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Original Research Article

Assessing the physiological properties of baker's yeast based on single-cell Raman spectrum technology

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ABSTRACT

With rapid progress in the yeast fermentation industry, a comprehensive commercial yeast quality assessment approach integrating efficiency, accuracy, sensitivity, and cost-effectiveness is required. In this study, a new yeast quality assessment method based on single-cell Raman technology was developed and contrasted with traditional methods. The findings demonstrated significant associations (Pearson correlation coefficient of 0.933 on average) between the two methods in measuring physiological indicators, including cell viability and intracellular trehalose content, demonstrating the credibility of the Raman method compared to the traditional method. Furthermore, the sensitivity of the Raman method in viable but non-culturable cells was higher in measuring yeast cell viability (17.9 % more sensitive). According to the accurate quantitative analysis of metabolic activity level (MAL) of yeast cells, the cell vitality was accurately quantified at population and single-cell levels, offering a more comprehensive assessment of yeast fermentation performance. Overall, the single-cell Raman method integrates credibility, feasibility, accuracy, and sensitivity in yeast quality assessment, offering a new technological framework for quality assessments of live-cell yeast products.

1. Introduction

Saccharomyces cerevisiae, known as Baker's yeast, is a fungal species widely used in the food industry and everyday life due to its role in fermentation [1]. In industrial production, baker's yeast strains with stable fermentation performance and flexibility to diverse fermentation conditions are key to attaining optimal economic benefits [1]. The industrial yeast market is highly competitive, with various brands of baker's yeast of different qualities. Therefore, the rapid assessment of yeast products is necessary for production and fermentation companies to cultivate optimal yeast strains to enhance commercial competitiveness.

The primary quality parameters of yeast products include cell viability [2], cryopreservation survival rate [3], intracellular trehalose content [4], intracellular ATP content [5], and cell vitality [6]. Measuring these parameters allows the yeast to be assessed and differentiated by different companies, and can be better applied in production. In traditional methods, measurements are primarily performed by biochemical cultivation and chemical detection [6]. Specifically, cell viability is the proportion of active yeast cells in yeast products [2], making it an essential parameter for yeast fermentation performance. This parameter can be measured by plate counting and total cell

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counting, which are reliable but require complex experimental conditions and extended durations [7]. Additionally, plate counting, which uses colonies rather than cell numbers [9], cannot account for cells that are sublethally damaged, injured, inhibited, dormant, or inactive [10]. In industrial production, yeast fermentation dough is typically subjected to a freezing process to prolong its shelf life [3], making the cryopreservation survival rate a crucial parameter of yeast cells. The cryopreservation survival rate is the proportion of yeast cells that remain active following freezing, and plate counting is required to measure this parameter. Trehalose is a stable polysaccharide with a symmetrical structure of two glucose molecules [11] that can maintain cell vitality [12], making it a critical parameter in assessing yeast freezing tolerance. The mechanism by which trehalose protects cells includes the trehalose glass hypothesis [13] and lipid-specific binding hypothesis [14]. Intracellular trehalose content can be measured using the anthrone-sulfuric acid method [8], relying on colorimetric measurement with complex experimental steps. Intracellular ATP is critical in yeast fermentation, impacting several key processes, including energy supply, metabolic regulation, cell growth, and stress response [5]. Intracellular ATP content is closely tied to the activity of metabolic enzymes in yeast cells, such as ATP synthase, phosphofructokinase, pyruvate kinase, and enzymes associated with the TCA cycle [15]. Cell vitality reflects the actual fermentation performance of baker's yeast and is typically measured via the dough gas production capacity [6], which approximates the actual fermentation of yeast, and the results can be observed easily with high credibility. However, it can only measure the vitality of yeasts at the population level, while the vitality of single cells is usually overlooked.

Given the characteristics and limitations of traditional methods approaches for measuring baker's yeast, a more advanced strategy integrating efficiency, sensitivity, and cost-effectiveness must be developed for yeast assessment. Recently, yeast cell physiological performance, through non-destructive approaches, has been achieved via diverse approaches, including electrochemical microscopy (SECM), flow cytometry, and Raman spectroscopy. SECM is an advanced technology founded on the electroporation of living yeast cells, enabling precise measurement of electron transfer efficiency under specific conditions, assessing the metabolic activity and physiological condition of yeast cells [16]. Flow cytometry provides rapid multi-parametric analysis of single cells in solution according to scattered fluorescent light signals that can be converted into electronic signals, allowing for rapid and high-throughput analysis of individual yeast cells without damage, providing detailed information regarding cell vitality, cell cycle status, and protein expression levels [17]. Raman spectroscopy technique is a light scattering technique that detects alterations caused by collisions between incident photons and matter in molecular vibrational or rotational energy levels [18], enabling qualitative and quantitative analysis of substances.

Single-cell Raman spectrum (SCRS) is derived from Raman techniques, and cells can be detected and identified at the single-cell level with advantages like rapidity and low costs [19]. SCRS is considered to be a novel technology for single-cell phenotype identification and has been widely used in scientific research across various fields, including food inspection [20], microplastic identification [21], bacteria identification [22], drug analysis [23], and diagnosis [24]. Studies have demonstrated that SCRS is more precise at the single-cell level, and quantitative analysis of substances in a sample can be achieved. Avetisyan A et al. achieved quantitative determination of sugar uptake by a single bacterium to track changes in the bacterial system [25]. Moreover, the single-cell Raman technique based on D_2O-labeling was extensively applied. J Zhang et al. have established a strategy in probiotic product assessment based the SCRS technique, achieving 93 % accuracy in species-level identification of cells extracted from probiotic products, and cell vitality and heterogeneity were quantified through the C-D band [26].

In this study, the physiological parameters of baker's yeast (high-sugar and low-sugar yeast) were assessed using traditional methods based on traditional experimental approaches like plate counting and chemical reactions, as well as the Raman method based on the SCRS technique. Statistical analysis was conducted to contrast both methods, as well as the feasibility and credibility of the Raman methods. Moreover, baker's yeast is typically composed of yeast strains and additives to enhance fermentation performance. Therefore, an assessment of yeast pure strains isolated from yeast products must be conducted, and the results of yeast products and pure strains were comprehensively examined to explore the influences of additives in yeast products.

2. Materials and methods

2.1. Yeasts and culture medium

Eight *S. cerevisiae* strains were extracted from widely used yeast products purchased from a market (Tianjin, China), and all yeasts were commercial baker's yeast. Strains A-D were isolated from commercial low-sugar yeast products, and Strains E-G were isolated from commercial high-sugar yeast products.

Low-sugar yeasts were grown on YEPD medium containing 10 g/L yeast extract powder, 300 g/L sucrose, and 20 g/L peptone and fermented on LSMLD medium [27] with 5 g/L urea, 5 g/L Na_2HPO_4, 33.25 g/L maltose, 5 g/L glucose, 2.5 g/L $(NH_4)_2SO_4$, 0.6 g/L $MgSO_4$, 16 g/L KH_2PO_4, 2.5 mg/L vitamin B_1, 22.5 mg/L vitamin B_3, 1 mg/L vitamin B_2, 1.25 mg/L vitamin B_6, 5 mg/L vitamin B_5, and 0.5 mg/L vitamin B_9 at 30 °C. High-sugar yeasts were grown on high-sugar medium containing 10 g/L yeast extract powder, 20 g/L glucose and 20 g/L peptone and fermented on H-LSLMD medium containing 5 g/L urea, 5 g/L Na_2HPO_4, 300 g/L sucrose, 2.5 g/L $(NH_4)_2SO_4$, 0.6 g/L $MgSO_4$, 16 g/L KH_2PO_4, 2.5 mg/L vitamin B_1, 22.5 mg/L vitamin B_3, 1 mg/L vitamin B_2, 1.25 mg/L vitamin B_6, 5 mg/L vitamin B_5, and 0.5 mg/L vitamin B_9 at 30 °C. In pure strain experimentation, yeast strains that were isolated from products were grown on YEPD or high-sugar medium and transferred to molasses medium containing 10–12 Brix molasses, 5 g/L yeast extract powder, and 0.5 g/L $(NH_4)_2SO_4$. All media was sterilized at 115 °C for 30 min (LSMLD and H-LSMLD were filter sterilized).

2.2. Cultivation and fermentation of yeast cells

Pure strain experimentation was conducted as follows [28]: for low-sugar yeasts, strains were grown on YEPD medium, and single clones were chosen and grown on YEPD medium overnight. After centrifugation (10000 rpm for 3 min), cells were transferred into molasses medium at a ratio of 1:10 and cultured for 12 h. Then, they were transferred into LSMLD medium and fermented for 25 min. The cell suspension was removed for measurement at 0, 5, 10, 15, and 25 min. An additional portion of the cell suspension was harvested, washed, and stored at −20 °C for seven days for identical measurement. For high-sugar yeasts, YEPD medium and LSMLD medium were substituted with high-sugar medium and H-LSMLD medium, respectively, and the fermentation time points were harvested at 0, 10, 20, 30, and 50 min, while the other steps mirrored the low-sugar yeasts. The experiment was repeated two times.

The yeast product experiments proceeded as follows: strains from yeast products were fermented in LSMLD or H-HSMLD medium, and other steps mirrored the pure strain experiments. The experiment was repeated two times.

2.3. Collection of SCRS

SCRS of yeast cells proceeded as follows: for low-sugar yeasts, LSMLD medium was prepared, substituting D_2O for H_2O at different concentrations (50 %, 75 %, and 100 %), and the concentration at which yeast cells grew optimally was selected for subsequent culture. Yeast cells were grown in LSMLD (H_2O replaced with D_2O) medium at 30 °C and with 180 rpm shaking for 2–6 h. The cells were rinsed three times

with sterile water to remove residual D_2O. The cells were diluted, and an appropriate amount of cell suspension was placed onto glass slides for single-cell Raman spectroscopy. The parameters were established as follows: laser wavelength of 532 nm, power of 100 MW, pinhole of 25 μm, acquisition time of 2 s, and 120–150 SCRS of yeast cells were obtained. SCRS of yeast cells that were not incubated with D_2O were harvested following the same steps as the control group. Then, the collected SCRS of yeast cells were subjected to baseline correction and normalization using Labspec software (version 5.58.25, Jobin-Yvon), and thereby, the final SCRS was acquired. According to the SCRS acquired, a concentration of D_2O and cultivation time were chosen for the following experiments. For high-sugar yeasts, LSMLD (H_2O replaced with D_2O) medium was substituted with H-LSMLD (H_2O replaced with D_2O) medium. The experiment was repeated 2 two times.

SCRS of trehalose was performed as follows: A gradient concentration of trehalose strand solution was prepared, and an appropriate amount of solution was placed onto glass slides for single-cell Raman spectroscopy for characterization under the same conditions as collecting yeast cells SCRS. The experiment was repeated two times.

2.4. Measurement of cell viability and cryopreservation survival rate

For the traditional method, cell viability was measured via the number of viable cells and total cells in the same sample. Standard plate count (SPC) followed the following steps [29]: to determine the number of viable cells, the cell suspension was diluted and cultured on a YEPD medium. The plate was cultured in an incubator at 30 °C for 1–2 days until colonies could be counted. Total cell count was determined as follows (TCC) [30]: the cell suspension was diluted and added to a hemocytometer for microscopic observation to evaluate the total cells, including active and inactive cells. Cell viability can be computed using the formula: Cell viability = (Live cells count by SPC)/(Total cells count by TCC) × 100 %. The measurement was repeated two times.

For the Raman method [26], cell viability was measured by the proportion of cells with the C-D band in the SCRS. During cellular metabolism, D_2O was utilized by active cells, leading to the appearance of a C-D band (2040 cm^{-1} to 2300 cm^{-1} Raman shift), and D_2O could be used to probe the viability of yeast cells. The measurement was repeated two times.

Cryopreservation survival rate was measured by the SPC of viable cells before and after freezing, and the results were computed using the formula: Cryopreservation survival rate = (Live cells before freezing)/(Live cells after freezing). The measurement was repeated two times.

2.5. Measurement of intracellular trehalose content

Traditionally, the intracellular trehalose content was measured by the anthrone-sulfuric acid method described previously [8]. A gradient concentration of trehalose solutions was prepared and combined with an anthrone-sulfuric acid solution (80 % sulfuric acid, 1.6 g/L anthrone), and the mixture underwent reaction in a boiling water bath for 10 min and rapidly cooled to room temperature. Absorbance was measured at 630 nm using a visible spectrophotometer, and a standard curve of trehalose was constructed containing absorbance at 630 nm on the y-axis and trehalose concentration on the x-axis. Yeast cells were resuspended in a trichloroacetic acid solution (5 g/L trichloroacetic acid) and crushed in an ice bath for 1 h, oscillating every 15 min, and the suspension was rinsed three times with ultrapure water. The supernatant was obtained and mixed with an anthrone-sulfuric acid solution, and the mixture was reacted in a boiling water bath for 10 min, before being rapidly cooled to room temperature in an ice bath. The absorbance was evaluated at 630 nm using a visible spectrophotometer. The measurement was repeated two times.

For the Raman method, intracellular trehalose content was measured by analyzing the SCRS of yeast cells and trehalose. Studies demonstrate that trehalose has several characteristic peaks (485, 536, 851, 920, 1130, 1360, and 1465 cm^{-1} were observed) in the fingerprint region of SCRS, and the height of the peaks was correlated with the trehalose content [25]. Monitoring trehalose uptake and conversion of individual bacteria through Raman spectroscopy has been achieved [25]. The Raman standard curve of trehalose was developed with a peak area of 1130 cm^{-1} on the y-axis and trehalose concentration on the x-axis, as the peak area can more accurately quantify the substance than the peak height. The average SCRS of yeast cells was acquired using R software (version 4.0.1; R Core Team). The average SCRS was associated with the Raman standard curve, and quantitative analysis of the intracellular trehalose content of yeast cells was achieved.

2.6. Measurement of yeast fermentation capacity, intracellular ATP content, and cell vitality

Yeast fermentation capacity was measured by the volume increment (VI) of the fermented dough in a cylinder [31]. For low-sugar yeasts, the dough was composed of 300 g of standard flour, 9 g of fresh yeast, 1.5 g of salt, and 156 mL of sterile water at 30 °C for 5 min and divided into 50 g pieces. The dough was placed into a 250 mL measuring cylinder, compacted, and incubated at 30 °C for 180 min to ferment. The volume of the dough in the cylinder was recorded every 15 min until 180 min of fermentation was reached. Another dough sample was prepared following the same procedure and stored at −20 °C for seven days. For high-sugar yeast, the dough was composed of 300 g of standard flour, 9 g of fresh yeast, 1.5 g of salt, 90 g of sucrose, and 156 mL of sterile water. The subsequent steps were the same as outlined above. The measurement was repeated three times.

Intracellular ATP content was measured using an ATP assay kit from Beyotime (Product code: S0026), which was performed according to the manufacturer's instructions. The yeast cells were resuspended with lysis buffer and vortexed to lyse. The supernatant was reacted with a working solution in a white 96-well plate, and the luminescence was documented in a luminometer. The measurement was repeated three times.

The cell vitality was measured by the MAL of yeast single cells [26] which is evaluated by the SCRS of yeast cells. The cells with high MAL had high-efficiency metabolic processes, demonstrating higher efficiency in D_2O use and, therefore, higher C-D peaks. MAL of single-cell yeast can be computed by analyzing collected yeast cell SCRS [26].

2.7. Statistical analysis

SPSS (version 25.0, IBM Corp) software was employed for statistical analysis. For the data comparison before and after freezing, *t*-test analysis was performed, and $p < 0.05$ was considered statistically significant, while $p < 0.01$ was considered statistically highly significant.

Pearson correlation analysis was performed, and Pearson correlation coefficient (r) was estimated to determine the linear association between the traditional method and the Raman method; $r > 0.7$ was considered statistically significant, and $r > 0.9$ was considered statistically highly significant [32]. In this study, both the traditional methods and the Raman method contained ten samples each for the same parameter, and the sample data were normally distributed with a linear relationship.

3. Results

3.1. The SCRS of yeast products

The SCRS of eight yeast strains from strain A to strain H before and after freezing are presented in Fig. 1. The fingerprint region, typically ranging from 400 to 1800 cm^{-1}, contains unique peaks corresponding to the vibrational modes of molecular bonds, enabling the identification of specific substances [25]. Carbohydrates can be identified due to characteristic peaks from 1030 to 1130 cm^{-1}, such as C–C, C–O, and C–O–H deformation modes [25], and trehalose exhibits a strong peak in the 1130 cm^{-1} of Raman shift (Fig. 1A), making trehalose quantification in

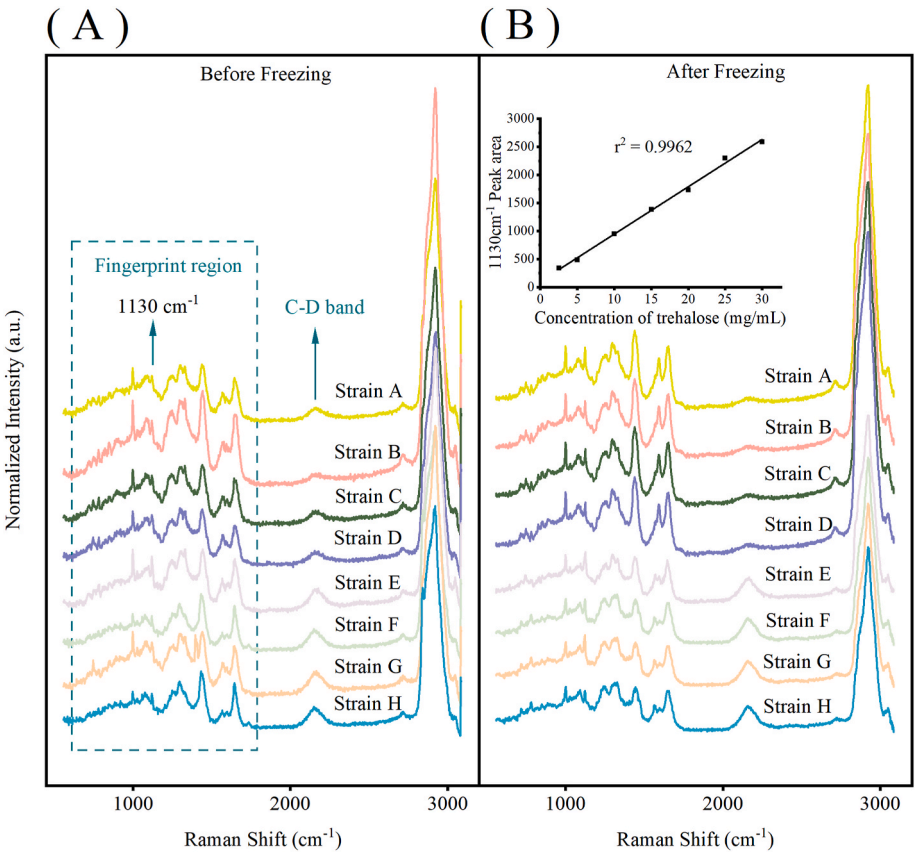

Fig. 1. Single-cell Raman spectrum (SCRS) of eight yeast strains before and after freezing, and the standard curve of the trehalose. (A) SCRS of eight yeast strains before freezing. (B) SCRS of eight yeast strains after freezing and the trehalose standard curve.

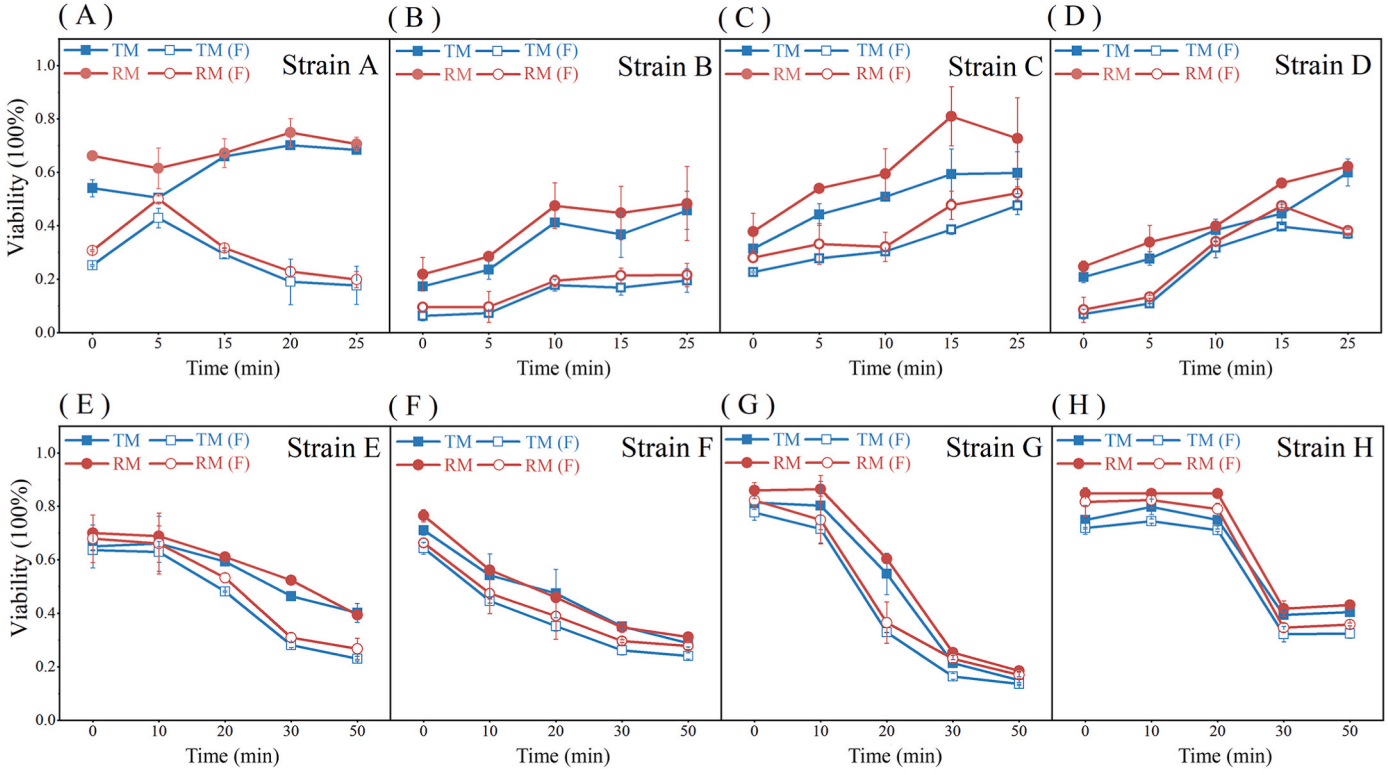

Fig. 2. Cell viability of eight yeast strains measured by the traditional method and the Raman method before and after freezing. TM, traditional method before freezing; TM (F), traditional method after freezing; RM, Raman method before freezing; RM (F), Raman method after freezing.

yeast cells feasible. The C-D band range from 2000 to 2300 cm^{-1} stems from D_2O uptake by the cells, quantifying the vitality of yeast cells. The SCRS after freezing is presented in Fig. 1B, and the standard curve of trehalose is necessary for the Raman method to quantify intracellular trehalose content. The trehalose standard curve presents the relationship between the characteristic peak area of trehalose and trehalose concentration, with an R^2 value of 0.9962, indicating a representative linear relationship.

3.2. Cell viability of yeast products

Cell viability before and after freezing was measured to analyze fermentation performance and freezing tolerance. As fermentation time increased, cell viability of low-sugar yeast strains exhibited an upward trend (Fig. 2A–D), while high-sugar yeast strains showed a downward trend, suggesting the high stress of high-osmolarity environments (Fig. 2E–H).

Before freezing, the traditional method of examining low-sugar yeasts (Fig. 2A–D) shows that after 25 min of fermentation, the highest viability was attained by strain A (68.4 %), and the second-highest viability was achieved by strain C (59.8 %), while the lowest viability was observed in strain B (45.9 %). The cell viability from the Raman method was consistent with the traditional method, and the Pearson correlation coefficients (r) were A-0.763, B-0.985, C-0.996, and D-0.965 ($p < 0.05$ for all values). The correlation between the two methods was significant in strain A, and highly significant in strains B, C, and D. In terms of high-sugar yeasts (Fig. 2E–H), the traditional method demonstrates that after 50 min of fermentation, the highest viability was achieved by strain H (40.5 %), followed by strain E (39.1 %), whereas the lowest viability was observed in strain G (16.8 %). The cell viability from the Raman method was highly correlated with the traditional method, with all strains showing a high Pearson correlation coefficient ($r > 0.9$).

Following freezing, the cell viability of all strains showed a general decrease, whereas low-sugar yeast strains showed a greater decrease ($p < 0.05$). The traditional method indicates that the cell viability of low-sugar yeasts was reduced by 39.8 % on average, while that of high-sugar yeasts was decreased by an average of only 17.6 %, indicating that high-sugar yeast strains had higher freezing tolerance compared to low-sugar yeast strains. Among the strains, strain B exhibited the greatest decrease by 58.8 % (Fig. 2B) on average following freezing, while strain H exhibited the smallest decrease at only 11.2 % (Fig. 2H) on average. Pearson correlation analysis indicates that the two methods remain highly correlated in measuring yeast cell viability after freezing.

Notably, the cell viability results from the Raman method were 10–20 % higher than those acquired via the traditional method. This is attributed to the Raman method, which relies on detecting D_2O activity at the single cell level, and therefore, yeast cells with low vitality can be

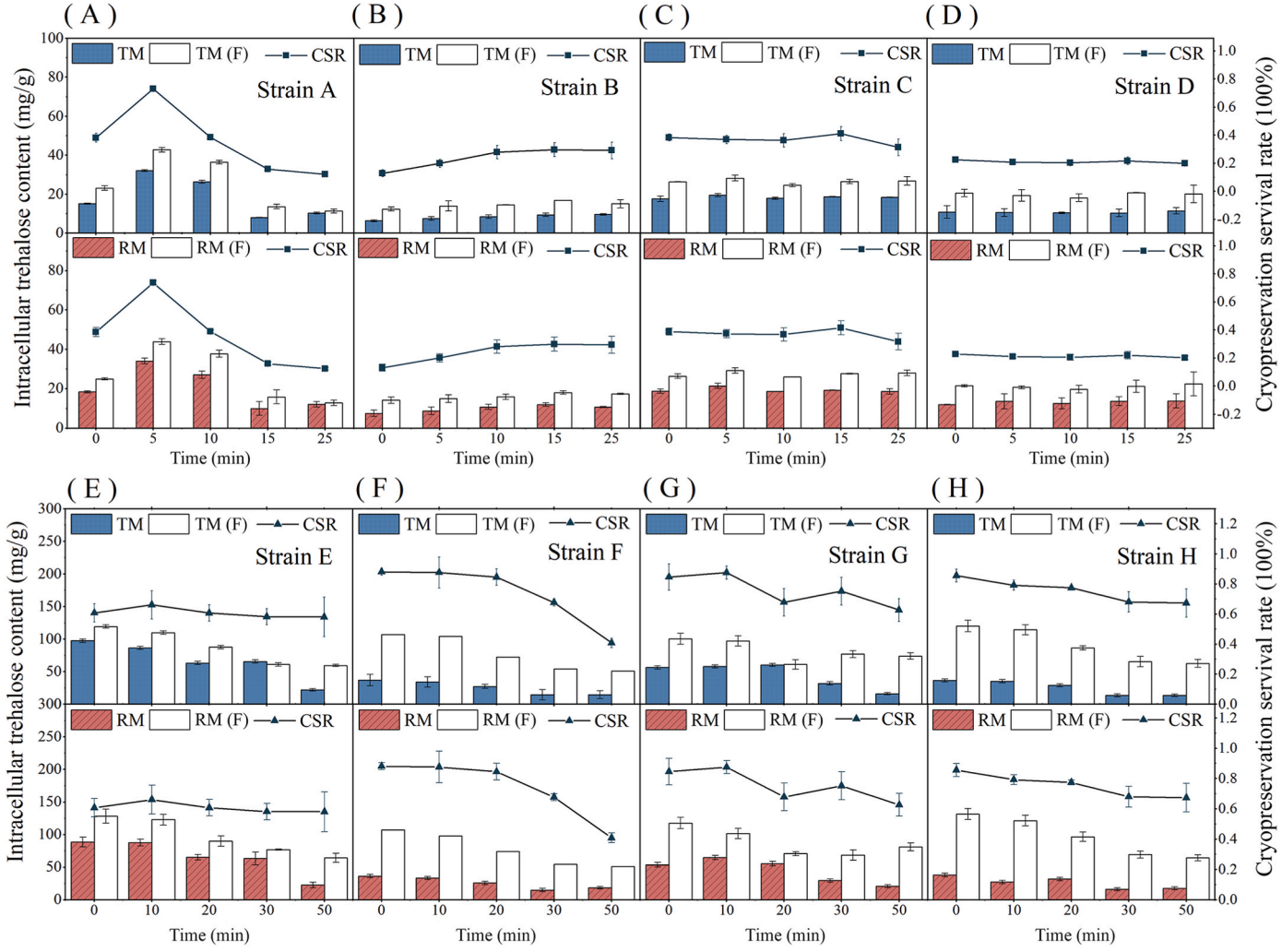

Fig. 3. Intracellular trehalose content and cryopreservation survival rate of eight yeast strains measured by traditional method and Raman method before and after freezing. TM, traditional method before freezing; TM (F), traditional method after freezing; RM, Raman method before freezing; RM (F), Raman method after freezing; CSR, cryopreservation survival rate.

identified, while they cannot be detected by traditional methods. Therefore, the Raman methods have the advantages of high sensitivity and accuracy in measuring yeast cell viability, enabling accurate analysis and assessment of yeast fermentation performance.

3.3. *The intracellular trehalose content and cryopreservation survival rate of yeast products*

The intracellular trehalose content and cryopreservation survival rate of eight strains were measured before and after freezing using the Raman method (Fig. 3A–H lower panel) and traditional method (Fig. 3A–H top panel) to assess the influence of the freezing process on intracellular trehalose content of yeast cells, the correlation between the intracellular trehalose content and the cryopreservation survival rate, as well as the correlation between the two methods.

The intracellular trehalose content of each yeast strain was generally increased after the freezing process ($p < 0.05$), as shown in Fig. 3. This is attributed to endogenous trehalose biosynthesis, one of the main pathways for cells to resist external pressure. Specifically, high-sugar yeast strains have higher freezing tolerance compared to low-sugar yeast strains ($p < 0.05$), with the highest intracellular trehalose content for strain A at 40.1 mg/g (Fig. 3A) and the highest intracellular trehalose content for strain E at 139.5 mg/g (Fig. 3E). The cryopreservation survival rate of high-sugar yeast strains was typically higher than low-sugar yeast strains ($p < 0.05$), consistent with cell viability. Moreover, high-sugar yeast exhibited higher trehalose synthesis performance. Before freezing, strain H had an intracellular trehalose content of 25.5 mg/g on average, which increased to 75.2 mg/g following freezing, increasing by 294 %. This increase indicates that trehalose can be biosynthesized rapidly under freezing conditions by high-sugar yeast strains such as strain H, which means that strain H could have improved fermentation performance compared to those yeast strains that cannot biosynthesize trehalose rapidly.

The Raman method exhibits equivalent feasibility and credibility to traditional methods in assessing intracellular trehalose content. Prior to freezing, the traditional method (Fig. 3A–H upper panel) demonstrates that strain A had the highest intracellular trehalose content at 20.1 mg/g on average, followed by strain C at 18.4 mg/g, whereas strain M exhibited the lowest at 8.3 mg/g, and the results acquired by the Raman method (Fig. 3A–H blow) were consistent. The Pearson correlation coefficients (r) between these two methods were A-0.975, B-0.955, C-0.971, D-0.981, E−0.922, F-0.931, G-0.957, and H-0.945, indicating a highly significant correlation between the two methods in measuring the intracellular trehalose content of yeast strains.

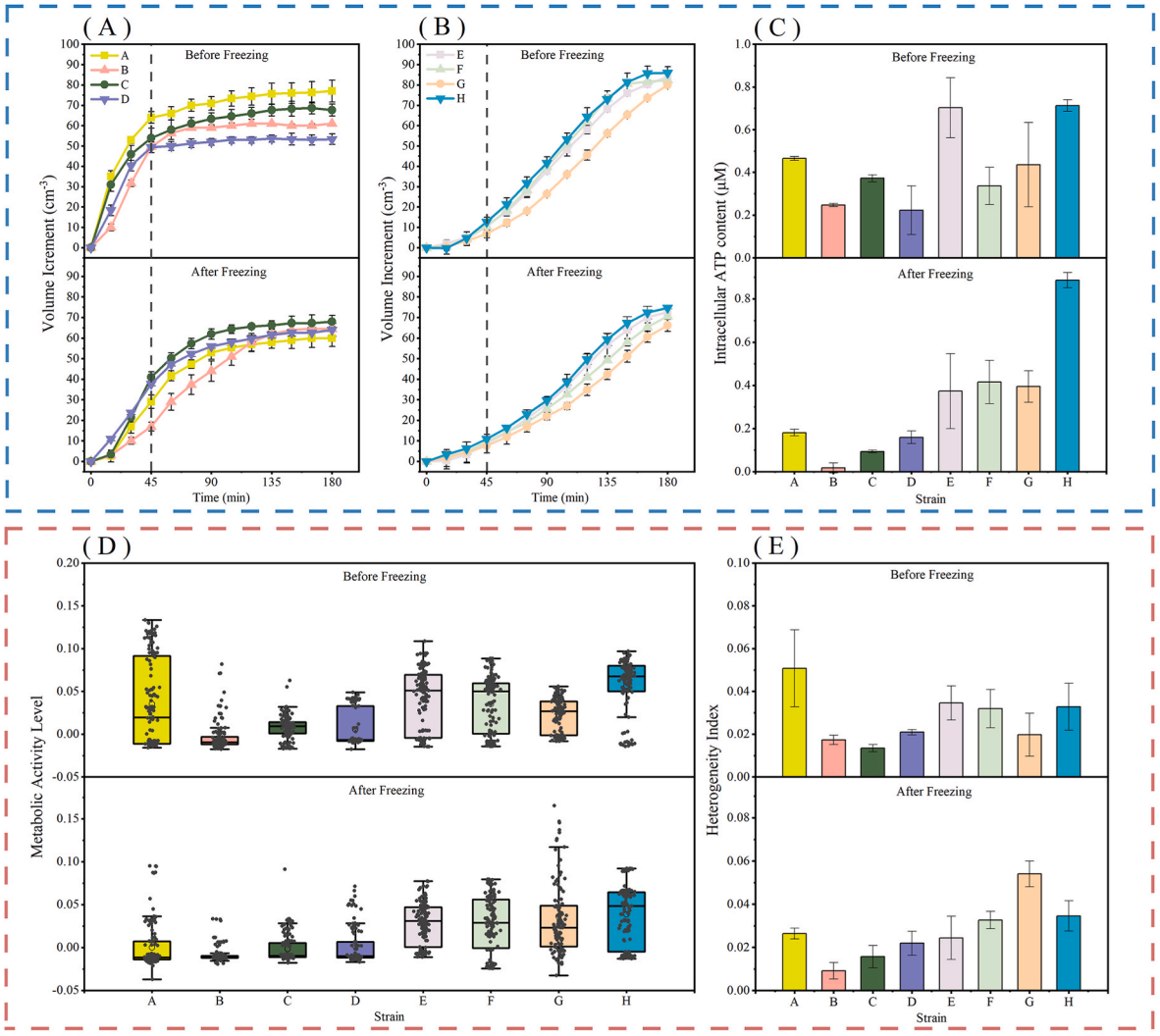

Fig. 4. Volume increment, metabolic activity level, intracellular ATP content, and heterogeneity index of yeast products before and after freezing. (A) Volume increment of four low-sugar yeasts. (B) Volume increment of four high-sugar yeasts. (C) Intracellular ATP content of eight yeast strains. (D) Metabolic activity level of eight yeast strains. (E) Heterogeneity index of eight yeast strains.

3.4. The fermentation capacity, intracellular ATP content, and cell vitality of yeast products

Traditionally, the fermentation capacity of baker's yeast can be measured by the dough gas production capacity and intracellular ATP content, but only at the population level. In contrast, the Raman method measures the MAL and enables the measurement of fermentation capacity and cell vitality at the population and single-cell levels [26]. In this study, dough gas production capacity was measured using the VI of the dough in the cylinder within 180 min of fermentation, and the VI at 45 min, commonly used in dough fermentation [3]. As for the Raman method, the fermentation capacity was measured according to the MAL of yeast strains.

Before freezing, the VI of high-sugar yeast strains (Fig. 4B upper panel) at 45 min was lower ($p < 0.05$) compared to low-sugar yeast (Fig. 4A upper panel) for the high osmolarity fermentation environment but higher at 180 min, suggesting the higher fermentation capacity of high-sugar yeast. The intracellular ATP content exhibited similar trends to high-sugar yeast strains, generally higher than the low-sugar yeast strains (Fig. 4C, $p < 0.05$). The VI of four low-sugar yeast strains (Fig. 4A upper panel) show a pronounced difference ($p < 0.05$), with strain A, exhibiting the highest VI at 64.0 cm^3, followed by strain C at 54.1 cm^3, whereas strain B exhibited the lowest at 49.7 cm^3. Meanwhile, the four high-sugar yeast strains (Fig. 4B upper panel) behaved uniformly in fermentation, with strain G exhibiting the lowest VI at 6.8 cm^3 while the others were over 10 cm^3. Correspondingly, the MAL (Fig. 4D), which represents the fermentation capacity of yeast strains measured by the Raman method, was consistent with trends presented in Fig. 4A and B. Fig. 4D depicts the MAL of yeast strains, with the box representing the pullulation level and the dots representing single yeast cells. At the population level, strain A exhibited the highest MAL with the box positioned highest among four low-sugar yeast strains, suggesting the highest cell vitality, while strain B exhibited the lowest, and strain G exhibited the lowest MAL among the four high-sugar yeast strains, consistent with the results from the traditional method (Fig. 4A and B). Notably, strain H, which showed small differences from other strains according to the traditional method (Fig. 4D upper panel), exhibited a higher MAL (Fig. 4D), indicating its higher fermentation capacity not identified by the traditional method.

After freezing, the VI of all yeast strains decreased (Fig. 4A and B lower panel), while high-sugar yeast strains exhibited a smaller decrease ($p < 0.05$), indicating higher freezing tolerance of high-sugar yeast strains, consistent with the results outlined above. Fig. 4C indicates that low-sugar strains had a strong decrease in intracellular ATP content, and high-sugar strains decreased slightly, attributed to the higher freezing tolerance of high-sugar yeast strains. Notably, the intracellular ATP content of strains F–H decreased very slightly; strains F and H showed an increase, while strain E suffered a strong decrease. For yeast cells, trehalose synthesis requires ATP to provide energy for UDP-glucose formation. Strains F–H synthesize a large amount of trehalose at the initial period of freezing to resist the freezing environment, and therefore ATP was synthesized to provide energy. These findings were consistent with Fig. 3F–H.

Among the strains, strain A exhibited the greatest VI decrease, from 64 cm^3 before freezing to 28.7 cm^3, decreasing by 55.2 %. This can be attributed to the heterogeneity of yeast cells and the additives in yeast products. Fig. 4E presents the heterogeneity index (HI) of each yeast strain, representing the degree of heterogeneity among yeast cells [26]. Strain A exhibited a high HI, which may have a negative effect on its freezing tolerance. Additionally, additives included with yeast products may also impact the properties of yeast strains [33].

3.5. Credibility of the Raman method in assessing yeast pure strains, and the influence of additives on yeast products

Yeast product experiments demonstrated that the Raman method had the same feasibility and credibility as traditional methods in assessing baker's yeast products. Furthermore, the Raman method had advantages at the single-cell level. Further experiments should be conducted on pure yeast strains isolated from yeast products. The feasibility and credibility of the Raman method in assessing yeast pure strains can be analyzed. Conversely, the influence of the additives added to yeast products on yeast fermentation performance can be examined through yeast pure strain experiments.

In these experiments, the cell viability and intracellular trehalose content of eight pure yeast strains before and after freezing were measured by Raman and traditional methods, and the Pearson correlation analysis of both methods was performed. There was still a highly significant correlation (Fig. 5A) ($p < 0.05$) between the two methods in assessing pure strains, indicating that the Raman method has the same feasibility and credibility as traditional methods in assessing pure yeast strains.

However, there were differences to consider between the MAL results of yeast products and pure strains. The MAL of yeast products and pure strains before freezing (Fig. 5B) demonstrates that although yeast products exhibited higher HI compared to yeast pure strains (Fig. 5C), the yeast products still exhibited a higher MAL ($p < 0.05$), which represents a higher fermentation capacity. This could be attributed to the inclusion of emulsifiers and antioxidants in yeast products [33], which may increase the stability and antioxidation during dough fermentation. The primary emulsifier added is sorbitan monostearate (SPAN60), whose molecular structure contains both hydrophobic and hydrophilic groups that can enhance dough properties, promoting gas dispersion during fermentation [34]. The main antioxidant is vitamin C, which could inhibit the oxidation of other components in dough by oxygen during fermentation, improving the fermentation efficiency of yeast cells [35].

4. Discussion

Raman methods are comprehensive approaches for assessing the fermentation performance of the baker's yeast, integrating rapid measurement of cell viability, intracellular trehalose content, and cell vitality at the levels of population and single cell, which could address the pressing challenges in quality assessment of yeast products. Instead of waiting for cell culture and complex experimental operations, the Raman method utilizes information-rich SCRS based on D_2O probes from yeast cells to more efficiently assess the fermentation performance of yeast strains. Specifically, the fingerprint region facilitates the precise quantification of certain substances within yeast cells [26], including fermentation-relevant components like intracellular trehalose, while the C-D band allows for accurate quantification of cell viability and vitality through MAL analysis.

The Raman method exhibited notable advantages in feasibility, credibility, sensitivity, efficiency, and cost-effectiveness in assessing yeast products. Based on our estimations of experimental duration and consumable costs (Table 1), the Raman method requires approximately 5 h and $4.39 for a comprehensive quality assessment, including cell culture, sample preparation, and acquisition of SCRS, and takes an additional 10 h for data analysis. In contrast, traditional methods take 9–11 days and $33.24 for live cell counting, total cell counting, and trehalose extraction and detection, alongside an additional two days for data processing and analysis. The Raman method can be ten times faster and reduces consumable costs by an order of magnitude.

The Raman method based on SCRS can detect active and inactive yeast cells, providing yeast production companies with a more effective method of assessing yeast fermentation performance. It is also significant in the food industry, especially for detecting pathogenic bacteria in food products. For instance, the viable but non-culturable (VBNC) state is a common survival approach employed by microorganisms against environmental stress such as osmotic pressure and extreme temperatures [36]. In this state, microbial cells maintain metabolic vitality but

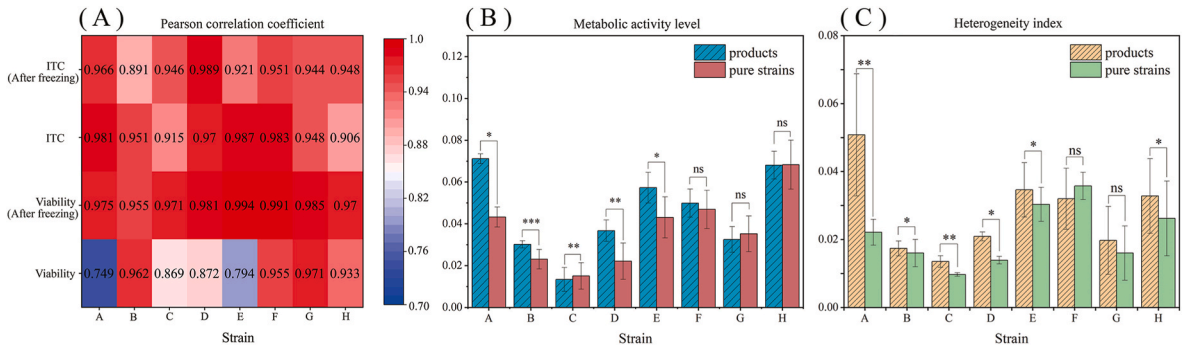

Fig. 5. The correlation between traditional and Raman methods in assessing yeast pure strains, and the fermentation performance of yeast pure strains. (A) The Pearson correlation coefficient between traditional and Raman methods in measuring cell viability and intracellular trehalose content of yeast pure strains. (B) Metabolic activity level of eight yeast pure strains. (C) Heterogeneity index of eight yeast pure strains. ITC, intracellular trehalose content.

Table 1
Comparison of quality-assessment parameters for yeast products between traditional methods and the Raman method. SCRS, single-cell Raman spectrum.

Methods	Cost ($)	Duration
Traditional methods		
Cell viability		
Hemocytometer	3.35	3 days
Plate colony	7.16	10 h
Intracellular trehalose content		
Trehalose extraction	8.33	2 days
Microplate reader	9.20	5 h
Cell vitality		
Dough gas production	5.20	5 h
Raman method		
Cell viability and vitality + intracellular trehalose content		
SCRS-based methods	4.39	5 h

cannot be cultured, and VBNC microorganisms can be resuscitated under favorable conditions and regain pathogenicity [37]. Current food safety standards like the Food Safety Modernization Act (FSMA) and General Food Law Regulation (Regulation (EC) No 178/2002) for detecting pathogenic microorganisms rely on CFUs on agar plates, which may fail to identify some pathogens [38], compromising public food safety. Recent studies have documented the application of Raman technology in identifying pathogenic bacteria in the VBNC state. Fan et al. [39] employed D_2O-labeled single-cell confocal micro-Raman spectroscopy to detect *Rhodococcus biphenylivorans* in the VBNC state under anaerobic stress, revealing a significant decrease in metabolic vitality in the VBNC state. As outlined, the Raman method could be promising in pathogenic bacteria detection.

Although single-cell Raman technology in this study has demonstrated numerous advantages in assessing yeast products, further development is required to explore its further prospects. For instance, there is only one single yeast strain, *Saccharomyces cerevisiae*, to be assessed, while other yeast strains such as *Kluyveromyces marxianus*, *Kluyveromyces lactic*, *Debaryomyces hansenii*, and *Torulaspora delbrueckii* could be assessed. Moreover, the Raman technology can be integrated with other advanced technologies to offer a powerful approach to investigating the physiological performance of yeast cells. SECM enables the precise delivery of electrical pulses to transiently permeabilize cell membranes, enabling the entry of diverse molecules into cells [40]. This process can be monitored using single-cell Raman spectroscopy, offering detailed information on the biochemical composition and metabolic activity of yeast cells and allowing researchers to explore the cellular responses to the electroporation at the molecular level [41], encompassing changes in protein expression and metabolic shifts. Furthermore, high-throughput Raman flow cytometry (FCM), such as FlowRACS [42], can enhance SCRS collection speed, improving assessment efficiency. Several quality assessment systems have been established based on the FCM. Zhang et al. employed the SCRS-based SCIVVS system to evaluate 21 statutory probiotic species and establish a novel assessment system for probiotic products [26]. In summary, single-cell Raman spectrum technology can be combined with various techniques to enable comprehensive and in-depth research on yeast fermentation.

5. Conclusion

As technology develops, traditional methods for yeast product measurement have reached technical maturity. However, it faces several challenges, including high costs, long durations, and cumbersome operations. In this study, both traditional methods and the Raman method were utilized to measure the core parameters of yeast products. Statistical analysis of the traditional and Raman methods revealed a significant correlation between the two methods in measuring the viability and intracellular trehalose content of yeast cells. The analysis of cell vitality by comparing dough gas production capacity, intracellular ATP content, and MAL of yeast cells demonstrated the feasibility and credibility of the Raman method in assessing yeast products, as well as pure yeast strains.

Notably, the Raman method exhibited higher sensitivity and accuracy in measuring viability than the traditional method. These advantages stem from the detection principle based on D_2O labeling, enabling yeast cells with low vitality that cannot grow into colonies on plates to be characterized through the Raman method, which could have more applications in the food industry, such as pathogenic bacteria detection. Moreover, MAL enables the analysis of yeast cell fermentation performance from a population and single-cell level, allowing for the assessment of fermentation performance. Furthermore, the Raman method offers cost and efficiency advantages, with multiple parameters measured in a single detection of yeast cells SCRS, making it a more attractive method for yeast companies. In conclusion, the Raman technology, combining high sensitivity, precision, and accuracy, can be widely applied in the future rapid assessment of yeast products.

CRediT authorship contribution statement

Xi Sun: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Writing – review & editing. **Xin Zhou:** Data curation, Formal analysis, Investigation, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ran Yu:** Resources. **Xiaofang Zhou:** Resources. **Jun Zhang:** Project administration. **Teng Xu:** Data curation, Formal analysis, Software. **Jianmei Wang:** Formal analysis. **Mengqi Li:** Data curation. **Xiaoting Li:** Data curation. **Min Zhang:** Resources. **Jian Xu:** Resources. **Jia Zhang:** Conceptualization, Funding acquisition, Project administration, Resources, Writing – review & editing.

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.

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