ground into powder, and mixed with potassium bromide at a mass ratio of 1: 100 prior to being used as test samples. Thereafter, the polysaccharides were placed in the sample area, and the crystal structure of the sample was obtained using a powder X-ray diffractometer (Empyrean, PANalytical B.V., Netherlands). The test conditions were 45 kV of voltage and 40 mA of current. The resulting powder had a 120° diffraction intensity from 5° scanning to 60° (2 θ angle range). Besides, the step size and 121 step rate were set to 0.013 and 0.3 s/step, respectively.

2.4. Preparation of yogurts

A well-growing bacteria-free CV-SPPW was selected and homogenized at 5000 rpm/min for 5 min. Each 100 mL of yogurt consisted of skimmed milk powder (14 g/100 mL), cane sugar (7 g/100 mL) and CV-SPPW (different volume ratios), of which <100 ml consisted of water. CV-SPPW dosage was divided into 5 different ratios: 0% (0 mL/100 mL), 5% (5 mL/100 mL), 10% (10 mL/100 mL), 15% (15 mL/100 mL), and 20% (20 mL/100 mL). Then, the mixture of skimmed milk powder, cane sugar, and CV-SPPW was heated at 90 °C for 10 min. After the temperature of the solution was cooled to 42 °C–45 °C, 0.4 g of starter culture was inoculated into yogurt (*Streptococcus thermophilus* and *Lactobacillus bulgaricus* in the starter culture are 1:1). Finally, the yogurt was fermented at 42 °C for 7 h. After the fermentation, the yogurt was refrigerated at 4 °C for 24 h to obtain the finished yogurt.

2.5. Principal component analysis of yogurts

The total protein was determined by the Kjeldahl method (AOAC 988.05). The fat content was obtained using the Soxhlet extraction method. The total solids content of yogurt was determined with reference to the national standard method. (GB5413.39—2010). Other assays and methods were the same as 2.3.2.

2.6. Structural characteristics of yogurts

2.6.1. Textural characterization

The TA11/1000 probe was used. The yogurt texture was determined under the following conditions: measurement speed of 0.50 mm/s, pre-test speed of 2 mm/s, post-test speed of 0.50 mm/s, and trigger point load of 7 g.

2.6.2. Rheological characteristics

A DHR-2 (TA Instruments, USA) rheometer was employed to characterize the rheological properties of yogurts. The yogurt samples were placed in two parallel plates with a size of 1 mm, and scanned with a 2° cone (40 mm diameter) at a frequency of 1 Hz. Then, the samples were heated from 5 °C to 40 °C at a heating rate of 1 °C/min, during which, the machine ran at a shear rate of 1/s. Following the above process, the

energy storage modulus (G') and loss modulus (G") of the samples were measured.

2.6.3. Scanning electron microscopy

The yogurt samples were fixed with 2.5% glutaraldehyde solution for 12 h, and subsequently rinsed with phosphate buffer solution (pH 6.8). Thereafter, the samples were dehydrated by gradient dehydration with ethanol solutions of 50%, 70%, 80%, 90%, and 100% by volume, respectively. The dehydrated and lyophilized samples were scanned at a voltage of 10.0 kV using a scanning electron microscope (SU8010, Hitachi, Japan) after gold spraying.

2.6.4. X-ray diffraction analysis

Yogurt samples were lyophilized and later passed through an X-ray diffractometer (Model Empyrean, PANalytical B.V., Netherlands) at the 40 kV operating voltage and the 40 mA current for characterization. The diffraction intensity of the collected powder was scanned from 5° to 60° (2θ angular range), and the step size was set to 0.013° with the step rate being set to 0.3 s/step.

2.6.5. Fourier transform infrared spectroscopy

Yogurt samples were freeze-dried through a 200-mesh sieve by the same method described in 2.3.2.

2.7. Storage stability of yogurt at 4 °C for one week

See 2.6.1 for details.

2.8. Sensory evaluation of yogurts

Sensory evaluation of yogurts was conducted with reference to the method of Sulieman et al. (Sulieman et al., 2019a) after making some adjustments. The sensory evaluation of yogurt samples in the current experiment covered five domains, including color, flavor, texture, sweetness and sourness, and organizational status. For arrangement of marks, each part was worth 20 marks out of 100. Besides, 20 well-trained postgraduate students from our college were recruited as the volunteers.

2.9. LC-MS analysis of yogurts

The LC analysis was performed on the ACQUITY UPLC System (Waters, Milford, MA, USA). Chromatography was conducted with the ACQUITY UPLC ® HSS T3 (150 × 2.1 mm, 1.8 μm) (Waters, Milford, MA, USA), at the flow rate of 0.25 mL/min and the column temperature of 40 °C. The supernatant obtained after centrifugation (10 min at 12,000 rpm at 4 °C) was filtered through a 0.22 μm membrane, the filtered liquid was transferred to the detection bottle, and 2 μL of each sample was taken. Data were collected separately under both positive and negative ion modes. The measurement conditions included: positive ion spray voltage of 3.50 kV, negative ion spray voltage of −2.50 kV, sheath gas velocity of 30 arb, auxiliary gas flow rate of 10 arb, and capillary temperature of 325 °C. The resolutions of the first and second levels were 70,000 and 17,500, respectively, the primary ion scanning range was 100–1000 *m/z*, the secondary cleavage was conducted using HCD rows with a collision energy of 30 eV, and the final 10 ions were fragmented before signal acquisition.

2.10. Statistical analysis

All tests were conducted three times (except where specified). SPSS statistics 26.0. Origin 2021, Metabolic Analyzer Analysis was used for statistical analysis and drawing. $p < 0.05$ indicated the statistically significant difference. Data were expressed as mean ± standard deviation (SD) from triplicate measurements.

3. Results and discussion

3.1. Preparation and principal component analysis of CV-SPPW

3.1.1. Basic substance analysis of CV-SPPW

As shown in Table 1, after the fermentation, the polysaccharide content increased by 4.79 times, soluble protein increased by 10.8 times, and the reducing sugar content increased by 2.1 times compared with those before fermentation. The yield of polysaccharide produced by CV-SPPW was higher than that produced by the *C. versicolor* culture in the stirred tank bioreactor (0.74 ± 0.12 g/L) (Duvnjak et al., 2016). This was probably because that the SPPW contained certain proteins and carbohydrates, providing nutrients and promoting the fermentation of *C.versicolor* to produce more metabolites. The increased edible mushroom proteins and polysaccharide after fermentation can form the structurally stable complexes (Yan et al., 2023). Polysaccharide can provide electrostatic repulsion and spatial site resistance for casein micelles to ensure their dispersion, therefore maintaining the homogeneity and fluidity of the acidified milk beverages (Sun et al., 2023b) and providing theoretical support for the structural stability of yogurts.

3.1.2. Monosaccharide fractions

Fig. 1A and Fig. 1B show the high performance liquid chromatograms of monosaccharide standards and monosaccharide detected in CV-SPPW, respectively. The results of the monosaccharides composition are displayed in Table S1. Clearly, the polysaccharides of CV-SPPW were mainly composed of five monosaccharides, namely, fucose, arabinose, galactose, glucose and mannose. Glucose and mannose are the main monosaccharide components, which are different from the monosaccharide fractions of *C.versicolor* polysaccharide reported by Yongshuai Jing et al. (Jing et al., 2022). Relative to the determination of polysaccharide in sweet potato wastewater fermentation bioflocculants, this study produced monosaccharides such as glucose and mannose, which are more suitable for the applications in the food industry (Guo et al., 2018). SPPW contains a certain amount of carbohydrates and protein, which is more beneficial for the liquid fermentation of *C.versicolor* (Cheng et al., 2015b). The result is consistent with the increasing contents of polysaccharide, reducing sugars and soluble proteins in SPPW after fermentation. Previous studies have analyzed the network structures of glucose, arabinose, galactose, mannose and other polysaccharide through a coiled network, revealing that these carbohydrates can establish corresponding structural characterization patterns due to their structural variability, forming more complex network structures (Sabater et al., 2020). This provides reference for the structural quality stability of yogurts after fermentation.

3.1.3. FT-IR analysis

Fig. 1C presents the FTIR spectra of CV-SPPW polysaccharide. The results demonstrated that the relatively broad and strong absorption peak near 3412 cm^{-1} was a stretching vibration of the non-free O—H

Table 1
Comparison of basic components of sweet potato pulp water before and after fermentation.

The basic ingredients of SPPW and CV-SPPW before and after fermentation			
Group	Crude polysaccharide(g/L)	Reducing sugars (g/L)	Soluble protein(g/L)
SPPW	0.86 ± 0.06[a]	7.02 ± 0.03[a]	1.42 ± 0.02[a]
CV-SPPW	4.98 ± 0.245[b]	21.76 ± 0.6[b]	16.76 ± 0.6[b]

Note: Before fermentation means sweet potato pulp water. After fermentation means CV-SPPW. Values are expressed as means ± standard deviation ($n = 5$). Lack of letters in different letters indicates statistically significant differences (Duncan's t-test. $p < 0.05$) for comparison of treatment means between different substrates.

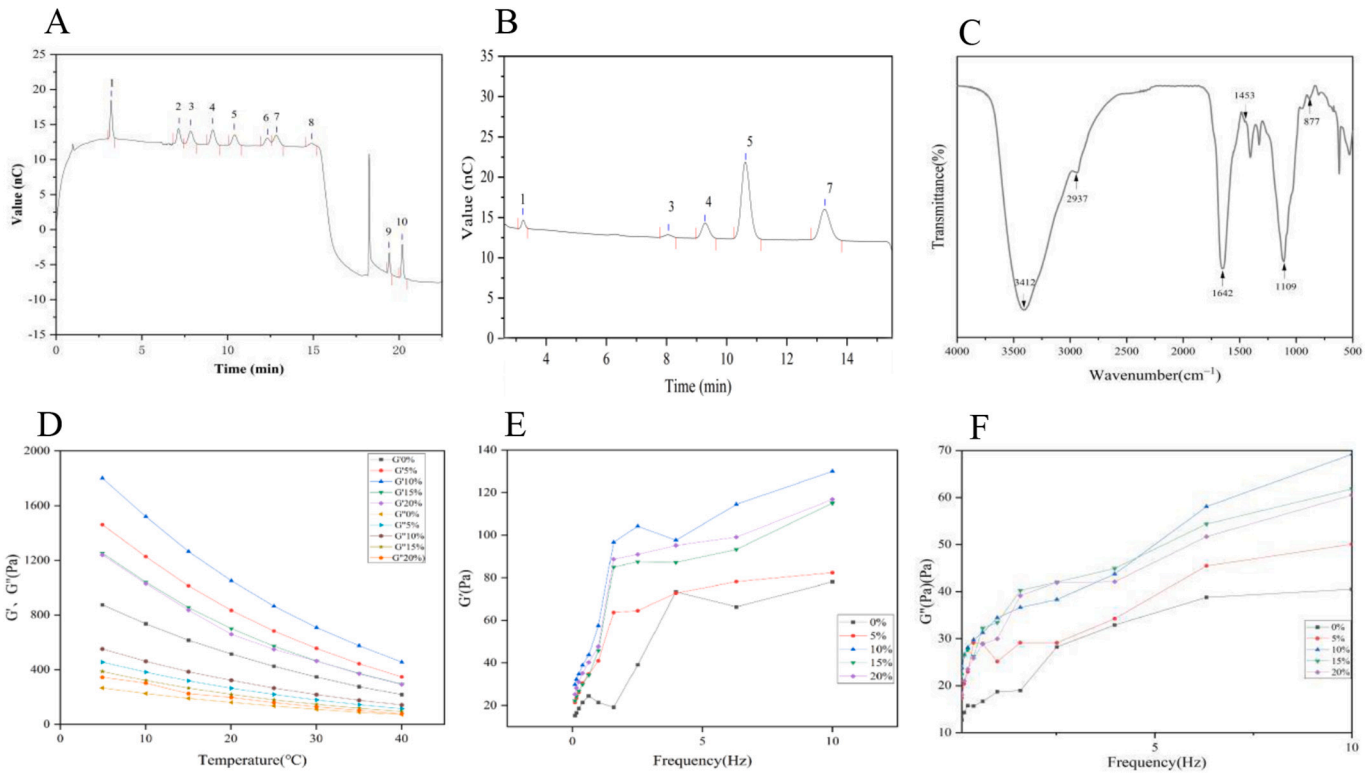

Fig. 1. Fig. 1A high performance liquid chromatogram of a monosaccharide standard. Note: The peak of 1A monosaccharide labels are (1) fucose, (2) rhamnose, (3) arabinose, (4) galactose, (5) glucose, (6) xylose, (7) mannose, (8) fructose, (9) galacturonic acid, (10) glucuronic acid. The peaks in Fig. B are: (1) fucose, (3) arabinose, (4) galactose, (5) glucose, (7) mannose. Fig. 1B is the peak of the polysaccharide of sweet potato pulp water fermented by *C. versicolor* (CV-SPPW), and Fig. 1C is the infrared spectrum of monosaccharide. Fig. 1D shows the storage modulus (G') and loss modulus (G") of CV-SPPW yogurt with different proportions. Fig. 1E shows the change of storage modulus (G') of CV-SPPW with frequency. Fig. 1F shows the variation of loss modulus (G") with frequency of CV-SPPW yogurt with different proportions. 0%, 5%, 10%, 15%, and 20% represent different amounts of CV-SPPW added, respectively. All experiments were repeated 3 times and plotted with average values.

bond of the polysaccharide chain, indicating the existence of intermolecular hydrogen bonding. This strengthened the intermolecular force between polysaccharide and proteins. The absorption peak near 2937 cm^{-1} was caused by the glycan chain C—H bond stretching vibration, while that near 1642 cm^{-1} belonged to a C═O characteristic absorption peak, that near 1453 cm^{-1} was a -CH2 deformation absorption vibration peak, and that near 1109 cm^{-1} was attributed to glycosidic bond C-O-C stretching vibration (Yang et al., 2023). Pai Peng et al. (Peng et al., 2022b) investigated the dynamic interactions between proteins and carbohydrates, finding that the introduction of glycosidic and hydrogen bonds made the complex structures of proteins and carbohydrates more stable. This conclusion lays a foundation for the introduction of CV-SPPW into the yogurt system, contributing to the structural stability of yogurts. Moreover, vibrations in the range of 1000–1200 cm^{-1} (1109 cm^{-1}) were caused by the ester sugar groups (C-O-C) and (C-O-H) of the pyranose ring, demonstrating the presence of pyranose in the polysaccharide (Yang et al., 2022). Pyranose facilitates the synthesis of aldoses, the valuable raw materials for the food industry, and can be taken as emulsifiers and acidifiers (Hu, Xiang, & Lu, 2019a).

3.2. Principal component analysis of yogurts

As displayed in Table S2, the crude polysaccharide content in yogurt increased significantly from 10.41 g/L to 20.50 g/L ($p < 0.05$) with the addition of CV-SPPW. There existed no significant difference in fat content or total solids content among the five groups. The reducing sugar was featured with a trend of first increasing and then decreasing. The first increase in reducing sugar content was probably because that the addition of a certain level of CV-SPPW induced the enhanced metabolism of *LAB* to produce more reducing sugars, whereas an excessive amount of CV-SPPW exceeded the metabolic range of *LAB* and caused the decline in *LAB* metabolism. Compared with 0%, the total protein and soluble protein contents tended to firstly decrease and then increase, probably because that the *LAB* protein hydrolyzing enzyme decomposed the protein and provided abundant nitrogen source materials for *LAB* fermentation (Kieliszek et al., 2021). When the addition was over 10%, the enzyme activity decreased, thus increasing the contents of total protein and soluble protein. In general, the addition of CV-SPPW affected the metabolism of *LAB* and the interaction between the carbohydrates and proteins produced. Besides, it also affected the substance content and the structure of yogurts. When the addition amount exceeded 10%, the binding ability was weakened and the contribution to the bundle gel structure of yogurt decreased, which might be resulted from the change in the charge density of the polysaccharide-protein polymer during the interaction between polysaccharide and proteins. Moreover, the charge was imbalanced (Li et al., 2016), which exceeded the binding ability of the casein matrix, leading to the free state of the remaining CV-SPPW.

3.3. Structural characterization of yogurts

3.3.1. TPA analysis

The structural characteristics of yogurts containing 0%–20% CV-SPPW are displayed in Table S3. There were significant differences in elasticity, cohesion and chewiness between the yogurts with and without CV-SPPW ($p < 0.05$), but not in viscosity ($p > 0.05$). The elasticity, hardness and cohesion of 5% and 10% yogurts increased with the addition of CV-SPPW, which might be caused by the covalent and non-

covalent interactions in the whole yogurt system (Jaros et al., 2006). Furthermore, studies have shown that moderate addition of carbohydrates enhances the disulfide bonds, hydrogen bonds, and β folding forces in the carbohydrate-protein complexes, thereby enhancing structural properties such as viscosity of the product (Peng et al., 2022b). In general, adding CV-SPPW at an appropriate amount is beneficial for improving the yogurt structure, while the excessive addition amount destroys its structure. Chewiness indicates the amount of energy and time required to chew and swallow food. The significant increase in chewiness may be the consequence of an increase in the viscoelasticity of yogurt (Olawuyi & Lee, 2019).

3.3.2. Dynamic rheological properties

As shown in Fig. 1D, the G' and G" values of yogurts in each group decreased with the increasing temperature, indicating that the yogurt structure was also changing with the change in temperature. Therefore, temperature change caused certain changes in the yogurt internal organization. In addition, it was observed from Table S2 that the addition of CV-SPPW changed the contents of sugars and proteins in yogurts and improved their structural properties, consistent with the previous study (Lin et al., 2022). As the addition amount of CV-SPPW increased, the G' and G" values gradually increased, which peaked at 10% of the addition amount, and subsequently decreased with the further increase in the addition amount. Therefore, it is suggested that the moderate addition of CV-SPPW significantly improved the yogurt structure. This may be due to the fact that the disulfide, hydrogen and β-folding forces in the yogurt system after the addition of CV-SPPW further promote the cross-linking of casein to form a specific network structure, consequently enhancing the structure of yogurt (Peng et al., 2022b).

As displayed in Fig. 1EF, the G' and G' values of yogurt increased with the increasing scanning frequency. Compared with the 0% group, the addition of CV-SPPW led to the increases in G' and G“ values of yogurt, while the 10% group had a higher G" value. When the CV-SPPW addition amounts were 15% and 20%, the G' and G“ values of yogurt decreased relative to the 10% group, which suggested that the addition of 10% CV-SPPW improved the viscoelasticity of yogurt, thereby enhancing the network structure and making it have a better thick and silky taste. In all frequencies, the G' value of all groups was greater than the G" value, indicating that the yogurts were mainly elastic and tended to be solid. The result corresponded to the data obtained by TPA. This may be due to the fact that appropriate levels of CV-SPPW can form strong interactions with proteins and enhance the network structure, where electrostatic bonding contributes to the formation of a dense protein network structure and aggregated particles, causing the more elastic rather than the viscous flow behavior (Sulieman et al., 2019b). However, excess CV-SPPW can form flocs with proteins, influencing the yogurt structure.

3.3.3. SEM analysis

As shown in Fig. 2, 0% of yogurt grain was relatively finely crushed. From the figure, an uneven structure was observed, and there were many pores in the microstructure of yogurt. With the increasing CV-SPPW addition amounts to 5% and 10%, the microstructure showed that the granularity was weakened. The pore size and number were more uniform, and the structure of the yogurt gradually became homogeneous and exhibited an ordered state. The Fig. 2 showed that the structures of yogurts with 15% and 20% CV-SPPW addition were not orderly, but more compact, increasing the flocculation state of yogurts.

These differences are mainly related to the compactness of the protein matrix and the amount of CV-SPPW. As shown in Fig. 0%, the protein matrix of 0% group consisted of aggregated casein micelle particles. The microstructure change from 0% to 10% yogurt was caused by the addition of CV-SPPW, which added biomolecules including proteins and polysaccharides to the yogurt. Food macromolecules such as polysaccharide and proteins increased their affinity with water molecules to a great extent due to a large number of hydroxyl groups. The natural whey protein acted as a framework structure to support the whole network structure, while the CV-SPPW functioned as a filler to make the structure of yogurt denser and more compact. Owing to the formation of the more stable and ordered bundled gel structure, the microstructure of yogurt improved its hardness and cohesion.

3.3.4. XRD analysis

As shown in Fig. 3A, there were no wide diffraction peaks within the diffraction angle range of 10°-55°, and there were obvious strong diffraction peaks. Compared with the diffraction peak at 20.05 of 0% group, the diffraction peak intensities the other groups increased with the addition of CV-SPPW, and shifted from 20.05 to 19.40. These results suggested that the addition of CV-SPPW not only changed the crystal structure of yogurt, but also affected its amorphous structure (Liu et al., 2017).

In general, the image results of X-ray diffraction peaks showed that the intensities of the peaks reflected the grain size of the crystalline region inside the yogurt; and that the size of the grain was proportional

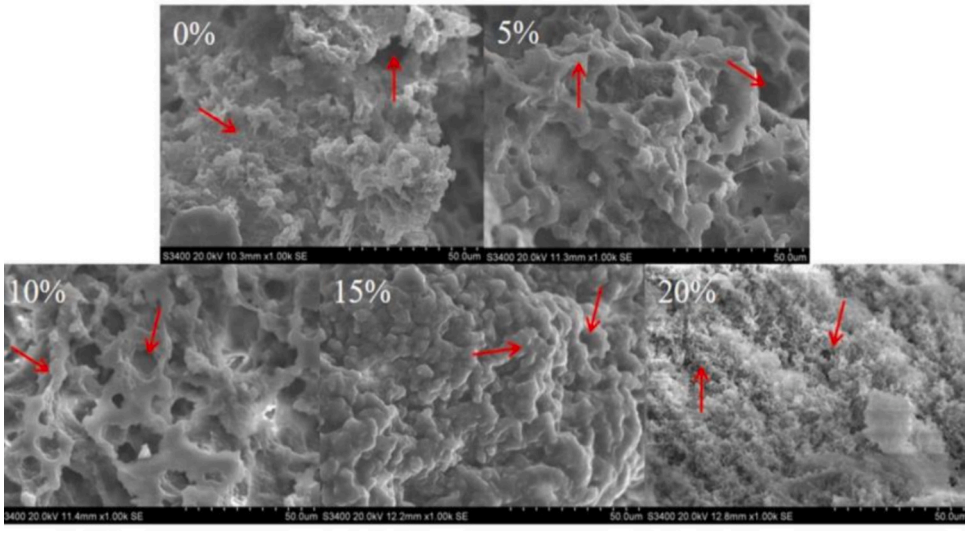

Fig. 2. The scanning electron microscope (SEM) shown in Fig. 2 shows the microstructure of different amounts of yogurt with a magnification of 1 K. Note: 0% 5%, 10%, 15% 20% represent different amounts of CV-SPPW added yogurt.

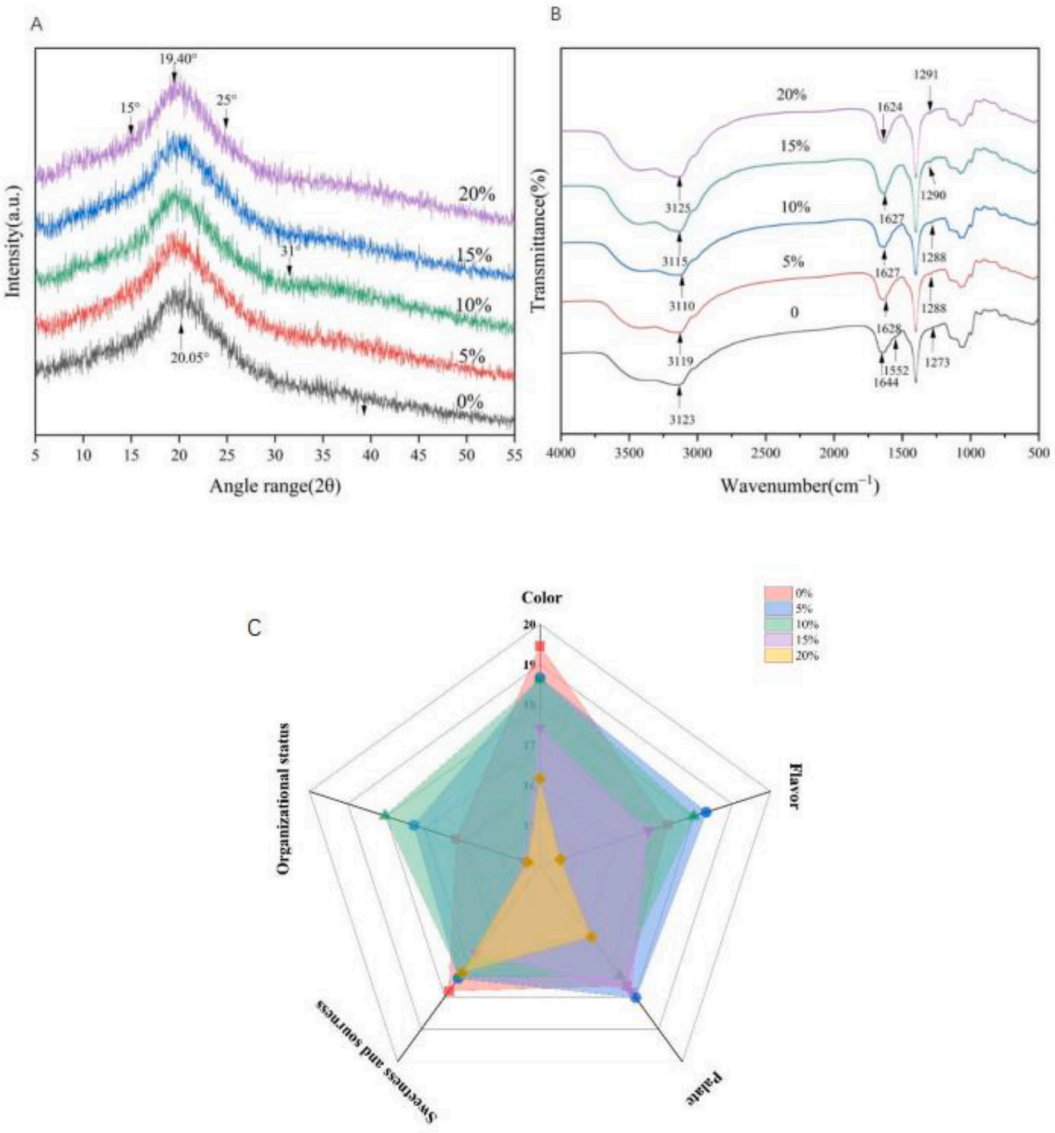

Fig. 3. Fig. 3A shows X-ray diffraction patterns of CV-SPPW yogurt added at different scales, and Fig. 3B shows Fourier infrared (FT-IR) analysis of CV-SPPW yogurt with different scales.0%, 5%, 10%, 15% 20% represent different amounts of CV-SPPW added yogurt. The sensory characteristics of the yogurt sample group with 0%, 5%, 10%, 15% and 20% CV-SPPW addition shown in Fig. 3C. Different the colors represent different amounts of yogurt added to CV-SPPW.

to the intensity of the diffraction peak. This phenomenon reveals that the proteins and CV-SPPW rearrange to form larger particles upon interaction, and the formation of a more stable structure leads to a significant increase in the hardness and elasticity of yogurt. Obviously, when the amounts of CV-SPPW were 5% and 10%, the crystalline structure of yogurt gradually became uniform, dense and orderly. When the amount of CV-SPPW was changed, the crystallization degree of yogurt also changed, which became a tighter agglomerate polymer, increasing the grain size of the internal crystalline area of yogurt. The XRD peak shown by yogurt with different amounts of CV-SPPW had different degrees of enhancement. This conforms to the findings by Zhenzhen Huang et al. (Huang et al., 2022), finding that the diffraction peaks decreased after replacing the polysaccharide by agar in the protein system, and that two smaller diffraction peaks appeared after the polysaccharide was added again to form a more stable emulsion.

3.3.5. FT-IR analysis

Fig. 3B shows the FTIR spectra of yogurt with different ratios of CV-SPPW. In the 0% yogurt, the amide I band of the protein appearing near 1644 cm^{-1} was attributed to the stretching of C=O; the amide II band located at the center of 1522 cm^{-1} was generated by the stretching and bending of C—N, and the C—N characteristic absorption peak of the amide 3 band existed near 1291 cm^{-1} (Zhang, Li, Wang, Zhang, Feng & Zhang, 2019). With the increasing content of CV-SPPW, the intensity of the O—H characteristic absorption peak of the polysaccharide around 3123 cm^{-1} was significantly enhanced, and the peak shifted to the low band, indicating the presence of hydrogen bond interaction between the protein and the polysaccharide, conforming to the occurrence of hydrogen bonds in the FT-IR analysis of CV-SPPW polysaccharides. The absorption peak of the amide II band disappeared after the addition of CV-SPPW. This conforms to the decrease in the standardized areas of amide I and II bands, while the amide triple band gradually appears. Guerrero et al. (Guerrero et al., 2014). investigated the change in the infrared spectrum of soybean protein caused by carbohydrates, which reduced the areas of amide I and II bands and eliminated the carboxylic acid band, revealing that the interaction between protein and carbohydrate enhanced the stability of the mixture.

The addition of CV-SPPW significantly affects the secondary structure of proteins and the stretching vibrations of certain motifs, which may be due to the interaction between polysaccharide and protein

complexes on the β-folding, β-turning angle of proteins (Nawrocka et al., 2018). This result demonstrated that the addition of CV-SPPW to yogurt enhanced the stability of yogurt structure and the strength of intramolecular hydrogen bonds, which formed an effective gel structure and a more stable yogurt system, and provided a valuable explanation for TPA results.

3.4. Storage stability of yogurt at 4 °C for one week

Table S4 displays the changes in the texture characteristics of yogurt with different amounts of CV-SPPW during 7 days of storage at 4 °C. As shown in the table, the hardness of yogurt in the four groups of 5%, 10%, 15% and 20% was significantly higher than that of the 0% group ($p < 0.05$) on the 7th day of storage at 4 °C, indicating that the addition of CV-SPPW could improve the hardness of yogurt, and the hardness of yogurt also exhibited an upward trend with the increase of CV-SPPW addition. The reason for this result may be due to the interaction of CV-SPPW with proteins and other substances in yogurt, enhancing the structural stability of yogurt. Shen et al. (Shen et al., 2023) proposed that to maintain the good taste experience of yogurt, the hardness of yogurt must be controlled within an appropriate range, because too high hardness will cause yogurt to lose its unique viscosity, and too low hardness will result in a decrease in coagulation degree and unstable texture characteristics. However, the sensory results of the yogurt showed that the experimenters accepted the effect of CV-SPPW on the tissue structure of the yogurt. With the increase of CV-SPPW addition, the viscosity, cohesion and elasticity of yogurt first increased and then decreased, and this trend remained within 7 days of storage. Cohesiveness reflects the degree of deformation of the yogurt sample before rupture and is directly related to the internal strength of the yogurt architecture (Basiri et al., 2018) During the storage period, the cohesion of yogurt after the addition of CV-SPPW was significantly higher than that in the 0% group ($p < 0.05$), which may be associated with the change of the crystallinity of yogurt after the addition of CV-SPPW. For example, the elasticity of yogurt in the 3 groups of 5%, 10% and 15% was significantly higher than that in the 0% group ($p < 0.05$) during 7 days of storage at 4 °C; nevertheless, when the addition amount of CV-SPPW was 20%, the elasticity of yogurt would show a downward trend, and thus an appropriate amount of CV-SPPW needed to be added to the yogurt system. In summary, after the addition of CV-SPPW, CV-SPPW changed the structure of yogurt, which exerted a certain effect on improving the structural properties of yogurt, making it stronger and more cohesive, and also improving the viscoelasticity of yogurt, and thus the yogurt showed more stable texture characteristics at different storage temperatures. This is similar to the conclusion of Zheng Shasha (Zheng, 2023b) that the combination of *C. versicolor* fermentation broth and yogurt found that it could improve the fermentation matrix of yogurt and improve the storage stability of yogurt.

3.5. Analysis on sensory characteristics of yohurt

According to sensory analysis (Fig. 3C), the 5% and 10% CV-SPPW groups showed better yogurt texture and organizational structure scores. However, with the further increase in CV-SPPW addition amount, the organizational structure and taste score of yogurt significantly decreased. In addition, the organizational structures of yogurts of 15% and 20% addition groups declined. Through the taste of yogurt by the experimenters, we found that the experimenters were capable of accepting the yogurts very well. In addition, we derived the sensory ratings of different groups of yogurts. From 0% to 20%, the sensory scores of the five groups were 88.51 ± 3.22^{ab}, 89.75 ± 2.05^{a}, 90.41 ± 1.96^{a}, 83.18 ± 2.96^{b}, and 78.49 ± 1.48^{c}. A higher taste score indicated that the experimenter received the unique taste of yogurt with CV-SPPW.

3.6. LC-MS analysis of yogurt

Based on the above results, CV-SPPW had a significant effect on yogurt quality. Therefore, metabolomics were used to clarify the mechanism of action of the effect of CV-SPPW on the structural substances of yogurt. Metabolites with $p \leq 0.05$, and fold change ≥ 1.5 or ≤ 0.8 were selected as the objects of metabolic analysis. The results of PCA are shown in Fig. 4A, where SP1 represents the CV-SPPW group, SP2 stands for the yogurt group with 0% CV-SPPW addition, and SP3 indicates the yogurt group with 10% CV-SPPW addition. LC-MS analysis identified totally 552 compounds across all samples. Three parallel samples were clustered together in the PCA score plot, demonstrating excellent experimental reproducibility and data reliability. The total contribution of the first two principal components was 91.3% (PC1 = 72.36% and PC2 = 22.56%), revealing the similarity and difference between different samples. Based on from the aggregation and dispersion of samples, SP1 and SP3 groups were closer to each other, while SP2 group was farther away from SP1 group, suggesting the similarities in the types and quantities of metabolites between SP1 and SP2 groups. The comparative speculation of the three groups of SP1, SP2, and SP3 indicated that the addition of CV-SPPW changed the metabolic environment of *LAB*, affected the metabolic pathways of *LAB*, and altered the metabolites in yogurt, consistent with the conclusion reached by Fan Xiankang et al. (Fan et al., 2023c).

To further understand the metabolic differences between the yogurt group with 10% CV-SPPW and the yogurt group with 0% CV-SPPW, the volcano plot of differential metabolite screening was plotted, as shown in Fig. 4B. A total of 552 differential metabolites were detected, including 155 up-regulated metabolites, 272 down-regulated metabolites, and 125 with no statistically significant differences. As presented in Fig. 5A, amino acid metabolism and fatty acid degradation were significantly enhanced, indicating that *LAB* had a strong ability to hydrolyze proteins and convert pentoses and glucosinolates, which lowered the pH of the yogurt and enhanced its network structural properties. Fig. 5B showed that phenylalanine, threonine, tyrosine, α-ketoglutaric acid, 2-ketoglutaric acid, and succinic acid were produced by lactobacilli during fermentation, and entered the TCA cycle in the presence of enzymes, thus synergistically promoting the production of sugar compounds in yogurts. In addition, tyrosine exerted a positive effect on the growth of *LAB* (Yonezawa et al., 2010).

Fig. 6A exhibits a heatmap of cluster analysis of metabolites. d-xylose, cellobiose, maltitol, d-alginate, and d-maltose contents increased by 23.56, 12.33, 1.98, 1.59, and 2.27 times, respectively. d-xylose can reduce intestinal pH, inhibit the reproduction of other harmful bacteria, and selectively promote the proliferation of beneficial bacteria including intestinal *bifidobacteria*; as a result, they become the dominant intestinal flora, aiming to regulate the intestinal micro-ecological balance, and promote intestinal health (Hu et al., 2023). d-xylose is a water-soluble dietary fiber, which can be added to yogurt to improve its viscoelasticity and enhance its structural properties (Fan et al., 2023c). N Paßlack et al. (Tan et al., 2022) considered that cellobiose formed a more stable conformal structure with proteins through intermolecular interaction forces, while the glucose molecules of cellobiose were connected through β-1,4-glycosidic bonds, which were fermented by microorganisms in the human large intestine, and showed some probiotic potential (Paßlack et al., 2020). With wide application in the food field, maltitol can stabilize the natural structure of whey protein, and provide the desired texture for food (Kim et al., 2020). According to a previous report, d-trehalose has the specific ability to bind to proteins, forming the complex stabilizing structures (Herman et al., 2007). The 2.72-fold increase in d-glucuronic acid may be caused by the fact that the monosaccharide component of the polysaccharide produced by the addition of CV-SPPW contains pyranose, enhancing the emulsifying properties of yogurt and improves its structural stability (Hu, Xiang, & Lu, 2019b). In general, the addition of CV-SPPW changes the metabolic environment of *LAB* and produces sugars improving the quality and

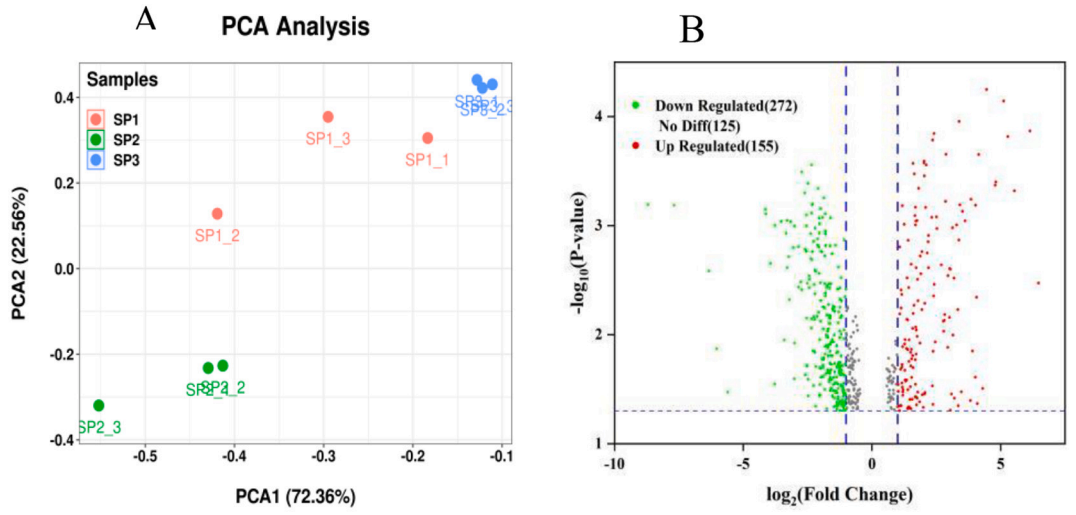

Fig. 4. Fig. 4A is the PCA scoring chart, where SP1 is the CV-SPPW yogurt group, SP2 is the blank group, SP3 is the CV-SPPW group. Fig. 4B shows a metabolic volcano diagram.

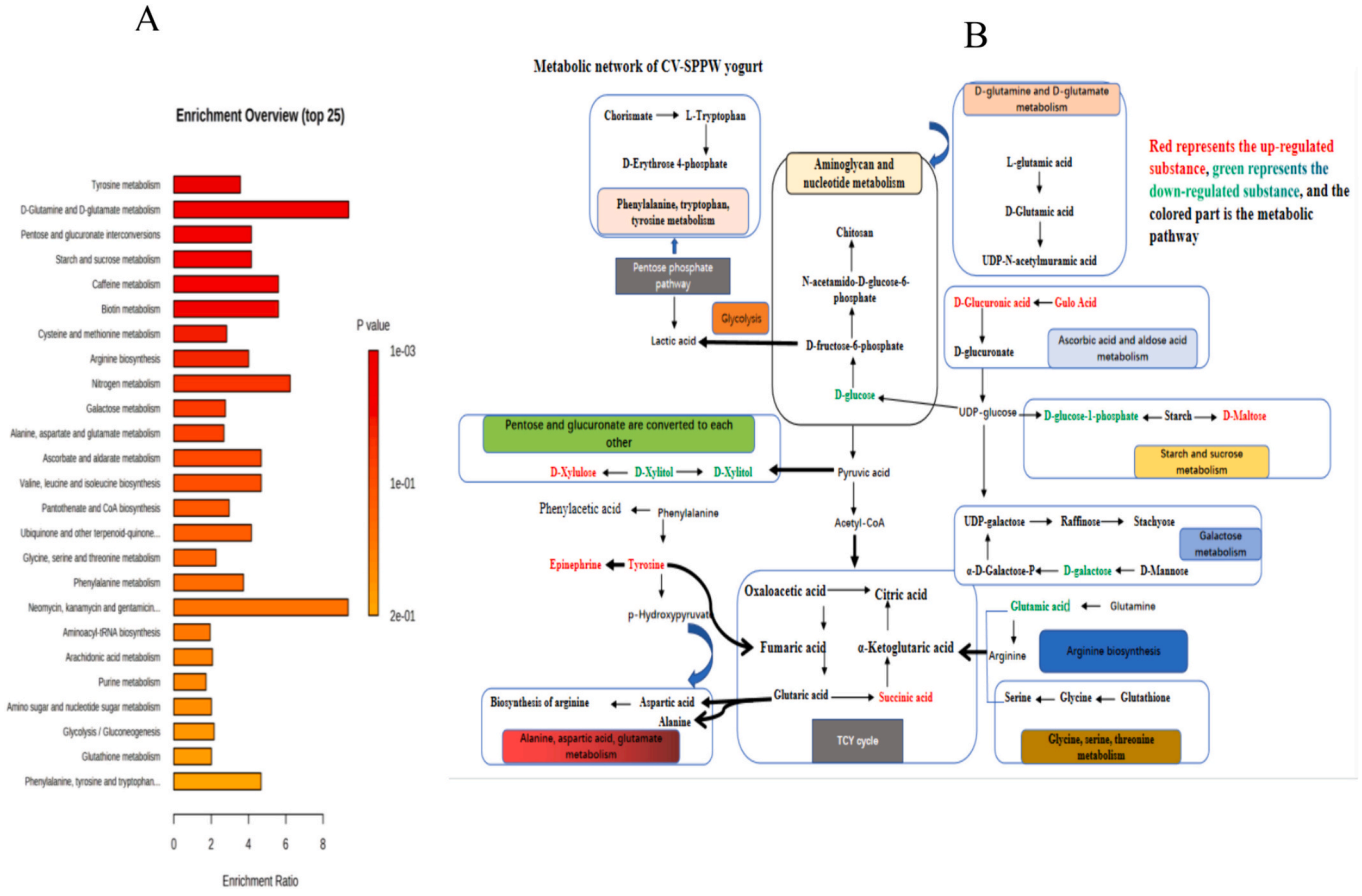

Fig. 5. Fig. 5A shows the 25 metabolic pathways of lactic acid bacteria after the addition of CV-SPPW, and Fig. 5B represents the metabolic network between the added CV-SPPW yogurt-specific metabolite and the added Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway.

structure of yogurt to varying degrees.

3.7. Correlation analysis between structural properties and some compounds

More insight into the correlation between indicators might be obtained by visualizing the correlation analysis graph (Fig. 6B). Crude polysaccharide content was negatively ($p < 0.05$) related to soluble protein content, and positively correlated with the structural properties of yogurt (like hardness, elasticity, chewiness, stickiness and cohesiveness). Moreover, it was revealed in this study that, the crude polysaccharides content in yogurt increased by the addition of CV-SPPW, and crude polysaccharides were capable of improving the structural properties such as elasticity and viscosity of yogurt to varying degrees in

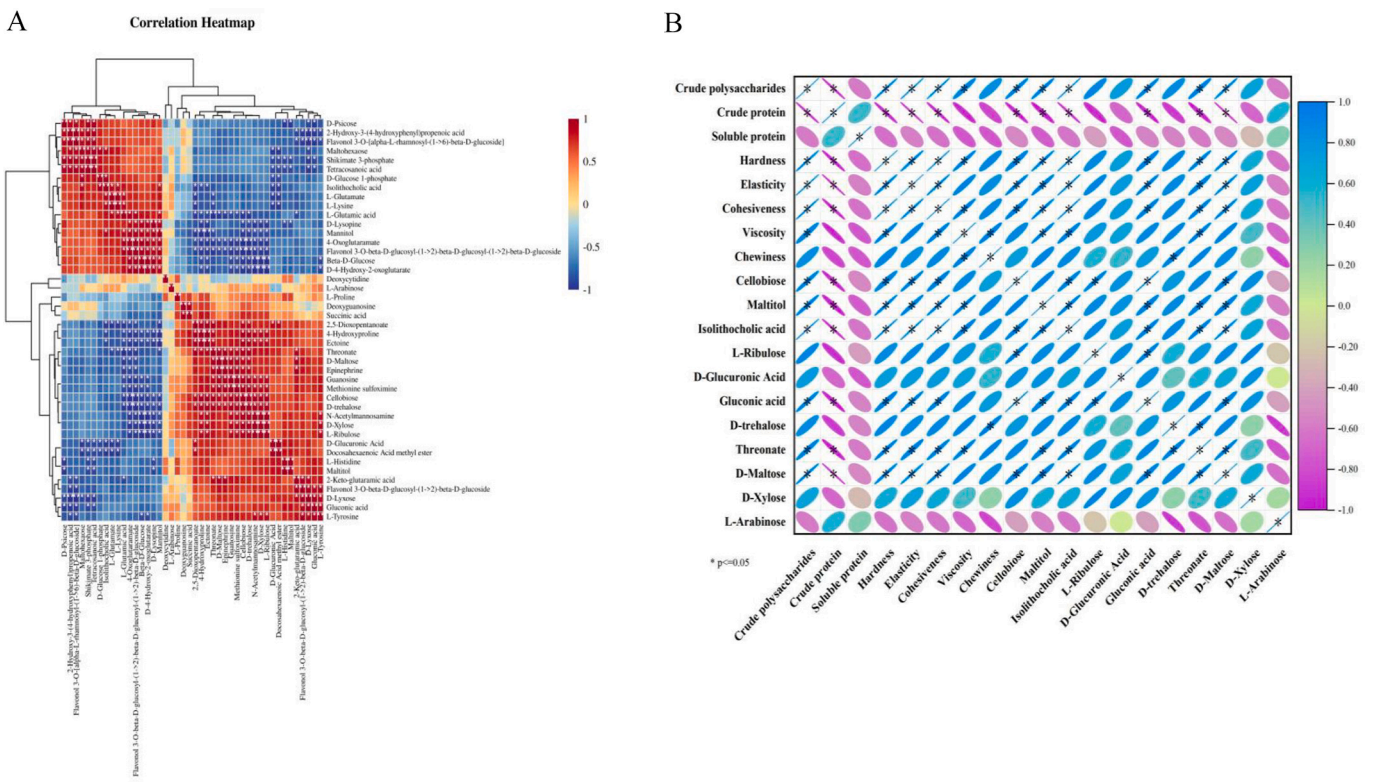

Fig. 6. Fig. 6A. the heat map visualization of the differential metabolites in the CV-SPPW fermentation group and the natural fermentation group. Fig. 6B. The correlation between 0% and 10% texture of CV-SPPW and some compounds was significant, and the correlation was significant at the level of $p < 0.05$.

the yogurt system. In addition, the crude polysaccharide content showed a significant positive correlation with the carbohydrate contents of d-xylose, cellobiose, maltitol, d-alginate, and d-maltose. The positive correlation between structural properties and some compounds may be explained by the following facts. Firstly, the addition of CV-SPPW affected the metabolic environment of *LAB*, providing more energy for the metabolism of *LAB* and promoting the enhancement of TCA. The addition of CV-SPPW also changed the metabolic pathway of *LAB* and enhanced the amino acid and sugar metabolic pathway of *LAB*; thus, *LAB* metabolism produced more carbohydrates such as d-xylose, cellobiose, and maltitol. Moreover, the content of carbohydrates including d-xylose, cellobiose, maltitol, d-alginate, and d-maltose showed a significant positive correlation with the structural properties of yogurt (including hardness, elasticity, chewiness, stickiness and cohesiveness). After the addition of CV-SPPW, *LAB* metabolism produced more carbohydrates like d-xylose, cellobiose, maltitol, d-alginate, and d-maltose, improving the viscosity, elasticity, emulsification, and stability of yogurt. As shown in Table S3, the addition of 10% CV-SPPW significantly improved the hardness, viscosity, elasticity and cohesion of yogurt when compared with those of the group with 0% CV-SPPW ($p < 0.05$). Besides, d-glucuronic and gluconic acids produced by *LAB* metabolism contributed to the structural stability of yogurt. Overall, *LAB* metabolism produces more carbohydrates interacting with the casein in yogurt to form a more stable structure.

4. Conclusion

In conclusion, this study indicated that CV-SPPW improved the fermentation environment of *LAB*. The addition of CV-SPPW increased the orderliness of the yogurt structure and the strength of intramolecular hydrogen bonding, thus improving the texture of yogurt. The addition of CV-SPPW enhanced the sugar metabolism and amino acid metabolism of *LAB*. After the addition of CV-SPPW, *LAB* metabolism produced more carbohydrate-protein interactions. Cellobiose, maltitol, d-trehalose and d-maltose generated during the metabolism of yogurt with the addition of CV-SPPW were found to be key contributors to the changes in the structural characteristics of yogurt. Therefore, the addition of CV-SPPW is expected to be a potential additive to enhance the sensory and textural profile of yogurt. However, in this study, the changes in yogurt structure after the addition of CV-SPPW were only observed through the microstructure, so the specific mechanism of the change of yogurt structure by CV-SPPW at the molecular level can be further investigated in the future studies.

CRediT authorship contribution statement

Jiamin Li: Methodology, Formal analysis, Data curation. **Tingting Zhou:** Visualization, Data curation. **Zhengbin Yang:** Software, Resources. **Qin Cen:** Validation, Methodology. **Rui Zhang:** Validation. **Fuyi Hui:** Writing – review & editing, Resources. **Hongyan Chen:** Writing – review & editing, Resources. **Ziru Dai:** Writing – original draft. **Xuefeng Zeng:** Writing – review & editing, Supervision, Methodology.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101582.

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