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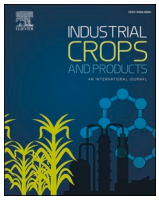

Enzymatic catalytic dynamics of lactic-acetic acid co-fermentation: Effect of cellulase on the physicochemical, phytochemicals, volatiles, and antioxidant activity of jujube puree extracts

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ABSTRACT

The processing of jujube (*Ziziphus jujuba*) is imperative due to its esteemed status and rich pharmacological properties despite its sensory challenges and short shelf-life. Traditional drying methods preserve jujube quality but complicate bioactive compound extraction due to the conjugated polysaccharide matrix. This study addresses the gap in research on enzymatic pretreatment by investigating the use of cellulases to break down the rigid cellulose structure, thus enhancing the efficiency of subsequent fermentation processes. Besides, this research explored the catalytic dynamics of cellulase pretreatment in lactic-acetic acid co-fermentation to improve jujube puree's functional and quality properties. Methodologies involved applying cellulase at concentrations of 1 %, 3 %, and 5 % (v/v) at 40 °C for 4 hours, followed by fermentation with *Lactobacillus plantarum* CGMCC 3346 and *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41). Results indicated that fermentation and enzyme hydrolysis significantly influenced jujube puree's physicochemical and sensory attributes. Fermentation increased densities and moisture content, while unfermented and enzyme-treated samples had higher total soluble solids (TSS) values (11.00–11.50 °Brix). Enzyme concentration affected the reducing sugar and protein content, with higher enzyme concentrations (5 %EF) producing lighter products ($L^* = 52.00$). Total phenolic content increased with fermentation, particularly in the 3 %EF treatment (351.89 mg GAE/100 g FW), while flavonoid and flavonol contents peaked in the 5 %EF (426.33 mg RE/100 g FW) and 3 %EF (25.80 mg QE/100 g FW) treatments, respectively. Proanthocyanidin content was highest at moderate enzyme concentrations, notably in the 3 %EF treatment (715.95 mg CE/100 g FW). Fermentation also enhanced antioxidant properties and altered flavor profiles by decreasing acetoin levels and increasing acid and ester production. This study highlights the significance of the combinative effect of enzyme concentration and fermentation processes in augmenting jujube puree extracts' nutritional and sensory qualities.

1. Introduction

Jujube (*Ziziphus jujuba*), esteemed as one of mainland China's top five fruits, encapsulates not only a profound cultural heritage but also a wealth of pharmacological benefits attributable to its functional components (Li et al., 2022; Rashwan et al., 2020). Despite its esteemed status, this multifaceted fruit confronts inherent challenges, including organoleptic limitations such as persistent bitterness in its dried forms (Kong et al., 2024; Pu et al., 2017; Zhu et al., 2024), which are predominantly processed or consumed and a short shelf-life in its fresh state due to its high water and sugar content (Pan et al., 2022). Consequently, concerted efforts have emerged to unlock jujube's biotechnological

Abbreviations: VOCs, volatile organic compounds; TSS, total soluble solids; °Brix, degrees brix; PH, potential of hydrogen; G/L, grams per liter; °C, degrees Celsius; TE, Trolox equivalents; GAE, gallic acid equivalents; RE, rutin equivalents; QE, quercetin equivalents; CE, catechin equivalents; DPPH-SA, 2,2-diphenyl-1-picrylhydrazyl scavenging activity; FRAP, ferric reducing antioxidant power; ΔE, color difference; PCA, principal component analysis; PC, principal component; FW, fresh weight.

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potential, fostering exploration into innovative avenues for human consumption and therapeutic applications (Zhu et al., 2024).

Although drying is imperative for preserving the quality, color, aroma, and flavor of jujube (Rashwan et al., 2020), extant reports have highlighted that the polysaccharide constituents of dried jujubes—cellulose, hemicellulose, and lignin—become conjugated, thereby complicating the extraction of bioactive compounds (Rashwan et al., 2020; Ruan et al., 2022). The crystalline structure of cellulose, in particular, creates a complex matrix that poses significant challenges for its conversion into fermentable sugars and for the efficient release of bioactive components. Enzymatic pretreatment, especially with cellulase enzymes, offers a promising solution by effectively degrading the rigid cellulose structure into simpler sugars (Anuradha Jabasingh and Valli Nachiyar, 2011; Faria et al., 2020; Leite et al., 2019; M. Wang et al., 2012). This approach enhances the accessibility of the puree and augments the efficiency of subsequent fermentation processes. Recent research by Boasiako et al. (2024) highlights the presence of flavonoids and anthocyanins in dried jujube puree, although these are overshadowed by a significantly higher content of proanthocyanidins. This intricate matrix presents an intriguing opportunity for further exploration through enzymatic hydrolysis, as demonstrated by Liu et al. (2023), who showcased the efficacy of boiling water and steam treatments in enhancing juice yield, thereby facilitating lactic acid fermentation. However, studies on enzymatic interventions in jujube processing remain limited, highlighting the exigency for novel approaches.

Amidst this quest for solutions, enzyme pretreatment emerges as a promising green, non-thermal pre-processing technology, aligning with sustainability principles and offering pathways for enhanced agricultural productivity, food security, and environmental stewardship (Boasiako et al., 2024). Enzymatic hydrolysis, characterized by its simplicity, cost-effectiveness, and generation of bioactive peptides, stands out as a method of choice for optimizing jujube's biotechnological value. Building upon extant literature (Boasiako, Ekumah, et al., 2024; Boasiako, Yinka, et al., 2024), this study hypothesizes that enzyme pretreatments, particularly those involving cellulase, could significantly enhance raw jujube puree's functional and quality properties. The enzymatic preparation represents an innovative approach aiming to achieve superior enzyme hydrolysates and yields with minimal enzyme material. These findings complement ongoing explorations in jujube fruit processing, offering a promising avenue for enhancing both the functional and quality properties of jujube puree.

Furthermore, fermentation with beneficial microorganisms such as *Lactobacillus plantarum* and *Acetobacter pasteurianus* holds promise in altering the biochemical composition of fruit purees, enhancing their nutritional and functional profiles. While previous studies (J. Li et al., 2022; T. Li et al., 2021; G. Liu et al., 2023; Pan et al., 2022) have explored the impact of lactic acid bacteria (LAB) fermentation on the quality of jujube juices, there is limited information on how co-fermentation with acetic acid bacteria, combined with enzymatic hydrolysis, affects the quality characteristics of jujube puree hydrolysates. Considering the efficacious nature of *Lactobacillus plantarum* CGMCC 3346 in either bi- or tri-cultural fermentation with *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41) in our previous studies (Boasiako, Ekumah, et al., 2024; Boasiako, Yinka, et al., 2024), this study focuses on these two strains along with various cellulase hydrolysis and fermentation treatments on jujube puree extracts, evaluating their impact on physicochemical properties, phytochemical contents, volatiles, and antioxidant properties. By discerning the correlations between these parameters, this research aims to identify optimal processing conditions that maximize jujube puree's nutritional and sensory qualities, thereby contributing to the development of functional jujube-based products with enhanced health benefits and aesthetic appeal.

2. Experimental approach

2.1. Enzymes, microbial strains, chemicals and reagents

Cellulase (98 %, CAS number: 9012–54–8) and 2-Octanol ($\geq$ 99.5 %) were obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). *Lactobacillus plantarum* CGMCC 3346 was procured from Synbio Tech Inc. in Kaohsiung City, Taiwan, China. The strain of acetic acid bacteria (AAB), *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41), was acquired from Yishui Jinrun Biological Technology Co. Ltd. in Yishui, Shandong, China. All other chemicals were of analytical grade and procured from Sinopharm Chemical Reagent, China, without requiring further purification.

2.2. Enzyme activation, jujube puree, enzyme hydrolysis, centrifugation of extracts, and lactic-acetic acid co-fermentation

2.2.1. Enzyme activation and jujube puree preparation

Initially, 100 mL of a 0.2 M sodium acetate buffer at pH 6.26 was prepared. Subsequently, 1 g of cellulase enzyme was suspended in the buffer and stirred until fully dissolved. The enzyme solution was then stored at 4°C until further use. Immediately after preparing the enzyme solution, jujube puree was prepared according to the methodology described in Boasiako et al. (2024) protocol. Specifically, dry jujube samples with a moisture content of 24.58 $\pm$ 1.01 % (wb) were boiled in distilled water at a 1:5 (w/v) ratio for 10 minutes. After boiling, the ventrally grooved pits were removed. The jujubes were then combined with distilled water at a 1:2 (w/v) ratio and homogenized using a kitchen blender. The pH and °Brix of the resulting puree were adjusted to 5.5 and 11 °Brix, respectively, using food-grade Na_2CO_3 and distilled water.

2.2.2. Enzymatic hydrolysis

After cooling the puree to 25 °C, the pasteurized puree was used for enzymatic extraction. Cellulase enzyme was added to portions of the puree at concentrations of 1 %, 3 %, and 5 % (v/v). The containers were then sealed airtight and incubated (Qiang Le HYL-C2 rotary equipment) at 40 °C for 4 hours (Amulya and ul Islam, 2023), with a rotation speed of 100 rpm (Qiang Le HYL-C2). Following hydrolysis, the enzymes were deactivated by boiling the contents for 5 minutes (Amulya and ul Islam, 2023). The hydrolysates were then centrifuged at 4000 rpm for 10 minutes at 25°C to obtain the extracts. The supernatants (extracts) were collected and weighed, and the extraction yield (Amulya and ul Islam, 2023) was calculated as:

$$\textit{Extraction yield}, \quad \% = \frac{(\textit{weight of the extract})}{\textit{weight of sample used}} \times 100 \tag{1}$$

2.2.3. Lactic-acetic co-fermentation procedure

The extracts were used for lactic-acetic acid co-fermentation. The jujube extract was mixed with 1 % (v/v) of each respective activated inoculant (Boasiako, Ekumah, et al., 2024; Boasiako, Yinka, et al., 2024), and placed in a rotary shaking incubator (Qiang Le HYL-C2) at 37 °C for 48 hours to initiate anaerobic fermentation with *Lactobacillus plantarum* CGMCC 3346. This was followed by a 24-hour incubation at 30 °C (Xia et al., 2022) to support the activity of *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41). The unique combinations of enzyme starter cultures used to create lactic-acetified jujube hydrolysates (EF) were as follows:

A. 1 %E: Enzyme hydrolysate with 1 % cellulase concentration.
B. 3 %E: Enzyme hydrolysate with 3 % cellulase concentration.
C. 5 %E: Enzyme hydrolysate with 5 % cellulase concentration.
D. 1 %EF: Fermented enzyme hydrolysate with 1 % cellulase concentration, using *Lactobacillus plantarum* CGMCC 3346 and *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41).

E. 3 %EF: Fermented enzyme hydrolysate with 3 % cellulase concentration, using *Lactobacillus plantarum* CGMCC 3346 and *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41).
F. 5 %EF: Fermented enzyme hydrolysate with 5 % cellulase concentration, using *Lactobacillus plantarum* CGMCC 3346 and *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41).

EFCON consists of sterile extracted hydrolysates without any bacterial inoculants (pasteurized without inoculation). Each fermentation was conducted individually in 1000 mL Erlenmeyer flasks. The fermented samples and the control (EFCON) were stored at −40 °C. Before each analysis, the samples were thawed to room temperature, and various aliquots were used for the experiments.

2.3. Evaluation of sensory attributes

2.3.1. Panelist selection

For this study, 16 sensory panelists were carefully selected, with ages ranging from 23 to 61 years. the Society of Sensory Professionals (SSP) recommends four or more panelists, provided their training is adequate to justify lower numbers (Manual on Descriptive Analysis Testing for Sensory Evaluation, 2019). These individuals were recruited from the Food Fermentation Laboratory at Jiangsu University, ensuring that each panelist possessed considerable expertise and experience in the domain of fermented foods. The panel consisted of 56 % males and 44 % females, offering a diverse demographic suitable for sensory evaluation. Each panelist underwent extensive training, encompassing a total of 120 hours, designed to refine their sensory assessment skills and familiarize them with the specific attributes of the samples under evaluation. Before their involvement, all panelists provided informed consent, affirming their understanding of the study's nature and their voluntary participation.

2.3.2. Experimental design

The sensory evaluation was conducted in a controlled environment within the food laboratory at Jiangsu University. The laboratory was well-ventilated and illuminated by fluorescent lighting to ensure consistent lighting conditions throughout the evaluation. The temperature was maintained between 23 and 25 °C (Boateng et al., 2022). This standardized setting was crucial for minimizing external factors that could influence the panelists' sensory perceptions.

2.3.3. Sample preparation and presentation

The samples comprised lactic-acetic acid co-fermented jujube hydrolysates, including both fermented hydrolysate samples and unfermented enzyme hydrolysate (EFCON). Each sample was meticulously prepared to ensure uniformity and consistency in presentation. The samples were dispensed in 15 mL disposable plastic cups, each containing 10 mL of hydrolysate. To maintain sample anonymity and mitigate bias, each cup was labeled with a unique, randomly generated three-digit code (Boateng et al., 2022). This random coding ensured that panelists could not identify the samples based on their labels.

2.3.4. Sensory evaluation protocol

Panelists were instructed to evaluate the samples based on a predefined list of sensory descriptors encompassing four main categories: appearance, aroma, texture, taste, and flavor.

Appearance: Panelists assessed the color and clarity of each sample.

Aroma: Floral and fruity aromas were evaluated for intensity and quality.

Texture: The mouthfeel and thickness of the purees were examined.

Taste and Flavor: Attributes such as off-flavors, sourness, sweetness, acidity, and astringency were assessed.

Each attribute was rated using a nine-point hedonic scale, which ranged from "dislike extremely" (1) to "like extremely" (9). This scale provided a structured framework for panelists to quantify their sensory perceptions and ensured consistency in the evaluation process. To minimize potential biases, the presentation sequence of the samples was randomized for each panelist. This randomization prevented any order effects that could arise from the sequence in which samples were presented, allowing each sample to be assessed independently.

2.3.5. Data collection and analysis

During the sensory evaluation, panelists documented their ratings for each attribute on standardized score sheets. These sheets were designed to capture both quantitative scores and qualitative comments, thus providing a comprehensive overview of the sensory characteristics of each sample. The collected data were then subjected to statistical analysis to identify significant differences between the treated samples and the control. Descriptive statistics—mean scores and standard deviations—were calculated for each attribute. An analysis of variance (ANOVA) was conducted to determine the statistical significance of the differences in sensory attributes among the samples. Principal component analysis and graphical representations were performed using OriginPro 2021 (OriginLab®, Northampton, MA, USA). Further statistical analysis was conducted to explore potential correlations between various sensory attributes.

2.4. Volatile analysis

2.4.1. Analysis of volatile compounds through HS-SPME-GC-MS

2.4.1.1. Sample extraction procedure. The extraction procedure utilized the headspace solid-phase microextraction (HS-SPME) technique (Kwaw et al., 2018). A 5 mL sample aliquot was placed in a 15 mL glass vial with 1.5 g of NaCl to enhance extraction efficiency. An internal standard (10 μL of 800 μg/L 2-octanol) was added to ensure accurate quantification. A 50/30 μm DVB/CAR/PDMS fiber was conditioned in the gas chromatography (GC) injection port at 250 °C for 30 minutes before use. The sample vial was incubated at 40 °C for 10 minutes to equilibrate volatile compounds, and the SPME fiber was exposed to the headspace for 30 minutes with continuous stirring at 2.5 Hz.

2.4.1.2. HS-SPME-GC-MS chromatographic analysis. Post-extraction, GC-MS analysis was conducted using a DB-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness) with helium as the carrier gas at 1.0 mL/min. The temperature program began at 40 °C (2-minute hold), ramped to 250 °C at 10 °C/min, and held at 250 °C for 5 minutes. The injection port was set to 250 °C and operated in splitless mode. Electron ionization was performed at 70 eV, scanning a mass range of 35–500 *m/z*. A similarity index (SI) threshold of >85 % was used for compound identification (Song et al., 2019), and compounds with peak areas below 0.1 % of the total ion chromatogram were excluded. Identification was based on retention indices and mass spectra compared to the NIST 17 library (version 4.52). Quantitative analysis (Li et al., 2021) employed 2-octanol as an internal standard, with equal response and calibration factors (1.0) assumed for both the standard and target compounds.

$$VC\left(\frac{ng}{g}\right) = \frac{peak\ area\ ratio \times 10\ \mu L(ISD) \times 0.8\left(\frac{ng}{\mu L}\right)(ISD)}{equivalent\ mass\ of\ volume\ used(EF)} \quad (2)$$

2.5. Physicochemical metrics

2.5.1. pH, total soluble solids (TSS), titratable acidity (TA), moisture content, protein, reducing sugars

pH measurements were obtained using a pH meter (LIDA Instrument PHS-3 C). Total soluble solids (TSS) were measured with an Atago digital refractometer and reported in °Brix (Ekumah et al., 2021). Total acidity (TA) was assessed through titration with 0.1 N NaOH to the phenolphthalein endpoint (pH 8.2 ± 0.1) (Ekumah et al., 2021). The TA values (Liu et al., 2023) were calculated and expressed as grams of malic

acid and acetic acid per liter of enzyme hydrolysate and fermented hydrolysates.

$$Titratable\ acidity, TA\left(\frac{g}{L}\right) = \frac{(C \times (V_1 - V_2) \times 0.060 \times D)}{V} \times 1000 \quad (3)$$

In the equation, C (mol/L) represents the concentration of NaOH; V_1 (mL) denotes the volume of NaOH utilized in the experimental test; V_2 (mL) signifies the volume of NaOH used in the blank test; D stands for the dilution factor of the sample; and V (mL) indicates the sample volume. The conversion coefficients for malic and acetic acids are also 0.1341 and 0.060, respectively.

Moisture content was determined gravimetrically (AOAC, A. of O. A. C. 1984) and expressed on a wet basis (wb). Protein content was measured via the Biuret spectrophotometric method (Tsuyoshi and Barry, 1978), with Bovine Serum Albumin (BSA) as the standard. Results were presented in grams of BSA per 100 g of enzyme hydrolysate (EF).

The reducing sugar (RS) was assessed according to Fischer and Stein (1961), and RS content was expressed in grams of glucose per liter of EF.

2.5.2. Color analysis

A colorimeter (Minolta CR-400, Tokyo, Japan) was used for color assessment (lightness-darkness (L^*), redness-greenness (a^*), and yellowness-blueness (b^*)). The total color change (ΔE), Hue angle (H^o) and chroma (C) were determined using the equations below (Boateng et al., 2022).

$$\Delta E = \sqrt{(L_o^* - L^*)^2 + (a_o^* - a^*)^2 + (b_o^* - b^*)^2} \quad (5)$$

$$C = \sqrt{(a^*)^2 + (b^*)^2} \quad (6)$$

$$H^o = \tan^{-1}\left(\frac{b*}{a*}\right) \quad (7)$$

Where L^*, a^*, b^* denotes the color attributes of the EF and L_o^*, a_o^*, b_o^* represent the control.

2.6. Polyphenolic contents

2.6.1. Total phenolic content (TPC)

The TPC of the treated samples and control was determined using the Folin–Ciocalteu method (Ekumah et al., 2021). Results were expressed as milligrams of gallic acid equivalent per 100 g of fresh weight (FW) of the sample.

2.6.2. Total flavonoid content (TFC)

Total flavonoid content (TFC) was measured according to Guo et al. (2023), and expressed as milligrams of rutin equivalents per 100 g of fresh weight (FW) of the enzyme hydrolysate (EF) samples.

2.6.3. Total flavonol content (TFLC)

Total flavonol content (TFLC) was measured according to Kumaran and Karunakaran (2007), and expressed as milligrams of quercetin equivalents (QE) per 100 g of fresh weight (FW) of the enzyme hydrolysate (EF) samples, based on the quercetin regression equation.

2.6.4. Total proanthocyanidin content (TPAC)

Proanthocyanidin content in the fermented juices was determined using the method of Saifullah et al. (2019). The procedure involved mixing 1.5 mL of concentrated HCl and 3 mL of vanillin solution (4 % v/v) with 0.5 mL of sample or standard. After a 15-minute incubation at room temperature, absorbance was measured at 500 nm using a UV spectrophotometer (UV-1600). Results were expressed as milligrams of catechin equivalents per 100 grams of fresh weight (FW) of enzyme hydrolysate (EF).

2.7. Antioxidant activities

2.7.1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant activity of the samples was evaluated with the DPPH assay (Li et al. (2022). Trolox was used as the reference standard, and results were reported as milligrams of Trolox Equivalent (TE) per 100 g of fresh weight (FW) of the EF.

2.7.2. Ferric reducing antioxidant power (FRAP) capacity

The FRAP assay was done per the procedure outlined by Feng et al. (2019) and the results were expressed as mg TE/100 g EF.

2.8. Morphology using scanning electron microscopy (SEM)

The methodology followed was based on our previous study (Boasiako et al., 2024) with slight modifications. Among the fermented enzyme hydrolysates, 3 % enzyme hydrolysate (3 %EF) demonstrated the most improved functional properties. Therefore, we focused on assessing the extent of fermentation in 3 %EF. Microstructural analysis was carried out using a JSM-7001 F Scanning Electron Microscope (Jeol Ltd., Japan) with 15 kV accelerating voltage, 3.5 spot size, 10 μm objective aperture, and 6 mm working distance.

2.9. Statistical analysis

Experiments were conducted in triplicate, and results were expressed as the mean ± standard deviation. Statistical analysis was carried out using one-way ANOVA with Minitab version 18 (Minitab, USA), and Tukey's test was used for significance with p-values ≤ 0.05. Pearson's correlations assessed parameter relationships, while OriginPro2021 (OriginLab®, Northampton, MA, USA) was employed for principal component analysis and graphical representations.

3. Results and Discussion

3.1. Sensory analysis of lactic-acetic acid co-fermented jujube enzyme hydrolysates

In examining the sensory characteristics of jujube puree extracts treated with varying concentrations of cellulase enzyme, we investigated samples fermented with enzyme hydrolysates at 1 %, 3 %, and 5 % (v/v) concentrations (designated as 1 %EF, 3 %EF, and 5 %EF, respectively) and compared them to an unfermented control (EFCON) as shown in Table 3 and Fig. 1. 1 % EF sample had a uniform sensory profile, with scores averaging 6–7 across most evaluated attributes. Increasing enzymatic fermentation to 3 % resulted in marginal improvements in sensory quality, particularly in fruitiness and texture (Table 3). The 5 % EF sample was the superior variant, achieving the highest scores across all sensory attributes. This sensory superiority may be ascribed to the significant degradative effect of cellulase at elevated concentrations on the cell wall, leading to the release of inherent soluble macromolecules, such as sugars (Anuradha Jabasingh and Valli Nachiyar, 2011; Faria et al., 2020; Leite et al., 2019; M. Wang et al., 2012), and amino acids (Boateng et al., 2024), which are subsequently enhanced during the co-fermentation phase. The unfermented control sample (EFCON) displayed inferior sensory characteristics, with excessive bitterness (Pu et al., 2017) and sweetness (Rashwan et al., 2020), and diminished acidity and sourness.

The sensory assessment score for the samples was subjected to analysis of variance (ANOVA) as shown in Table 3. Statistically, sourness scores of the fermented hydrolysates were significantly different ($p < 0.05$) from the control. The 1 %EF (6.56), 3 %EF (6.59), and 5 %EF (6.85) samples (Table 3) had higher scores than EFCON (2.49) ($p < 0.05$), indicating a more pronounced sourness in the fermented samples

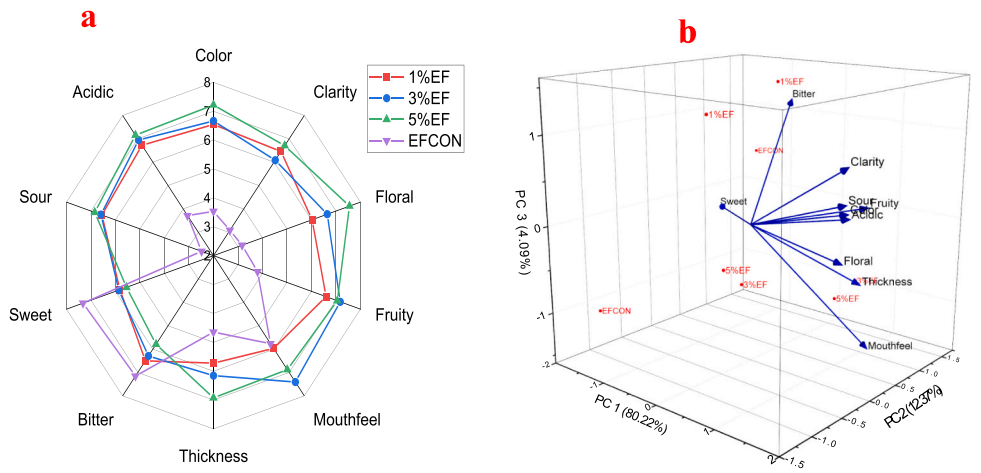

Fig. 1. Radar plot (a) and principal component analysis (b) of sensory descriptor scores of the fermented enzyme hydrolysates. Notes: 1 %EF- fermented enzyme hydrolysate with one percent cellulase concentration; 3 %EF- fermented enzyme hydrolysate with three percent cellulase concentration; 5 %EF- fermented enzyme hydrolysate with five percent cellulase concentration; EFCON- puree with no bacteria inoculants (unfermented). 1 = dislike extremely; 2 = dislike very much; 3 = dislike moderately; 4 = dislike slightly; 5 = neither like nor dislike; 6 = like slightly; 7 = like moderately; 8 = like very much; 9 = like extremely.

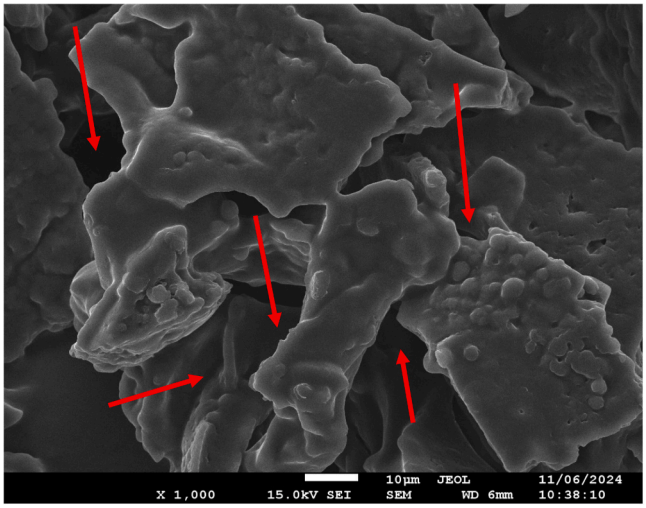

Fig. 2. Scanning electron micrographs (×1000, 15 kV) of 3 %EF- fermented enzyme hydrolysate with three percent cellulase concentration.

compared to the control. There was a significant difference ($p < 0.05$) in acidic attribute between the fermented samples and control, EFCON. The 1 %EF (6.72), 3 %EF (6.95), and 5 %EF (7.15) treatments scored higher than EFCON (3.71) ($p < 0.05$), indicating a more desirable acidity in fermented samples. It is conceivable that cellulase-mediated hydrolysis enhanced the availability of sugars (Anuradha Jabasingh and Valli Nachiyar, 2011; Faria et al., 2020; Leite et al., 2019; M. Wang et al., 2012), thereby promoting their more efficient conversion into organic acids by *Lactobacillus* and *Acetobacter* strains. This effect was particularly evident in the 5 % EF samples, as indicated in Tables 1 and 4. The bitterness among the fermented samples did not differ significantly ($p < 0.05$). Tukey's HSD test showed that the floral aroma scores for 5 %EF were significantly not higher than those for 3 %EF and 1 %EF but different from EFCON ($p < 0.05$). Also, the fruity aroma scores for 3 %EF and 5 %EF were significantly higher than those for 1 %EF and EFCON ($p < 0.05$). There was no significant difference between 3 %EF and 5 %EF, but both had significantly higher fruity scores compared to the control. Furthermore, the color score for 5 %EF was significantly higher than those for 3 %EF, 1 %EF, and EFCON ($p < 0.05$). There was no significant difference between 1 %EF and 3 %EF, but both had significantly higher color scores compared to EFCON. Enzymatic hydrolysis, in this instance, may have facilitated the release of specific color pigments by breaking down pectin and cellulose (Guo et al., 2018). This effect was significantly amplified by the synergistic actions of *Lactobacillus plantarum* CGMCC 3346 and *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41). The clarity score for 5 %EF was not significantly higher than those for 3 %EF and 1 %EF but differently from EFCON ($p < 0.05$). The comparative analysis reveals that the fermented samples, particularly those with higher enzyme concentrations, demonstrate superior sensory qualities compared to the unfermented control. The enhanced appearance, aroma, texture, and flavor profile observed in the fermented samples indicate that enzymatic treatment significantly enhanced the sensory attributes of jujube puree. These improvements are likely due to the enzymatic breakdown of cell wall components and the subsequent release of beneficial volatile compounds (Gómez-García et al., 2012; Patel et al., 2022). Consequently, increasing cellulase concentration yields a more desirable product. These findings are instrumental for product development, highlighting the benefits of fermentation and enzymatic treatment in improving the sensory properties of fruit-based products, thereby potentially increasing consumer acceptance and satisfaction. Furthermore, the study emphasizes the importance of optimizing enzyme concentrations to achieve the best sensory outcomes. However, limitations such as potential panelist variability and biases are acknowledged, suggesting that future research should address these factors for a more comprehensive understanding of optimizing sensory qualities in fermented fruit products.

Multivariate analysis was applied to overcome the difficulties and objectively assess the sensory scores (Boateng et al., 2024; Jere et al., 2020). Thus, Principal Component Analysis (PCA) was employed to explore the variation in sensory attributes of fermented jujube puree extracts treated with different cellulase concentrations and to identify the key characteristics distinguishing the samples. The first two principal components (PC1 and PC2) accounted for 92.59 % of the total variance, effectively summarizing the sensory data in a reduced-dimensional space. A cumulative principal component >85 % is high enough to explain the total variance of the dataset (Boasiako, Yinka, et al., 2024); thus, our selection of PC1–2 is justifiable for accurately differentiating sensory scores among groups, and there is no need to perform Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA), used to mitigate environmental and systematic errors (Boateng et al., 2024).

The PCA output revealed that the unfermented control (EFCON) was distinctly characterized by high sweetness and bitterness (Fig. 1b) (G. Liu et al., 2023; Rashwan et al., 2020), corroborating previous sensory

Table 1
Physicochemical and nutritional properties of enzyme-assisted co-fermented jujube hydrolysates.

Parameters	Enzyme hydrolysates			Lactic-acetic acid co-fermented enzyme hydrolysates			
	1 %E	3 %E	5 %E	1 %EF	3 %EF	%5EF	EFCON
Physicochemical properties:							
Yields from hydrolysates, %	86.99 ± 0.00^{c}	89.19 ± 0.00^{b}	91.52 ± 0.00^{a}	-	-	-	-
Densities, (g/mL)	0.87 ± 0.00^{c}	0.83 ± 0.00^{e}	0.85 ± 0.00^{d}	1.01 ± 0.01^{a}	0.98 ± 0.01^{b}	1.01 ± 0.01^{a}	0.85 ± 0.00^{a}
TSS (°brix)	11.50 ± 0.00^{b}	11.50 ± 0.00^{a}	11.00 ± 0.00^{d}	4.50 ± 0.00 ^{g}	4.50 ± 0.00 ^{f}	5.00 ± 0.00^{e}	11.00 ± 0.00^{c}
pH	5.50 ± 0.01^{c}	5.53 ± 0.00^{b}	5.57 ± 0.01^{a}	4.44 ± 0.01^{d}	4.33 ± 0.01^{e}	3.30 ± 0.01 ^{f}	5.50 ± 0.01^{c}
TA (g/L)	2.32 ± 0.01^{d}	2.28 ± 0.01 ^{f}	2.27 ± 0.01 ^{g}	8.27 ± 0.01^{b}	6.71 ± 0.01^{c}	36.97 ± 0.00^{a}	1.17 ± 0.00^{e}
m.c (%)	90.94 ± 4.28^{b}	90.70 ± 0.54^{b}	89.90 ± 2.53^{b}	97.01 ± 0.50^{a}	96.51 ± 0.48^{a}	97.01 ± 0.49^{a}	90.43 ± 0.29^{b}
Total reducing sugars (g/L)	34.01 ± 0.00^{c}	34.98 ± 0.00^{b}	34.13 ± 0.00^{c}	60.93 ± 0.00^{a}	24.60 ± 0.21^{e}	9.27 ± 0.00 ^{f}	27.13 ± 0.00^{d}
Total protein (g BSA/100 g FW)	0.32 ± 0.00^{e}	0.24 ± 0.00 ^{f}	0.21 ± 0.00 ^{g}	1.00 ± 0.00^{a}	0.46 ± 0.00^{d}	0.82 ± 0.00^{b}	0.61 ± 0.00^{c}
Colorimettric properties:							
L*	42.12 ± 0.46^{c}	40.00 ± 0.86^{d}	39.95 ± 0.63^{d}	47.75 ± 0.79^{b}	47.27 ± 0.45^{b}	52.00 ± 0.74^{a}	33.21 ± 0.60^{e}
a*	6.18 ± 0.12^{c,d}	5.70 ± 0.03^{d}	5.85 ± 0.08^{d}	6.56 ± 0.15^{b,c}	6.99 ± 0.11^{b}	5.67 ± 0.45^{d}	12.90 ± 0.17^{a}
b*	20.06 ± 0.81^{a}	17.61 ± 0.20^{a,b}	18.28 ± 0.39^{a,b}	19.14 ± 0.46^{a}	19.51 ± 0.40^{a}	15.77 ± 2.08^{b,c}	14.14 ± 0.88^{c}
C	20.99 ± 0.81^{a}	18.51 ± 0.20^{a,b}	19.19 ± 0.39^{a,b}	20.24 ± 0.47^{a}	20.73 ± 0.40^{a}	16.76 ± 2.11^{b}	19.15 ± 0.54^{a,b}
H	53.45 ± 1.29^{b}	59.23 ± 0.51^{a}	52.84 ± 1.07^{b}	53.45 ± 1.29^{b}	59.23 ± 0.51^{a}	52.84 ± 1.07^{b}	26.61 ± 4.24^{c}
ΔE	12.65 ± 1.32^{c}	10.51 ± 0.74^{d}	10.62 ± 0.87^{c,d}	16.06 ± 0.81^{b}	15.62 ± 0.04^{b}	19.66 ± 0.51^{a}	-

Data expressed as mean ± standard deviation. Means in the same row with different superscript letters are significantly different ($p < 0.05$).
Note
TSS- total soluble solids in degree brix; TA- titratable acidity in grams per liter; mc- moisture content in percentage; BSA- Bovine serum albumin; FW- fresh weight; L*- lightness-darkness; a*- redness-greenness; b*- yellowness-blueness; C- chroma; H°- hue angle in degrees; ΔE- the overall difference in color. 1 %E- enzyme hydrolysate with one percent cellulase concentration; 3 %E- enzyme hydrolysate with three percent cellulase concentration; 5 %E- enzyme hydrolysate with five percent cellulase concentration; 1 %EF- fermented enzyme hydrolysate with one percent cellulase concentration; 3 %EF- fermented enzyme hydrolysate with three percent cellulase concentration; 5 %EF- fermented enzyme hydrolysate with five percent cellulase concentration; EFCON- puree with no bacteria inoculants (unfermented).

Table 2
Phytochemical contents and antioxidant properties of enzyme-assisted co-fermented jujube hydrolysates.

Parameters	Enzyme hydrolysates			Lactic-acetic acid co-fermented enzyme hydrolysates			
	1 %E	3 %E	5 %E	1 %EF	3 %EF	5 %EF	EFCON
Phytochemicals:							
TPC (mg GAE/100 g, FW)	312.73 ± 1.90^{c}	297.91 ± 1.99^{d}	265.61 ± 0.00 ^{f}	337.07 ± 0.95^{b}	351.89 ± 0.00^{a}	212.61 ± 1.89 ^{g}	283.67 ± 0.00^{e}
TFC (mg RE/100 g, FW)	17.26 ± 0.00^{e}	13.53 ± 0.00 ^{f}	30.86 ± 0.32^{b}	19.16 ± 0.00^{d}	7.08 ± 0.00 ^{g}	426.33 ± 0.59^{a}	22.69 ± 0.00^{c}
TFLC (mg QE/100 g, FW)	3.50 ± 0.00^{e}	3.44 ± 0.01 ^{f}	3.60 ± 0.00^{d}	20.61 ± 0.01^{b}	25.80 ± 0.03^{a}	12.71 ± 0.04^{c}	3.22 ± 0.01 ^{g}
TPAC (mg CE/100 g, FW)	131.29 ± 3.37^{d}	119.90 ± 2.31^{e}	118.59 ± 1.30^{e}	564.95 ± 1.10^{b}	715.95 ± 1.96^{a}	292.19 ± 0.00^{c}	109.56 ± 4.70 ^{f}
Antioxidant capacities:							
DPPH$^{\bullet}$-SA (mg TE/100 g FW)	28.63 ± 0.02^{c}	30.21 ± 0.03^{b}	35.63 ± 0.00^{a}	21.27 ± 0.00^{e}	23.16 ± 0.05^{d}	21.18 ± 0.00^{e}	20.28 ± 0.03 ^{f}
FRAP (mg TE/100 g FW)	4.44 ± 0.00 ^{f}	4.94 ± 0.05^{e}	4.42 ± 0.00 ^{f}	10.63 ± 0.00^{b}	12.35 ± 0.07^{a}	7.74 ± 0.00^{c}	5.59 ± 0.04^{d}

Data expressed as mean ± standard deviation. Means in the same row with different superscript letters are significantly different ($p < 0.05$).
Note:
TPC- Total phenolic content milligram gallic acid equivalent/100 g, fresh weight; TFC- Total flavonoid content in milligram rutin equivalent/100 g, fresh weight; TFLC- Total flavonol content in milligram quercetin equivalent/100 g, fresh weight; TPAC- Total proanthocyanin content in milligram catechin equivalent/100 g, fresh weight; percentage scavenging activities; DPPH$^{\bullet}$-SA – 2,2-diphenyl-1-picrylhydrazyl in milligram Trolox equivalent per 100 gram fresh weight sample; FRAP – Ferric reducing antioxidant properties in milligram Trolox equivalent per 100 g fresh weight sample; 1 %E- enzyme hydrolysate with one percent cellulase concentration; 3 %E- enzyme hydrolysate with three percent cellulase concentration; 5 %E- enzyme hydrolysate with five percent cellulase concentration; 1 %EF- fermented enzyme hydrolysate with one percent cellulase concentration; 3 %EF- fermented enzyme hydrolysate with three percent cellulase concentration; 5 %EF- fermented enzyme hydrolysate with five percent cellulase concentration; EFCON- puree with no bacteria inoculants (unfermented).

Table 3
Analysis of variance (ANOVA) of mean sensory scores of the enzyme-assisted co-fermented jujube hydrolysates.

Samples	Appearance		Aroma		Texture		Flavor			
	Color	Clarity	Floral	Fruity	Mouthfeel	Thickness	Bitter	Sweet	Sour	Acidic
1 %EF	6.54 ± 0.06^{b}	6.46 ± 0.06^{a}	6.03 ± 0.45^{a}	6.60 ± 0.04^{b}	5.97 ± 0.26^{a}	5.72 ± 0.26^{a,b}	6.50 ± 0.61^{a}	5.82 ± 0.25^{a}	6.56 ± 0.28^{a}	6.72 ± 0.42^{a}
3 %EF	6.66 ± 0.23^{b}	6.08 ± 0.21^{a}	6.65 ± 0.91^{a}	7.17 ± 0.54^{a}	7.41 ± 0.67^{a}	6.16 ± 0.57^{a,b}	6.30 ± 0.62^{a}	5.87 ± 0.72^{a}	6.59 ± 0.54^{a}	6.95 ± 0.63^{a}
5 %EF	7.22 ± 0.60^{a}	6.70 ± 0.70^{a}	7.55 ± 0.22^{a}	7.06 ± 0.98^{a}	6.88 ± 1.04^{a}	6.92 ± 0.54^{a}	5.79 ± 0.42^{a}	5.55 ± 0.44^{a}	6.85 ± 0.79^{a}	7.15 ± 0.42^{a}
EFCON	3.53 ± 0.40^{c}	3.09 ± 0.63^{b}	3.17 ± 0.04^{b}	3.80 ± 1.06^{c}	5.77 ± 0.52^{a}	4.65 ± 0.42^{b}	7.15 ± 1.56^{a}	7.33 ± 0.46^{a}	2.49 ± 0.05^{b}	3.71 ± 0.06^{b}

Data expressed as mean ± standard deviation. Means in the same column with different superscript letters are significantly different ($p < 0.05$).
Note
1 %EF- fermented enzyme hydrolysate with one percent cellulase concentration; 3 %EF- fermented enzyme hydrolysate with three percent cellulase concentration; 5 %EF- fermented enzyme hydrolysate with five percent cellulase concentration; EFCON- puree with no bacteria inoculants (unfermented).

analyses which indicated significantly higher scores for these attributes in EFCON (Table 3) compared to the fermented samples. The unfermented control also exhibited poor visual quality and an imbalanced flavor profile, reinforcing its separation from the fermented samples in the PCA plot. The fermented hydrolysates (1 %EF, 3 %EF, and 5 %EF) were clustered together in one quadrant of the PCA plot, signifying their similar sensory profiles characterized by enhanced fruitiness, acidity, thickness, mouthfeel, sourness, floral notes, and improved color. This

Table 4
Quantitative amounts of volatile organic compounds identified in enzyme-assisted co-fermented jujube hydrolysates by HS-SPME-GC/MS method.

Volatile groups	SN	Compound name	CAS number	Concentration of fermented enzyme hydrolysates, ng/100 g FW (mean ± SD)			
				1 %EF	3 %EF	5 %EF	EFCON
Alcohols	ALC1	1-Butanol	71–36–3	3.09 ± 0.18^{a}	n.d	n.d	n.d
	ALC2	1-Butanol, 3-methyl-	123–51–3	693.81 ± 0.36^{a}	525.25 ± 0.14^{b}	275.36 ± 0.17^{c}	20.41 ± 0.06^{d}
	ALC3	1-Hexanol	111–27–3	n.d	n.d	n.d	0.76 ± 0.06^{a}
	ALC4	1-Hexanol, 2-ethyl-	104–76–7	n.d	n.d	2.62 ± 0.06^{a}	0.97 ± 0.05^{b}
	ALC5	1-Nonen−4-ol	35192–73–5	n.d	n.d	n.d	2.16 ± 0.00^{a}
	ALC6	1-Octen−3-ol	3391–86–4	n.d	n.d	n.d	25.35 ± 0.04^{a}
	ALC7	1-Propanol, 2-methyl-	78–83–1	142.39 ± 0.27^{a}	43.81 ± 0.15^{b}	17.43 ± 0.08^{c}	0.76 ± 0.04^{d}
	ALC8	2,3-Butanediol	513–85–9	11.21 ± 0.17^{a}	n.d	1.72 ± 0.10^{b}	n.d
	ALC9	2,4-Di-tert-butylphenol	96–76–4	n.d	n.d	2.07 ± 0.05^{a}	n.d
	ALC10	7-Octen−1-ol, 3,7-dimethyl-, (S)-	6812–78–8	n.d	2.26 ± 0.09^{a}	n.d	n.d
	ALC11	Benzyl alcohol	100–51–6	2.50 ± 0.11^{b}	5.26 ± 0.13^{a}	n.d	n.d
	ALC12	Phenylethyl Alcohol	60–12–8	469.91 ± 0.17^{a}	271.79 ± 0.17^{b}	n.d	n.d
	ALC13	Thymol	89–83–8	n.d	2.90 ± 0.05^{a}	n.d	n.d
		Subtotal		**1322.90 ± 0.85^{a}**	**851.27 ± 0.50^{b}**	**299.19 ± 0.41^{c}**	**50.42 ± 0.06^{d}**
Aldehydes	ALD1	Benzaldehyde	100–52–7	2.56 ± 0.15^{c}	26.34 ± 0.06^{a}	5.51 ± 0.12^{b}	5.55 ± 0.04^{b}
	ALD2	Benzaldehyde,2,4- dimethyl-	15764–16–6	n.d	2.72 ± 0.09^{b}	4.43 ± 0.10^{a}	n.d
	ALD3	Benzeneacetaldehyde	122–78–1	9.09 ± 0.12^{a}	7.76 ± 0.09^{b}	n.d	n.d
	ALD4	Butanal, 3-hydroxy-	107–89–1	n.d	3.47 ± 0.13^{a}	n.d	n.d
	ALD5	Heptanal	111–71–7	n.d	n.d	n.d	3.42 ± 0.07^{a}
	ALD6	Hexanal	66–25–1	n.d	n.d	n.d	7.33 ± 0.04^{a}
	ALD7	Hexanal, 5-methyl-	1860–39–5	n.d	n.d	0.72 ± 0.05^{a}	n.d
	ALD8	Nonanal	124–19–6	n.d	n.d	n.d	2.51 ± 0.03^{a}
		Subtotal		**11.64 ± 0.26^{c}**	**40.30 ± 0.34^{a}**	**10.65 ± 0.23^{d}**	**18.80 ± 0.11^{b}**
Acids	ACD1	2-Heptenoic acid	18999–28–5	n.d	n.d	n.d	2.43 ± 0.04^{a}
	ACD2	2-Hexenoic acid	1191–4–4	n.d	n.d	2.42 ± 0.07^{a}	2.16 ± 0.01^{b}
	ACD3	2-Octenoic acid	1470–50–4	n.d	n.d	n.d	4.05 ± 0.03^{a}
	ACD4	7-Octenoic acid	18719–24–9	2.12 ± 0.15^{a}	n.d	1.65 ± 0.08^{b}	1.70 ± 0.05^{b}
	ACD5	9-Decenoic acid	14436–32–9	n.d	n.d	2.25 ± 0.10^{a}	n.d
	ACD6	Acetic acid	64–19–7	n.d	92.37 ± 0.24^{b}	473.48 ± 0.08^{a}	45.56 ± 0.04^{c}
	ACD7	Benzoic acid	65–85–0	2.56 ± 0.34^{b}	n.d	6.11 ± 0.09^{a}	0.91 ± 0.02^{c}
	ACD8	Butanoic acid	107–92–6	2.00 ± 0.18^{b}	2.20 ± 0.19^{b}	2.26 ± 0.08^{b}	3.28 ± 0.04^{a}
	ACD9	Butanoic acid, 3-methyl-	503–74–2	n.d	n.d	29.34 ± 0.10^{a}	n.d
	ACD10	Crotonic acid	3724–65–0	n.d	n.d	n.d	1.85 ± 0.03^{a}
	ACD11	Dodecanoic acid	143–7–7	n.d	n.d	n.d	11.90 ± 0.03^{a}
	ACD12	Heptanoic acid	111–14–8	7.62 ± 0.30^{c}	6.48 ± 0.15^{d}	8.24 ± 0.05^{b}	10.31 ± 0.02^{a}
	ACD13	Hexanoic acid	142–62–1	39.85 ± 0.18^{d}	46.23 ± 0.23^{c}	46.92 ± 0.03^{b}	56.36 ± 0.02^{a}
	ACD14	Hydrocinnamic acid	501–52–0	n.d	n.d	1.34 ± 0.10^{a}	n.d
	ACD15	n-Decanoic acid	334–48–5	25.44 ± 0.17^{b}	n.d	5.24 ± 0.08^{c}	86.05 ± 0.03^{a}
	ACD16	Nonanoic acid	112–5–0	1.95 ± 0.09^{b}	n.d	2.11 ± 0.10^{b}	5.82 ± 0.04^{a}
	ACD17	Octanoic acid	124–7–2	n.d	16.88 ± 0.22^{b}	14.29 ± 0.07^{c}	26.15 ± 0.03^{a}
	ACD18	Pentanoic acid	109–52–4	2.93 ± 0.22^{c}	n.d	4.48 ± 0.07^{b}	4.85 ± 0.08^{a}
	ACD19	Pentanoic acid, 3-methyl-	105–43–1	13.49 ± 0.22^{a}	9.49 ± 0.20^{b}	n.d	5.12 ± 0.06^{c}
	ACD20	Propanoic acid	79–9–4	n.d	n.d	1.40 ± 0.07^{b}	2.68 ± 0.04^{a}
	ACD21	Propanoic acid, 2-methyl-	79–31–2	12.07 ± 0.27^{a}	7.16 ± 0.16^{c}	4.51 ± 0.11^{c}	n.d
		Subtotal		**110.02 ± 1.01^{d}**	**180.81 ± 1.05^{c}**	**606.05 ± 1.21^{a}**	**271.18 ± 0.43^{b}**
Ketones	KET1	2,3-Octanedione	585–25–1	n.d	n.d	n.d	0.88 ± 0.03^{a}
	KET2	5-Hepten−2-one, 6-methyl-	110–93–0	n.d	n.d	n.d	1.95 ± 0.05^{a}
	KET3	6-Methyl−3,5-heptadiene−2-one	1604–28–0	n.d	n.d	n.d	1.36 ± 0.04^{a}
	KET4	Acetoin	513–86–0	2.49 ± 0.10^{d}	4.42 ± 0.09^{b}	4.06 ± 0.07^{c}	20.62 ± 0.03^{a}
	KET5	Acetophenone	98–86–2	4.56 ± 0.32^{a}	n.d	n.d	n.d
		Subtotal		**7.05 ± 0.41^{b}**	**4.42 ± 0.09^{c}**	**4.06 ± 0.07^{c}**	**24.81 ± 0.15^{a}**
Esters	EST1	Acetic acid, 2-phenylethyl ester	103–45–7	41.26 ± 0.10^{b}	361.92 ± 0.21^{a}	14.47 ± 0.05^{c}	2.11 ± 0.03^{d}
	EST2	(*E*)−9-Octadecenoic acid ethyl ester	6114–18–7	11.08 ± 0.26^{a}	8.82 ± 0.11^{b}	n.d	n.d
	EST3	1-Butanol, 3-methyl-, acetate	123–92–2	22.26 ± 0.26^{a}	3.84 ± 0.18^{c}	18.17 ± 0.01^{b}	n.d
	EST4	7,10-Octadecadienoic acid, methyl ester	56554–24–6	2.04 ± 0.15^{a}	n.d	n.d	n.d
	EST5	7-Octenoic acid, ethyl ester	35194–38–8	3.24 ± 0.15^{a}	n.d	n.d	n.d
	EST6	Benzenepropanoic acid, ethyl ester	2021–28–5	18.85 ± 0.30^{b}	36.25 ± 0.00^{a}	3.05 ± 0.04^{c}	n.d
	EST7	Butanoic acid, 3-hydroxy-, ethyl ester	5405–41–4	75.15 ± 0.31^{a}	n.d	n.d	n.d
	EST8	cis−9-Tetradecenoic acid, propyl ester	0–0–0	n.d	2.33 ± 0.13^{a}	n.d	n.d
	EST9	Decanoic acid, ethyl ester	110–38–3	38.70 ± 0.18^{a}	5.43 ± 0.21^{b}	n.d	n.d
	EST10	Ethyl 9-decenoate	67233–91–4	6.16 ± 0.26^{a}	n.d	n.d	n.d
	EST11	Ethyl 9-hexadecenoate	54546–22–4	10.43 ± 0.31^{b}	16.10 ± 0.16^{a}	4.68 ± 0.05^{c}	n.d
	EST12	Ethyl tridecanoate	28267–29–0	n.d	10.11 ± 0.12^{a}	n.d	n.d
	EST13	Heptanoic acid, ethyl ester	106–30–9	2.04 ± 0.00^{b}	2.65 ± 0.22^{a}	n.d	n.d
	EST14	Hexanoic acid, ethyl ester	123–66–0	10.68 ± 0.18^{a}	7.00 ± 0.16^{b}	0.79 ± 0.06^{c}	n.d
	EST15	Isobutyl acetate	110–19–0	n.d	n.d	1.69 ± 0.12^{a}	n.d
	EST16	Tyrosol, acetate	58556–55–1	n.d	6.88 ± 0.10^{a}	n.d	n.d
		Subtotal		**241.89 ± 1.43^{b}**	**461.34 ± 1.52^{a}**	**42.86 ± 0.31^{c}**	**2.11 ± 0.03^{d}**
		Total		**1693.51 ± 2.30^{a}**	**1538.15 ± 3.42^{b}**	**962.81 ± 2.00^{c}**	**367.31 ± 0.67^{d}**

Data expressed as mean ± standard deviation. Means in the same row with different superscript letters are significantly different ($p < 0.05$).
Note
HS-SPME-GC/MS- headspace solid-phase microextraction gas chromatography-mass spectrometry; CAS- Chemical Abstracts Service; n.d- not detected; 1 %EF-

fermented enzyme hydrolysate with one percent cellulase concentration; 3 %EF- fermented enzyme hydrolysate with three percent cellulase concentration; 5 %EF- fermented enzyme hydrolysate with five percent cellulase concentration; EFCON- puree with no bacteria inoculants (unfermented).

spatial grouping suggests that enzymatic treatment, irrespective of cellulase concentration, imparts similar positive sensory attributes to the jujube puree extracts.

Analyzing the loadings for PC1 and PC2, it was found that positive loadings on PC1 were heavily associated with fruitiness, floral aroma, and improved color, whereas negative loadings correlated with sweetness and bitterness. PC2 differentiated the samples based on thickness, mouthfeel, sourness, and acidity. The fermented samples' grouping in the quadrant with positive loadings for PC1 and PC2 emphasizes their enhanced sensory attributes related to aroma and taste, which are desirable for improved consumer acceptance. In conclusion, the PCA provided a clear visualization of the sensory differences between the control and fermented samples, accentuating the effectiveness of enzymatic treatment in enhancing the sensory qualities of jujube puree. This analysis highlights the potential for targeted enzyme treatments to develop jujube-based products with superior sensory profiles.

3.2. Volatile organic compounds in jujube puree extracts using HS-SPME-GC/MS method

The examination of volatile organic compounds (VOCs) in fermented jujube puree extracts subjected to different enzyme concentrations revealed significant variations in the profiles of alcohols, aldehydes, acids, ketones, and esters (Table 4). These variations indicate the impact of enzymatic fermentation on the volatile composition, influencing the flavor and aroma of the jujube puree. Enzymatic hydrolysis, particularly with cellulases, modifies the volatile profiles in fruit purees by disrupting cell walls and releasing glycosidically bound aroma compounds (Sowbhagya and Chitra, 2010).

Lotfy et al. (2021) highlighted that enzyme concentrations affect the release and formation of volatile compounds, evident by the changes observed in jujube puree extracts. For example, 1-Butanol was present only in 1 %EF (3.09 ng/100 g) and absent in other samples. 1-Butanol, 3-methyl- was detected in all samples but decreased with increasing cellulase concentration: 693.81 ng/100 g in 1 %EF, 525.25 ng/100 g in 3 %EF, 275.36 ng/100 g in 5 %EF, and 20.41 ng/100 g in EFCON. This suggests that higher enzyme concentrations lead to reduced alcohol content. This trend suggests that lower enzyme concentrations facilitate the breakdown of complex substrates into simpler compounds, including 1-Butanol, 3-methyl-, whereas higher enzyme concentrations may result in substrate depletion and altered reaction dynamics (Shuddhodana et al., 2018; Yang et al., 2011), thus reducing alcohol levels. Additionally, microbial activity from *Lactobacillus* and acetic acid bacteria might contribute to reduced alcohol content as these microorganisms convert alcohols into acids and other volatile compounds under high cellulase activity conditions (Tammali et al., 2003; Xia et al., 2022).

1-Hexanol, 2-ethyl- was found in 5 %EF and EFCON but absent in 1 %EF and 3 %EF, suggesting that higher cellulase concentrations are required for its synthesis. In contrast, 1-Hexanol was exclusively detected in EFCON, with 1-Hexanol at 0.76 ng/100 g in EFCON and 1-Hexanol, 2-ethyl- at 2.62 ng/100 g in 5 %EF indicating that 1-Hexanol is only present under control conditions without enzymatic treatment. Compounds like 1-Octen-3-ol and 1-Nonen-4-ol were found only in EFCON at 25.35 ng/100 g and 2.16 ng/100 g, respectively, implying that fermentation might suppress their formation. 2,3-Butanediol was detected in 1 %EF (11.21 ng/100 g) and 5 %EF (1.72 ng/100 g), but not in 3 %EF or EFCON, indicating its potential association with lower enzyme concentrations. Phenylethyl Alcohol was prominently observed in 1 %EF (469.91 ng/100 g) and 3 %EF (271.79 ng/100 g) but was absent in 5 %EF and EFCON, suggesting that lower enzyme concentrations favor its production. The comparison of volatile profiles from the control (EFCON) to fermented samples shows a significant decrease in alcohols like 1-Butanol and Phenylethyl Alcohol in fermented samples compared to the control. Liu et al. (2023) reported that increased enzymatic hydrolysis leads to lower alcohol concentrations. Additionally, Guo et al. (2018) demonstrated that enzymatic treatments facilitate the release of alcohols such as 1-butanol and phenylethyl alcohol, thereby contributing to the development of complex flavor profiles in fermented fruit products.

Aldehydes, essential for potent aromas, generally increase with enzymatic fermentation (Li et al., 2023). Benzaldehyde was present in all samples, with the highest concentration in 3 %EF (26.34 ng/100 g), followed by EFCON (5.55 ng/100 g), 5 %EF (5.51 ng/100 g), and 1 % EF (2.56 ng/100 g). This aldehyde, known for its almond-like scent (Li et al., 2023), appears to be enhanced by moderate enzyme concentrations. Wang et al. (2019) found that moderate enzyme concentrations resulted in higher benzaldehyde levels, effectively depolymerizing the cellulose content of the puree extracts observed in the 3 %EF treatment. Benzeneacetaldehyde was found only in 1 %EF (9.09 ng/100 g) and 3 % EF (7.76 ng/100 g), but absent in 5 %EF and EFCON, suggesting its association with lower fermentation levels. Hexanal and Heptanal were only detected in EFCON, with Hexanal at 7.33 ng/100 g and Heptanal at 3.42 ng/100 g, indicating their presence in unfermented conditions. The alteration of aldehydes like Benzaldehyde and Hexanal during fermentation are expected as similar modifications in these aldehydes during fermentation have been documented (Liu et al., 2023; Song et al., 2019).

Acids play a significant role in flavor and preservation. Acetic acid, with its vinegar-like aroma, was significantly higher in fermented samples, especially in 5 %EF (473.48 ng/100 g), compared to 3 %EF (92.37 ng/100 g) and EFCON (45.56 ng/100 g). This increase demonstrates enhanced acetic acid production with higher enzyme concentrations. Es-sbata et al. (2022) reported significant increases in acetic acid levels during fermentation, aligning with the high levels observed in 5 %EF. Hexanoic acid was present in all samples, with the highest concentration in EFCON (56.36 ng/100 g), indicating minimal impact from fermentation, as noted by Liu et al. (2023), who observed that some acids remain stable during fermentation. Benzoic acid was detected in 1 %EF (2.56 ng/100 g), 5 %EF (6.11 ng/100 g), and EFCON (0.91 ng/100 g), but absent in 3 %EF, suggesting that lower to moderate enzyme concentrations may favor its formation. The increase in acetic acid and other organic acids in fermented samples indicates an enhanced acidic flavor profile through fermentation.

Ketones, contributing to fruity and buttery notes, showed variation across treatments. Acetoin, associated with creamy flavors (Z. Xiao and Lu, 2014), was found in all samples with the highest concentration in EFCON (20.62 ng/100 g) and lower amounts in 1 %EF (2.49 ng/100 g), 3 %EF (4.42 ng/100 g), and 5 %EF (4.06 ng/100 g), suggesting reduced acetoin levels during fermentation. Acetophenone, detected only in 1 % EF (4.56 ng/100 g), might result from mild fermentation conditions. Esters, contributing fruity and floral aromas, varied among treatments. Acetic acid, 2-phenylethyl ester was present in high amounts in 3 %EF (361.92 ng/100 g), lower in 1 %EF (41.26 ng/100 g), and minimal in 5 %EF (14.47 ng/100 g) and EFCON (2.11 ng/100 g), indicating optimal enzyme levels for its formation. 1-Butanol, 3-methyl-, acetate varied with 22.26 ng/100 g in 1 %EF, 3.84 ng/100 g in 3 %EF, and 18.17 ng/100 g in 5 %EF, absent in EFCON, highlighting its formation during fermentation.

The variations in volatile compounds across jujube puree extracts suggest that cellulase concentrations significantly impact the profile and quantity of VOCs. Lower enzyme levels preserve certain alcohols and aldehydes, while higher levels enhance acid and ester formation, leading to a richer and more complex aroma and flavor profile. The observed evolution of the unique flavor profile suggests a highly efficient enzymatic hydrolysis of esterified metabolites (Boasiako et al., 2024). This process resulted in a significant increase in the production of acetic acid (Acosta-Estrada et al., 2014). Understanding these changes is crucial for

optimizing fermentation processes to achieve desired sensory attributes in jujube puree products.

3.3. Impact of varying cellulase enzyme concentrations and lactic-acetic acid co-fermentation on physicochemical properties of jujube puree extracts

The measured responses in this study included the mass of extracts, total soluble solids, pH, titratable acidity, moisture content, total reducing sugars, and total protein content. Increasing the cellulase concentration from 1 % to 5 % (v/v) in non-fermented treatments (1 % E, 3 %E, 5 %E) resulted in slight reductions in mass, total soluble solids, pH, titratable acidity, and moisture content (Table 1). This observation suggests that higher enzyme concentrations (Loman and Ju, 2016) facilitate the hydrolysis of complex carbohydrates into simpler sugars (Li et al., 2021). These simpler sugars may then be metabolized by endogenous microorganisms or other metabolic processes, thereby marginally decreasing the overall mass and soluble solids content. Fermentation notably influenced the physicochemical properties of the jujube puree extracts. A comparative analysis of non-fermented treatments (1 %E, 3 %E, and 5 %E) with their fermented counterparts (1 % EF, 3 %EF, 5 %EF) revealed several significant trends:

3.3.1. Density of extracts and moisture content

Fermentation has been shown to influence the physicochemical properties of food products significantly, as evidenced in this study where it increased the mass and moisture content of the extracts. The consistency of volume (1 mL) across all samples allowed for an accurate comparison of relative densities, where expressing density in terms of mass was appropriate. The fermented treatments (1 %EF, 3 %EF, 5 % EF) demonstrated higher mass values compared to the enzyme hydrolysate treatments, suggesting enhanced moisture retention due to fermentation. The highest mass was observed in the 1 %EF and 5 %EF treatments (1.01 g/mL), whereas the enzyme hydrolysate treatments displayed lower mass values, particularly the 3 %E treatment (0.83 g/mL). These findings indicate that fermentation processes contribute to increased microbial biomass and the accumulation of fermentation by-products, both of which add to the overall mass (Vassileva et al., 2021). Additionally, cellulase and microbial activity during fermentation can release bound water from the cellular matrix, thus increasing the moisture content (Xu et al., 2022). In this study, the fermented samples exhibited higher moisture content, with the 1 %EF, 3 %EF, and 5 %EF treatments showing no significant differences ($p < 0.05$) yet differing significantly from their non-fermented counterparts (1 %E, 3 %E, 5 %E). This observation supports the notion that fermentation enhances water retention in food matrices (T. Xiao et al., 2024). The enhanced moisture retention can be attributed to the structural changes in the cell walls and the production of exopolysaccharides by fermentative microbes, which are known to improve water-holding capacity (Du et al., 2018). Thus, the increased mass and moisture content in the fermented jujube puree extracts can be attributed to the synergistic effects of microbial biomass production and the associated biochemical transformations during the lactic-acetic acid co-fermentation.

3.3.2. Total soluble solids, pH and titratable acidity

Our study found TSS values in fermented samples as low as 4.50 °Brix, whereas the enzyme hydrolysate and control samples maintained higher TSS values ranging from 11.00 to 11.50 °Brix. This reduction is primarily attributed to the microbial utilization of available sugars by *Lactobacillus plantarum* CGMCC 3346 (Zhang and Vadlani, 2015) and *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41) (Xia et al., 2022) into organic acids, gases, and biomass (Li et al., 2021). Our results indicate a significant decrease in pH in fermented samples, with the lowest pH recorded in the 5 %EF treatment (3.30). In contrast, the enzyme hydrolysate and control treatments exhibited higher pH values, around 5.50–5.57. This decrease in pH is a well-documented phenomenon commonly associated with the production of organic acids during fermentation (Aregbe et al., 2019). For instance, Boblllo and Marshall (1992) observed similar pH reductions when using *Lactobacillus plantarum*, attributing the change to lactic acid production. Additionally, the production of lactic and acetic acids by *Lactobacillus* and *Acetobacter* species, respectively, is a primary factor in lowering pH during fermentation (Dysvik et al., 2020; Magala et al., 2015). The marked increase in titratable acidity (TA) in our fermented samples, particularly the 5 %EF treatment (36.97 g/L), indicates substantial acid production. The elevated titratable acidity (TA) levels reported can be attributed to the production of organic acids by fermentative microorganisms (Dysvik et al., 2020) at high cellulase concentrations. The enzyme hydrolysate treatments in our study showed lower TA values, with the control having the lowest (1.17 g/L), highlighting the role of fermentation in significantly enhancing the acidity of the puree. Overall, the pH showed a minor increase with higher cellulase concentration, while titratable acidity slightly decreased, suggesting a reduced production of organic acids or increased buffering capacity in the hydrolysate (Tammali et al., 2003).

3.3.2.1. Correlation and overall impact of total soluble solids, pH and titratable acidity. The interrelationship between these parameters is evident: fermentation leads to the consumption of soluble solids (primarily sugars), which are metabolized into organic acids. This metabolic activity results in a reduction of TSS and pH while significantly increasing TA (Table 1). The observed differences in TSS, pH, and TA between fermented and enzyme hydrolysate treatments highlight the substantial impact of microbial activity on these parameters. In the enzyme hydrolysate treatments, the slight increase in pH with higher cellulase concentration, along with a modest decrease in TA, indicates that while enzyme action may release sugars and other soluble compounds, it does not significantly contribute to organic acid production compared to fermentation. The trends observed in TSS, pH, and TA can be attributed to the synergistic metabolic activities of *Lactobacillus plantarum* CGMCC 3346 and *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41). These microorganisms utilize sugars and other soluble compounds, converting them into organic acids, gases, and biomass. This microbial activity accounts for the reductions in TSS and pH and the increase in TA (Ferreira and Mendes-Faia, 2020; T. Li et al., 2021).

3.3.3. Total reducing sugars and protein

Our findings (Table 1) revealed that fermentation led to an initial increase in total reducing sugars in the 1 %EF treatment, likely due to the enzymatic release of sugars from polysaccharides, which were then partially utilized by the fermenting microbes. This observation is attributed to enzymatic hydrolysis of complex carbohydrates, which leads to an initial surge in reducing sugars during the early stages of fermentation (Amezcua-Allieri et al., 2017). However, at higher enzyme concentrations (3 %EF, 5 %EF), the total reducing sugars decreased, indicating a more complete utilization of available sugars by the fermenting microorganisms: *Lactobacillus plantarum* CGMCC 3346 and *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41) at moderate to high cellulase concentrations. Additionally, total reducing sugars were highest in the 1 %EF treatment (60.93 g/L), indicating that lower enzyme concentration during fermentation preserves more sugars. In contrast, the 5 %EF treatment showed a significant decrease in reducing sugars (9.27 g/L), suggesting that higher enzyme concentrations during fermentation result in greater sugar consumption. This effect is likely due to the increased enzymatic activity, which facilitates more efficient polysaccharide breakdown (Zerva et al., 2021), thereby increasing sugar availability and promoting subsequent microbial utilization. Despite these changes, the total reducing sugars remained consistent across the different enzyme concentrations, indicating that the enzyme efficiency in hydrolyzing polysaccharides into simple sugars was relatively stable across the range of cellulase concentrations, provided the substrate is

not limiting (Shuddhodana et al., 2018).

Fermentation also increased the total protein content in the extracts (Table 1), likely due to the contribution of microbial biomass, which adds to the protein content of the puree. This effect was more pronounced in the 1 %EF and 5 %EF treatments, suggesting that microbial growth was influenced by the availability of nutrients and enzyme concentration. The 1 %EF treatment had the highest protein content (1.00 g BSA/100 g FW), primarily attributable to the significant enhancement of microbial biomass during fermentation (Haque et al., 2011). While this explanation supports our findings, the anomalously low protein concentration observed in the 3 %EF sample might indicate unusually moderate cellulase activity under lactic-acetic co-fermentation conditions. It is posited that at a moderate cellulase concentration, as in the 3 %EF sample, there may be more extensive protein breakdown into smaller peptides or amino acids (Hu et al., 2015). This could be due to the non-specific activity of cellulase on proteins, thereby reducing the measurable protein content. Furthermore, variations in the extraction or processing of the 3 %EF sample could influence the efficiency of protein recovery, potentially resulting in lower observed protein levels (Manzanilla-Valdez et al., 2024; Poms et al., 2004). Enzyme hydrolysate treatments generally had lower protein contents, indicating that fermentation may enhance protein preservation or synthesis (Nasri et al., 2022). The total protein content exhibited a slight decrease with higher enzyme concentrations, possibly due to protein degradation or changes in solubility (Haque et al., 2011). These findings suggest that by carefully adjusting cellulase concentration and deciding whether to ferment, one can tailor the characteristics of jujube puree extracts to meet specific desired outcomes, providing valuable insights for the food processing and fermentation industries.

3.4. Effects of varying enzyme hydrolysis treatments and fermentation on the colorimetric properties of jujube puree extracts

The key colorimetric properties measured in this study include lightness-darkness (L*), redness-greenness (a*), yellowness-blueness (b*), chroma (C), hue angle (H), and the overall difference in color (ΔE) (Table 1). The lightness (L*) of the jujube puree extracts significantly increased with fermentation, with the highest lightness observed in the 5 %EF treatment (52.00). This suggests that fermentation, especially at higher enzyme concentrations, results in a lighter product. The increase in lightness could be attributed to the breakdown of complex compounds into simpler molecules during fermentation (Kwaw et al., 2018), which may reflect more light. In contrast, non-fermented samples showed a slight decrease in lightness with increasing enzyme concentration, indicating that enzymatic hydrolysis alone may lead to minor darkening of the hydrolysate (Toy et al., 2022).

Fermentation generally reduced the redness (a*) of the jujube puree extracts compared to the unfermented control ($p < 0.05$). The unfermented control (EFCON) had the highest redness value (12.90), while fermented samples exhibited lower a* values, suggesting that fermentation might lead to a reduction in red pigments or a shift towards green hues (Lee et al., 2004). Among fermented samples, the 1 %EF treatment showed a higher redness value (6.56) compared to the 5 %EF treatment (5.67), indicating that higher enzyme concentrations might slightly reduce redness during fermentation (Rashwan et al., 2020), regarding color parameters. The yellowness (b*) values were lower in fermented samples compared to the unfermented control. The lowest yellowness value was observed in the 5 %EF treatment (15.77), with no significant differences ($p < 0.05$) from the control (EFCON). This suggests that fermentation at 1 %EF and 3 %EF may break down yellow pigments more effectively, especially at moderately lower enzyme concentrations as evident in the fermentation of fruit purees (Díaz et al., 2004).

Chroma (C) values, representing color vividness, were generally lower in fermented samples compared to the unfermented control, indicating that fermentation results in less vivid colors. The lowest chroma value was observed in the 5 %EF treatment (16.76). This reduction in vividness might be due to the degradation of color compounds during fermentation. Non-fermented samples also showed a decrease in chroma with increasing enzyme concentration, suggesting a similar impact of enzymatic hydrolysis on color vividness. Boateng et al. (2022) reported that variations in chroma could be due to the material's sensitivity to cellular-bound yellow pigmentation and the osmotic and hydrodynamic effects of certain pre-processing techniques, which provides a plausible explanation for this finding. Fermentation led to higher hue angles (H), indicating a shift towards yellower hues. The highest hue angle was observed in the 3 %E and 3 %EF treatments (59.23). The unfermented control had the lowest hue angle (26.61), indicating a more reddish hue. The hue angle changes suggest that both enzyme hydrolysis and fermentation shift the color balance towards the yellow spectrum (Rashwan et al., 2020), with fermentation having a more pronounced effect. This trend may be deemed acceptable as an enhancement in yellow pigmentation in the jujube purees might be associated with high H°, as greenness was consequently decreased (Boateng et al., 2022).

Fermentation resulted in significant overall color changes, with higher ΔE values compared to the unfermented control. The highest ΔE was observed in the 5 %EF treatment (19.66), indicating substantial color alteration. In non-fermented samples, increasing enzyme concentration led to lower ΔE values, suggesting that enzymatic hydrolysis alone causes less color change. However, fermentation amplified these changes, particularly at higher enzyme concentrations, and this trend of color alterations is reported in fermented fruit products. These findings highlight the importance of optimizing enzyme hydrolysis and fermentation conditions to achieve desired color attributes in jujube puree extracts. Controlled treatment conditions can be used to refine the visual qualities of the product, enhancing its aesthetic appeal for food processing and product development.

3.5. Effects of fermentation and enzyme hydrolysis on the polyphenols, and antioxidant properties of jujube puree extracts

3.5.1. Polyphenols

The Total Phenolic Content (TPC) values across the different treatments ranged from 212.61 to 351.89 mg GAE/100 g FW (Table 2). A notable increase in TPC was observed following fermentation, in contrast to enzyme hydrolysate treatments alone. Specifically, the 3 %EF treatment exhibited the highest phenolic content (351.89 mg GAE/100 g), while the 5 %EF treatment recorded the lowest (212.61 mg GAE/100 g) ($p < 0.05$). This trend suggests that higher TPC values correspond to a greater concentration of phenolic compounds in jujube fruits (Ali et al., 2019; Khadivi and Beigi, 2022; Wang et al., 2016). However, discrepancies in TPC values compared to previously published data may be attributed to variations in cultivar, material source, and regional conditions (Wang et al., 2016). The interaction between *Lactobacillus plantarum* CGMCC 3346 and *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41) in the enzymatic hydrolysates appears to be pivotal in the formation and hydrolysis of bound phenolics, as indicated by Acosta-Estrada et al. (2014). These interactions significantly influenced TPC levels through enzyme catalysis, highlighting the modulatory roles of the bacterial strains used. Phenolic compounds are well-documented for their antioxidant properties and have been associated with various health benefits, including anti-inflammatory and anticancer effects (Li et al., 2022; Rauf et al., 2019). The measured TPC values indicate the presence of these bioactive compounds, which contribute to the overall antioxidant capacity of the extracts.

The Total Flavonoid Content (TFC) values exhibited considerable variability, ranging from 7.08 to 426.33 mg RE/100 g FW. Notably, the 5 %EF treatment demonstrated a significantly higher flavonoid content (426.33 mg RE/100 g), implying that fermentation at higher enzyme concentrations enhances flavonoid content by facilitating the breakdown of polyphenolic compounds into more stable or detectable flavonoids (S. Chen et al., 2011; Huynh et al., 2014), thereby elevating the

measured flavonoid content and potentially affecting the levels of flavonols and proanthocyanidins (Table 2). Conversely, the 3 %EF treatment had the lowest flavonoid content (7.08 mg RE/100 g). The extensive variability in TFC values reflects the diversity of flavonoid compounds in jujube fruits, which may be largely influenced by geographical location and post-horticultural practices (Gao et al., 2011). The non-linear trend observed aligns with Patel et al. (2022), who noted that enzyme concentration and incubation temperature significantly affect phenolic fractions. This is further supported by the negative correlation between TPC and TFC ($r = -0.796$, Table 5). Previous research has established jujube as a rich source of flavonoids, such as quercetin, kaempferol, and rutin (Rauf et al., 2019), which possess potent antioxidant activity (Fu et al., 2021). The observed TFC values highlight the potential health benefits of jujube puree extracts due to their high flavonoid content, contributing to their antioxidant capacity.

Flavonols, including quercetin and kaempferol (Song et al., 2019), are abundant in jujube fruits and contribute significantly to their antioxidant capacity (Rauf et al., 2019). The Total Flavonol Content (TFLC) values in our study ranged from 3.22 to 25.80 mg QE/100 g FW. Fermentation notably increased the TFLC, with the highest value observed in the 3 %EF treatment (25.80 mg QE/100 g). Non-fermented treatments exhibited relatively low flavonol content, akin to the control. The notably low TFLC observed in 5 %EF may be attributed to compensatory mechanisms and matrix alteration that facilitate the breakdown of polyphenolic compounds, thereby leading to increased flavonoid contents (Acosta-Estrada et al., 2014; Scholz and Williamson, 2007), pronounced at higher cellulase concentration. The phenomenon of matrix breakdown is further supported by the findings of Kwaw et al. (2018), who proposed that the increase in soluble flavonols during fermentation may be attributed to the enzymatic deglycosylation of complex polyphenols. The presence of these deglycosylated molecules in jujube puree extracts can enhance their antioxidant capacity and potential health benefits.

Proanthocyanidins, recognized for their strong antioxidant activity, are oligomeric and polymeric flavonoids found in jujube fruits (Rauf et al., 2019). The Total Proanthocyanidin Content (TPAC) values indicate the presence of these complex polyphenolic compounds in the jujube puree extracts, contributing to their antioxidant capacity and potential health-promoting effects. TPAC values ranged from 109.56 to 715.95 mg CE/100 g FW, with the highest content in the 3 %EF treatment (715.95 mg CE/100 g), suggesting that moderate enzyme concentration during fermentation maximizes proanthocyanidin levels. The lowest TPAC was found in the 5 %E treatment (118.59 mg CE/100 g). Higher TPAC values suggest a greater concentration of proanthocyanidins, enhancing the antioxidant capacity of the extracts. The relatively lower proanthocyanidin levels observed compared to Boasiako et al. (2024) indicate that cellulase treatment might have effectively broken down these compounds, known to be condensed tannins (Rauf et al., 2019). This supports Acosta-Estrada et al. (2014), who proposed that various extraction techniques influence bound phenolic compound levels. Overall, fermentation generally increased the total phenolic, flavonoid, flavonol, and proanthocyanidin contents, with the most substantial enhancements observed in the 3 %EF treatment.

3.5.2. Antioxidant properties

The study aimed to investigate the antioxidant capacity and phytochemical content of jujube puree extracts through DPPH radical scavenging activity (DPPH-SA) and Ferric Reducing Antioxidant Power (FRAP) assays. Previous studies have demonstrated the efficacy of jujube extracts in scavenging free radicals and reducing oxidative stress (K. Chen et al., 2019; Lin et al., 2020). Therefore, it was relevant to evaluate this efficacy in our enzyme hydrolysates and lactic-acetic acid co-fermented hydrolysates. Our findings (Table 2) not only align with but also extend the understanding of the antioxidant properties of jujube extracts.

The DPPH radical scavenging activity (DPPH-SA) values in our study ranged from 20.28 to 35.63 mg TE/100 g FW. The highest DPPH-SA value was observed in the 5 %E treatment (35.63 mg TE/100 g), indicating enhanced antioxidant capacity in non-fermented samples with higher enzyme concentrations. Interestingly, fermentation led to lower DPPH-SA values. This decline can be attributed to factors such as the transformation or degradation of antioxidants, changes in pH, alterations in solubility and bioavailability, loss of antioxidant compounds, and microbial consumption during the fermentation process (Scholz and Williamson, 2007). Despite these potential reductions, it is noteworthy that the DPPH-SA values for both the enzyme hydrolysates and fermented hydrolysates were significantly higher than those of the unfermented sample (EFCON) ($p < 0.05$). These observations suggest that while fermentation might reduce certain antioxidants' immediate availability, it could also convert them into different forms with varying bioavailability and activity (Verni et al., 2019; Zhao et al., 2021). This transformation could be critical in understanding the bioactive potential of fermented jujube products and highlights the importance of optimizing fermentation conditions to preserve or enhance antioxidant activity. Considering the limitation of a single antioxidant assay, we also evaluated the FRAP assay to corroborate the findings of the DPPH assay. The FRAP assay measures the phenolic-reducing capacity of jujube purees (Li et al., 2022), largely attributed to the high polyphenolic contents with proton-donating properties which eventually form hydrazine. Our findings showed FRAP values ranging from 4.42 to 12.35 mg TE/100 g FW. The highest FRAP value was observed in the 3 %EF treatment (12.35 mg TE/100 g), demonstrating that fermentation with moderate enzyme concentration enhances antioxidant properties. The strong negative correlation between FRAP and DPPH assays ($r = -0.93$*, Table S1) suggests that these assays measure different aspects of antioxidant capacity. While the DPPH assay reflects the ability to neutralize free radicals directly, the FRAP assay indicates the reducing power and overall antioxidant potential, which could be influenced by the polyphenolic content (Clarke et al., 2013; Ditrych et al., 2015). Non-fermented treatments showed lower FRAP values, indicating that fermentation can significantly enhance the reducing power of jujube puree extracts. This enhancement might be due to the release of bound phenolics and the formation of new antioxidant compounds during fermentation (Gan et al., 2016). The higher FRAP values in the 3 %EF treatment highlight the effectiveness of moderate enzyme concentration in boosting the antioxidant potential of the extracts. The measured values emphasize the potential benefits of jujube consumption and support the development of jujube-based functional foods with enhanced nutritional and functional properties.

3.6. Microstructural analysis

Scanning electron microscopy (SEM) analysis was conducted to evaluate the structural modifications in enzyme hydrolysates subjected to co-fermentation, focusing on the sample 3 %EF, identified for its distinct characteristics through multivariate analysis. In our previous study (Boasiako et al., 2024), we observed that unfermented jujube puree exhibited a slightly curled, sheet-like structure with most sheets maintaining their integrity. The unfermented jujube puree had a slightly curled, sheet-like structure, which was attributed to the high sucrose content in dry jujube fruit. However, the co-fermentation process induced significant transformations in the surface morphology of the jujube puree. Upon fermentation, the surface of the jujube puree underwent significant changes, including the formation of minor holes and the breakdown of the rigid, curled sheet-like structure. This aligns with the general effects of fermentation on food matrices, where the breakdown of rigid structures and the formation of pores and cavities are common (Pan et al., 2023). The co-fermentation with *Lactobacillus plantarum* CGMCC 3346 and *Acetobacter pasteurianus* (Ap CICC 20001, CGMCC 1.41), along with the enzymatic activity of cellulase, led to the degradation of the matrix, transforming it into a more porous and coarser form. These structural changes not only affect texture but also

facilitate the interaction of microbial enzymes (Akbari et al., 2023) with food components, enhancing the overall fermentation process. The presence of elongated "sheets" in the SEM images of fermented jujube puree can be linked to the formation of polysaccharide networks (Pan et al., 2023), which result from the interaction between microbial metabolic products and food matrix components, such as undigested dietary fiber, which act as barriers for phenolic compounds (Boasiako et al., 2024; Krakowska-Sieprawska et al., 2020; Liu et al., 2023). These networks support the observed alterations in the structural integrity of the fermented puree hydrolysates, influencing its texture and functionality.

3.7. Multivariate analysis of color, polyphenols, and antioxidant properties

The comprehensive analysis of jujube puree extracts subjected to various enzyme hydrolysis and fermentation processes elucidates significant correlations among color attributes, phytochemical contents, and antioxidant properties (Table S1). Understanding these correlations is imperative for optimizing processing conditions to enhance both the visual and nutritional quality of jujube puree.

The chromatic properties of jujube puree extracts, specifically lightness (L*), redness (a*), and yellowness (b*), are influenced by the phytochemical composition of the samples. Notably, phytochemicals such as phenolics, flavonoids, and proanthocyanidins substantially contribute to the coloration of the extracts. The lightness of the puree was found to be higher in fermented treatments with elevated phenolic content. For instance, the 1 %EF and 5 %EF treatments exhibited the highest lightness values (47.75 and 52.00, respectively) and were associated with high phenolic content (337.07 and 212.61 mg GAE/100 g, respectively). This observation suggests that phenolics may play a role in preserving or enhancing the lightness of the puree during fermentation. Regarding redness (a*) and yellowness (b*), fermented samples demonstrated varied effects. Higher phenolic and flavonoid contents generally correlated with lower redness and yellowness values, as evidenced by the 5 %EF treatment, which had high flavonoid content (426.33 mg RE/100 g) but lower a* and b* values. This indicates that these phytochemicals might protect against the degradation of color pigments or affect color stability during fermentation.

Polyphenols, including total phenolics, flavonoids, and proanthocyanidins, play a pivotal role in determining the antioxidant properties of jujube puree extracts. The antioxidant capacity, assessed through DPPH scavenging activity (DPPH-SA) and FRAP, exhibited strong correlations with the levels of these phenolics. Higher TPC was positively correlated with enhanced antioxidant properties. For example, the 3 % EF treatment, with a phenolic content of 351.89 mg GAE/100 g, showed a high FRAP value (12.35 mg TE/100 g), indicating that phenolics significantly contribute to the antioxidant potential of the extracts. Flavonoids and flavonols, recognized for their antioxidant activity, showed a direct correlation with antioxidant properties. The 5 %EF treatment, which had the highest flavonoid content (426.33 mg RE/100 g), exhibited strong antioxidant properties, reinforcing the role of these compounds in augmenting the antioxidant capacity of the extracts. Proanthocyanidins, which are potent antioxidants, also demonstrated a positive correlation with antioxidant activity. The 3 %EF treatment, possessing the highest proanthocyanidin content (715.95 mg CE/100 g), displayed significant antioxidant activity, further corroborating the importance of these compounds.

The interplay between color attributes, phytochemical contents, and antioxidant properties underscores the complex relationships in jujube puree extracts. Extracts with higher antioxidant properties often exhibited enhanced lightness and reduced vividness. For example, treatments with high phenolic content not only demonstrated higher lightness but also stronger antioxidant capacities. This suggests that antioxidants help preserve color integrity by preventing the oxidative degradation of color pigments during fermentation. The elevated levels of phenolics, flavonoids, and proanthocyanidins in fermented samples contribute to better color stability. These phytochemicals function as natural stabilizers, protecting against color loss and degradation, thereby maintaining the visual quality of the puree. These findings suggest that optimizing enzyme concentration and fermentation conditions can enhance both the visual and nutritional quality of jujube puree extracts. Understanding these relationships provides valuable insights for developing functional food products with superior health benefits and aesthetic appeal.

3.8. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a robust statistical technique employed to identify patterns and relationships within multidimensional datasets (Boateng et al., 2022). In this study, PCA was applied to analyze the phytochemical contents, antioxidant properties, and color parameters of jujube puree extracts treated with various enzyme hydrolysates and lactic-acetic acid co-fermented enzyme hydrolysates. The eigenvectors extracted from PCA provide insights into the underlying factors driving variability among the different treatments. These eigenvectors (Table S2) represent the coefficients of the principal components (PCs) for each parameter, detailing their contribution to the overall variability observed in the dataset. The coefficients of the first principal component (PC1) are predominantly positive for most parameters, indicating a positive correlation with this component. Parameters such as lightness (L*), chroma (C), total phenolic content (TPC), and total proanthocyanidin content (TPAC) exhibit high positive coefficients, suggesting they significantly contribute to the variation explained by PC1. This indicates that variations in these parameters are the primary drivers of variability among the samples. Conversely, parameters with negative coefficients, such as redness (a*), yellowness (b*), and overall color difference (△E*), demonstrate a negative correlation with PC1 (Table S1, Fig. 3). This implies that these color attributes are inversely related to the variability explained by PC1. The second principal component (PC2) exhibits both positive and negative coefficients across different parameters, indicating a mixed influence on the variability it explains. Parameters with high positive coefficients, such as total flavonol content (TFLC) and ferric-reducing antioxidant power (FRAP), contribute positively to PC2. This suggests that variations in these parameters are important factors driving variability orthogonal to PC1. In contrast, parameters such as redness (a*) and hue angle (H) with negative coefficients are inversely related to PC2, indicating that variations in these color attributes are negatively associated with the variability explained by PC2 (Fig. 3). The third principal component (PC3) also shows a mix of positive and negative coefficients, indicating a diverse influence on the variability it explains. Parameters with high positive coefficients, such as total flavonoid content (TFC) and DPPH scavenging activity, contribute positively to PC3. This suggests that variations in these antioxidant-related parameters are significant factors driving additional variability orthogonal to PC1 and PC2. Conversely, parameters with negative coefficients, such as yellowness (b*) and overall color difference (△E*), are inversely related to PC3, indicating that these color attributes are negatively associated with the variability explained by PC3. Overall, the extracted eigenvectors from the PCA provide valuable insights into the interrelationships among phytochemical contents, antioxidant properties, and color parameters in jujube puree extracts treated with different enzyme hydrolysates and fermentation processes. Understanding these relationships can aid in optimizing processing conditions to enhance jujube products' nutritional and sensory qualities.

4. Conclusions

This study comprehensively evaluated the significant impact of cellulase enzyme hydrolysis and co-fermentation on the quality attributes of jujube puree extracts. By varying cellulase concentrations and

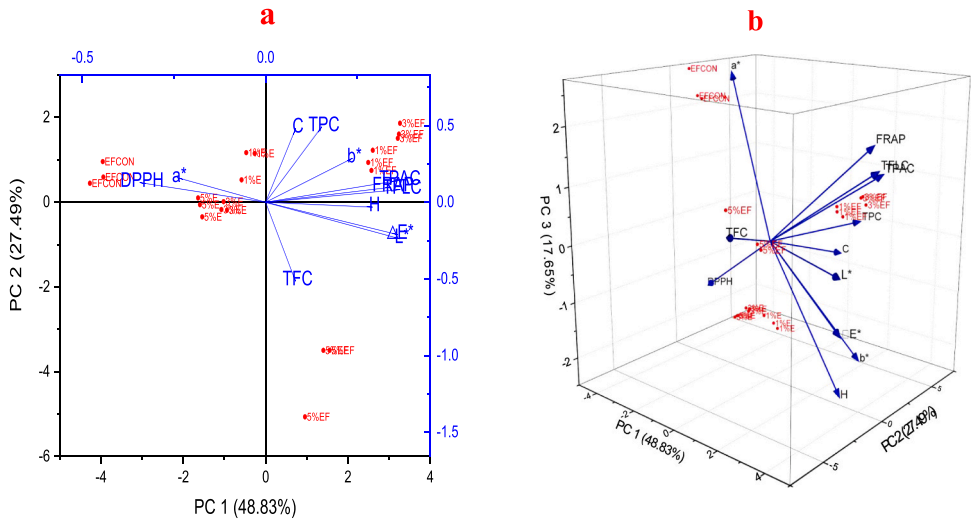

Fig. 3. Multivariate analysis: biplot (a) and 3D-loadings (b) of phytochemical contents, antioxidant properties, and color of enzyme hydrolysates and lactic-acetic acid co-fermented enzyme hydrolysates. 1 %E- enzyme hydrolysate with one percent cellulase concentration; 3 %E- enzyme hydrolysate with three percent cellulase concentration; 5 %E- enzyme hydrolysate with five percent cellulase concentration; 1 %EF- fermented enzyme hydrolysate with one percent cellulase concentration; 3 %EF- fermented enzyme hydrolysate with three percent cellulase concentration; 5 %EF- fermented enzyme hydrolysate with five percent cellulase concentration; EFCON- puree with no bacteria inoculants (unfermented).

applying lactic acid fermentation, the sensory, chemical, and nutritional profiles of the puree were significantly enhanced. Fermentation increased mass and moisture content, indicating microbial biomass production and moisture retention enhancement. The reduction in total soluble solids (TSS) following fermentation emphasizes microbial consumption of sugars and soluble compounds, leading to organic acid production and biomass formation. Fermentation also bolstered protein content, particularly evident in treatments with 1 % and 5 % enzyme concentrations, likely attributed to microbial biomass. Colorimetric analysis revealed a lactic-acetic acid co-fermentation-induced reduction in redness, possibly indicating a decline in red pigments or a shift towards greener hues. Higher enzyme concentrations during fermentation augmented the antioxidant capacity of the extracts, as indicated by elevated proanthocyanidin levels and enhanced DPPH scavenging activity and ferric-reducing antioxidant power (FRAP). Fermented samples, particularly those with moderate enzyme concentrations, exhibited superior antioxidant properties, suggesting their potential as functional food ingredients.

Future studies should explore the effects of other enzymes such as pectinases, amylases, and proteases, as well as combinations of enzymes, fermentation temperatures, and pH levels, and the impacts of longer fermentation durations on the sensory and nutritional quality of jujube puree. Long-term stability and shelf-life studies should evaluate how different storage conditions affect the stability of phytochemicals, color attributes, and antioxidant properties over time. Understanding these factors can lead to more robust product formulations. Additionally, conducting a techno-economic analysis will be essential to assess the economic feasibility and scalability of these processes, ensuring that enhancements in product quality can be achieved in a cost-effective manner.

Data and material availability

The authors have adequately explained the supporting information in the text.

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CRediT authorship contribution statement

Isaac Duah Boateng: Writing – review & editing, Visualization, Validation, Software. **Turkson Antwi Boasiako:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fengqiu Hua:** Methodology, Investigation, Data curation. **Xiong YuQing:** Validation, Methodology, Investigation, Data curation. **Yongkun Ma:** Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

No conflict of interest.

Data availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2024.119590.

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