



Research article

Synergistic effects of lactobacillus strains and *Acetobacter pasteurianus* on jujube puree's product functionality and quality

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ABSTRACT

Commercial lactic acid bacteria strains and indigenous Chinese acetic acid bacterium were co-cultivated bi- and tri-culturally in Junzao jujube puree for the first time to investigate their effects on physicochemical properties and quality attributes. Lactic-acetic acid bacteria co-fermentation was performed at 37 °C for 48 h during the anaerobic fermentation phase and at 30 °C for 144 h during aerobic fermentation. FTIR results showed that predominant wave numbers at 1716–1724 cm⁻¹ and 2922–3307 cm⁻¹ exhibited discernible alterations in the lactic-acetic acid co-fermented jujube purees compared to the control sample. Pearson correlation analysis showed that the flavonoid and flavonol contents were responsible for the enhanced 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl scavenging activities of the fermented jujube purees. Consequently, fermented jujube puree from tricultures of *Lactobacillus casei*, *Lactobacillus plantarum*, and *Acetobacter pasteurianus* gave the best results, with the highest phenolics, flavonoid, and flavonol contents and the most improved antioxidative properties and color. Overall, lactic-acetic acid bacteria co-culture holds significant promise in valorizing Junzao jujube purees for functional ingredient development, paving the way for further research into similar interactions with different food matrices or microbial strains.

1. Introduction

Jujube (*Ziziphus jujuba* Mill.) is used as a functional food because it contains many polysaccharides and polyphenols (phenolic acids and flavonoids) [1], both of which have strong antioxidant properties [2], and delightful flavor [3]. The functional food market is expanding due to consumer interest in healthy diets rich in probiotics, phytochemicals, and antioxidants [3]. Jujube is a dense phytochemical fruit [4]. The beauty of its functionality is its immense contribution to human health through certain pharmacological activities, such as hepatoprotection, anti-carcinogenicity, anti-cardiovascular, neuroprotection, antihyperglycemic prowess, and antioxidation [5,6]. These benefits of jujube are not intensely exploited and are shrouded by the fact that the dried forms lack organoleptic appeal with delayed bitterness [7]. In contrast, the fresh forms cannot be maintained for longer than ten days without

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List of abbreviations

| | |
|---------|---|
| LAB | lactic acid bacteria |
| AAB | acetic acid bacteria |
| Lc | <i>Lactobacillus casei</i> |
| Lh | <i>Lactobacillus helveticus</i> |
| Lp | <i>Lactobacillus plantarum</i> |
| Ap | <i>Acetobacter pasteurianus</i> |
| TPC | total phenolic content |
| TFC | total flavonoid content |
| TFLC | total flavonol content |
| ABTS-SA | 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity |
| DPPH-SA | 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity |
| FRAP | ferric reducing antioxidant power capacity |

regulated atmospheric conditions [1] and are liable to lose freshness because of long-distance transport and high sugar and water content [8]. For this purpose, there is a real need to enrich jujube through certain biotechnological means, such as lactic-acetic acid fermentation, to expand its diverse nature as a functional food (long shelf life, higher nutritional content, appealing characteristics, and potent pharmacological capacities).

Lactic acid bacteria (LAB) should be considered traditional cultures [9]. Lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus xylophilus*, *Lactobacillus lactis*, *Lactobacillus acidophilus*, and *Lactobacillus paracasei*) have been employed to enhance the functionality (for instance, sensory physiognomies-color) of inexhaustible arrays of fruits and vegetables [10–13] to boost yield while reducing by-product accumulation [14]. Most of these beneficial functionalities of LAB emanate from microstructural modification of the substrates, as revealed by scanning electron microscopy [15] and Fourier-transform infrared spectroscopy (FTIR) [10].

Some studies have reported the effects of LAB on jujube puree [3,8,16–18]. These studies are heavily concentrated on mono- or bi-cultures of LAB [8], resulting in a paucity of information on the corresponding mixed cultures with other potential probiotics, such as acetic acid bacteria [19]. Numerous microbial interactions may take place during multispecies food fermentation. The cohabitation of microorganisms in the same ecological niche can result in favorable or unfavorable interactions, impacting their growth patterns, capacity for adaptation, and capacity to manufacture metabolites [20].

Given the diverse behaviors of microbial strains in various food matrices and their distinct growth and bioconversion capacities, it is prudent to characterize the resulting lactic-acetic acid co-fermented jujube puree. To our knowledge, there is no research on bi- and tri-culture fermentation of jujube puree assessment of phytochemical content, antioxidant properties, color, and structural modifications. This study comprehensively examined the impact of bi- and tri-cultured lactic-acetic acid bacteria co-fermentation, employing three commercial LAB (*Lactobacillus plantarum*, *Lactobacillus casei*, and *Lactobacillus helveticus*) and one acetic acid bacterium (*Acetobacter pasteurianus*) on the functionality (phytochemical content, antioxidative properties) and quality (color attributes, structural changes via FTIR) of Junzao jujube puree. Phytochemical content, antioxidative capacity, color properties, and chemical structure were examined after eight days of fermentation. Furthermore, the relationship between fermented puree's color, phytochemical content, and antioxidant properties was evaluated. The findings provide valuable insights into the compositional changes of lactic-acetic acid co-fermented Junzao jujube puree, offering potential applications in commercial settings using these LAB and AAB combinations.

2. Materials and methods

2.1. Bacterial strains, chemicals, and reagents

Lactic acid bacteria (LAB): *Lactobacillus plantarum* Lp-28, *Lactobacillus casei* Lc-122, and *Lactobacillus helveticus* Lh-43 were purchased from Synbio Tech Inc. (Kaohsiung City, Taiwan), whereas the acetic acid bacteria (AAB) strain *Acetobacter pasteurianus* (Ap-As.1.41, HuNiang 1.01) was purchased from Yishui Jinrun Biological Technology Co. Ltd. (Yishui, Shandong, China).

Aluminum chloride, sodium acetate, 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, and TPTZ (2, 4, 6-tripryridyl-s-triazine) reagents were procured from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Folin-Ciocalteu's reagent was procured from Adamas Life Titan Scientific Co., Ltd. (Songjiang District, Shanghai, China). Distilled water and all additional analytical-grade chemicals were obtained from Sinopharm Chemical Reagent (Shanghai, China) without further purification.

2.2. Jujube fruit sample and activation of bacteria starter cultures

Matured and completely dried (intensely reddish-brown) *Ziziphus jujuba* Mill cv. Junzao fruits were obtained commercially from a fruit shop (Zhenjiang, Jiangsu Province, China) in May 2023. Samples with broken cell walls, mold infestation, and a darkish color were discarded. Ideally, the sampled fruits were washed in 0.02 % sodium hypochlorite solution and then thoroughly washed in submerged distilled water to eliminate surface microbial contaminants. Prior to jujube puree preparation, the treated and washed

samples were stored in clean plastic film at $-40\text{ }^{\circ}\text{C}$.

The protocol described by Kwaw et al. [11] was followed with major modifications to activate the bacterial strains. The four microorganisms (*Lactobacillus plantarum* Lp-28, *Lactobacillus casei* Lc-122, and *Lactobacillus helveticus* Lh-43, and *Acetobacter pasteurianus*, Ap-As.1.41, HuNiang 1.01) were individually activated. Briefly, *Lactobacillus plantarum* Lp-28 and *Lactobacillus casei* Lc-122 were activated in de Man Rogosa Sharpe (MRS) broth at $37\text{ }^{\circ}\text{C}$ for 24 h, and *Lactobacillus helveticus* Lh-43 was activated by subculturing in MRS broth at $37\text{ }^{\circ}\text{C}$ for 24 h. *Acetobacter pasteurianus*, Ap-As.1.41, HuNiang 1.01 were activated by subculturing in reinforced acetic acid-ethanol (RAE) broth at $30\text{ }^{\circ}\text{C}$ for 24 h. The cultures were centrifuged for 10 min at 4000 rpm, $25\text{ }^{\circ}\text{C}$, using a Ruijiang RJ-TDL-50A centrifuge (Ruijiang Analytical Instrument Co., Ltd., China). After removing the supernatant, the bacterial cells were washed in a sterile 0.1 % NaCl solution. Inoculum concentration was estimated and corrected to 10^8 CFU/mL using an XB-K-250 hemocytometer (Jianling Medical Device Co., Danyang, Jiangsu, China), and suspensions were used as starter cultures for fermentation of the Junzao jujube puree.

2.3. Jujube puree formulation and fermentation

Jujube puree was prepared according to the method described by Li et al. [16], with major modifications. Sterile frozen jujube samples were thawed to room temperature and boiled in distilled water (1:5, w/v) for 10 min. Subsequently, ventrally grooved pits were removed. Using a kitchen blender (JYLC91T; Joyoung Co., Ltd., Hangzhou, China), the jujubes were combined with distilled water in a 1:2 (w/v) ratio. The resulting puree (pH 5.13) was too viscous ($13\text{ }^{\circ}\text{Brix}$), and the pH and $^{\circ}\text{Brix}$ were adjusted to 5.5 and 11, respectively, using food-grade Na_2CO_3 and distilled water. Before fermentation, the puree was pasteurized at $70\text{ }^{\circ}\text{C}$ for 30 min. The pasteurized jujube puree was inoculated with 1 % (v/v) of each inoculant, cooled to room temperature, and then incubated (rotary shaking incubator, IS-RDD3, Crystal Technology and Industries, Jiangsu, China) for 48 h at $37\text{ }^{\circ}\text{C}$ during the anaerobic fermentation phase for optimum activity of lactobacillus strains and 144 h at $30\text{ }^{\circ}\text{C}$ during the aerobic fermentation phase as suitable conditions for *Acetobacter pasteurianus*.

The various starter culture combinations (Table 1) used to prepare the lactic-acetified jujube puree (JLAP) were as follows: *Lactobacillus casei*-*Acetobacter pasteurianus* (JLcAp), *Lactobacillus helveticus*-*Acetobacter pasteurianus* (JLhAp), *Lactobacillus plantarum*-*Acetobacter pasteurianus* (JLpAp), *Lactobacillus casei*-*Lactobacillus helveticus*-*Acetobacter pasteurianus* (JLcLhAp), *Lactobacillus casei*-*Lactobacillus plantarum*-*Acetobacter pasteurianus* (JLcLpAp), and *Lactobacillus helveticus*-*Lactobacillus plantarum*-*Acetobacter pasteurianus* (JLhLpAp). Sterile purees (pasteurized) without inoculation served as the control (JCON). All fermentations were performed independently in 1000 mL Erlenmeyer flasks.

2.4. Phytochemical contents

2.4.1. Total phenolic content (TPC) analysis

The total phenolic content of the fermented samples was determined using the Folin–Ciocalteu method described by Ekumah et al. [21]. Briefly, 2 mL of freshly prepared Folin–Ciocalteu reagent (1:1 v/v) was added to a 0.2 mL JLAP sample in a test tube, after which 2 mL of sodium carbonate (75 g/L) was added and vortexed for 30 s. The mixture was subsequently incubated for 40 min at room temperature ($25\text{ }^{\circ}\text{C}$), and the absorbance was measured at 760 nm using a UV spectrophotometer (UV-1600, Beijing Rayleigh Analytical Instrument, Beijing, China). TPC was expressed as milligrams of gallic acid equivalent per 100 g FW of JLAP.

2.4.2. Total flavonoid content (TFC) analysis

The aluminum chloride colorimetric method described by Guo et al. [22] for total flavonoid determination was used with slight modifications. The fermented sample (1 mL) was combined with 0.3 mL of 50 g/L NaNO_2 and 4 mL distilled water before vortexing for 1 min. One milliliter of AlCl_3 (100 g/L) was added, vortexed, and left to stand for 5 min. It was then followed by adding 2.4 mL of distilled water and 2 mL of a 1 M NaOH solution. After 2 min of intermittent shaking at 150 rpm (IS-RDD3, Crystal Technology, and

Table 1

Amount of inoculum of microbial strains used for the co-fermentation of purees.

| Treatments | <i>Lactobacillus casei</i> , Lc 122 | <i>Lactobacillus plantarum</i> , Lp 28 | <i>Lactobacillus helveticus</i> , Lh 43 | <i>Acetobacter pasteurianus</i> , Ap-As.1.41, HuNiang 1.01 |
|------------|-------------------------------------|--|---|--|
| | 100 mL | | | |
| JLcAp | 1 % | – | – | 1 % |
| JLhAp | – | – | 1 % | 1 % |
| JLpAp | – | 1 % | – | 1 % |
| JLcLhAp | 1 % | – | 1 % | 1 % |
| JLcLpAp | 1 % | 1 % | – | 1 % |
| JLhLpAp | – | 1 % | 1 % | 1 % |
| JCON | – | – | – | – |

Note.

This percentage is for 100 mL of Junzao jujube puree. JLcAp- *Lactobacillus casei*- *Acetobacter pasteurianus* puree; JLhAp- *Lactobacillus helveticus*-*Acetobacter pasteurianus* puree; JLpAp- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; JLcLhAp- *Lactobacillus casei*-*Lactobacillus helveticus*-*Acetobacter pasteurianus* puree; JLcLpAp- *Lactobacillus casei*- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; JLhLpAp- *Lactobacillus helveticus*-*Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; (JCON)- puree with no bacteria inoculants (unfermented).

Industries, Jiangsu, China), the mixture was incubated at room temperature. At 510 nm, using a UV spectrophotometer (UV-1600) against a blank, the absorbance was measured after 10 min. TFC was calculated as milligrams of rutin equivalents per 100 g FW of the JLAP samples.

2.4.3. Total flavonol content (TFLC) analysis

The concise protocol of Kumaran & Karunakaran [23] was used to determine the total flavonol content of JLAP. Briefly, 2 mL of sample was mixed with equal volumes of $AlCl_3$ (2 %) and 3 mL of sodium acetate solution (50 g/L). The final mixture was vortexed and maintained at 20 °C for 2.5 h. The absorbance of the sample was measured at 440 nm against a blank using a UV spectrophotometer (UV-1600), and the TFLC results were expressed as mg QE/100 g FW of JLAP using the quercetin regression equation.

2.5. Antioxidative activities

2.5.1. ABTS radical cation scavenging activity (ABTS-SA)

A slightly modified protocol of Kwaw et al. [11] was used to evaluate the 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging activity (ABTS^{•+}-SA) of the fermented puree. Briefly, a mixture of ABTS (7 mM) and potassium persulfate (4.95 mM) was prepared in a ratio of 1:1 (v/v) and stored at 25 °C in the dark for 16 h. The mixture was further diluted with methanol to obtain an absorbance value of 0.82 at 734 nm. After that, 0.06 mL of the JLAP sample (diluted 1:15, v/v) was added to 2.1 mL of the mixture and allowed to stand in the dark for 10 min. Absorbance was read at 734 nm using a UV spectrophotometer (UV-1600). The control group was treated with JLAP without ABTS. The percentage ABTS^{•+}-SA was calculated using equation (1) as follows:

$$\% \text{ ABTS}^{\bullet+} - \text{SA} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \dots \dots \dots [1]$$

% ABTS^{•+} - SA is percentage of ABTS radical cation scavenging activity.

A_{control} is the absorbance of the control; A_{sample} is the absorbance of the sample.

2.5.2. DPPH radical scavenging assay

A slightly modified protocol from Li et al. [16] was used to evaluate the radical-scavenging antioxidant activity of JLAP by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, 4.2 mL of 0.1 mM DPPH solution (made with methanol) was added to 0.12 mL of the fermented sample (diluted 1:15). After vortexing, the mixture was incubated for 30 min at 25 °C in the dark. The absorbance was measured using a UV spectrophotometer (UV-1600). The control group was treated with JLAP in the absence of DPPH. The results were expressed as the percentage of DPPH[•]-SA using equation (2), as follows:

$$\% \text{ DPPH}^{\bullet} - \text{SA} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100 \dots \dots \dots [2]$$

% DPPH[•] - SA is percentage DPPH radical scavenging activity.

A_{control} is the absorbance of the control; A_{sample} is the absorbance of the sample.

2.5.3. Ferric reducing antioxidant power (FRAP) capacity

The FRAP assay was performed according to the procedure outlined by Feng et al. [3] with a few minor modifications. Briefly, 0.2 mL was mixed with 3.8 mL of FRAP reagent (a mixture of 10 parts 300 mM sodium acetate buffer (pH 3.6), 1 part 10 mM TPTZ (2, 4, 6-tripyridyl-s-triazine) in 40 mM HCl, and 1 part 20 mM $FeCl_3 \cdot 6H_2O$). The solution was incubated for 30 min at 37 °C (IS-RDD3; Crystal Technology and Industries, Jiangsu, China). After that, absorbance was measured at 593 nm using a UV-1600 spectrophotometer against a blank. Using an iron (II) tetraoxosulphate (VI) ($FeSO_4$) standard curve produced under the same conditions, the results were reported in mM $FeSO_4$.

2.6. Color assessment

A HunterLab ColorQuest XE Spectrophotometer (Hunter Associates Laboratory, Virginia, USA) was used to measure the CIE color parameters of the samples (lightness (L^*), redness (a^*), and yellowness (b^*)). The overall difference in color (ΔE), the chroma (C), and the hue angle (H^a) [24] were determined by equations (3)–(5) as follows:

$$\Delta E = \sqrt{(L_o^* - L^*)^2 + (a_o^* - a^*)^2 + (b_o^* - b^*)^2} \dots \dots \dots [3]$$

$$C = \sqrt{(a^*)^2 + (b^*)^2} \dots \dots \dots [4]$$

$$H^a = \tan^{-1} \left(\frac{b^*}{a^*} \right) \dots \dots \dots [5]$$

Where L^* , a^* , b^* and L_o^* , a_o^* , b_o^* represent the chromatic values of (lightness-darkness), (redness-greenness), and (yellowness-blueness)

of the JLAP samples and control, JCON, respectively.

2.7. Fourier-transform infrared (FTIR) spectroscopy analysis

Fourier-transform infrared (FTIR) spectroscopy evaluated the chemical structure by elucidating the functional groups in the fermented jujube puree. The methodology outlined by Kafle et al. [25] was followed with slight modifications. Briefly, freeze-dried samples were sieved to obtain finer particles, and ~1 mg of the fermented freeze-dried samples was used. Before sample analysis, reference spectra were collected by taking measurements of the air background spectrum (spectra from the cleaned blank crystal). FTIR measurements were performed by compacting the sample into a 5.0 mm aperture with approximately 128 scans using a high-throughput screening eXTension (HTS-XT) unit coupled to a Tensor 27 spectrometer (Bruker Optics, Billerica, MA, USA). Spectra were recorded between 4000 and 500 cm^{-1} with a spectral resolution of 4 cm^{-1} .

2.8. Data processing and statistical analysis

The analyses were performed in triplicate and are reported as mean \pm standard deviation. One-way analysis of variance (ANOVA) was used to determine the mean differences. Tukey's test was used to evaluate statistical significance when the p-value was less than 0.05. Pearson's correlation and principal component analysis were used to establish and characterize the relationships between the selected parameters. Minitab version 18 (Minitab, LLC, Chicago, USA) and OringinPro version 2019 (OriginLab, Northampton, USA) were used in these analyses.

3.0. Results and discussion

3.1. Effect of lactic-acetic acid fermentation on the phytochemical contents of jujube puree

The total phenolic content (TPC), total flavonoid content (TFC), and total flavonol content (TFLC) of fermented jujube puree are presented in Table 2, and commensurate findings of Khadivi & Beigi [26] and Ali et al. [27]. The higher phytochemical concentrations in JLAP samples compared to JCON showed that lactic acid bacteria (LAB) and acetic acid bacteria (AAB) increased the phytochemical concentrations in the jujube puree during fermentation. The greater phytochemical concentrations in JLAP could be attributed to the ability of LABs and AAB to produce hydrolytic enzymes, such as (poly)-phenol oxidases [17], esterases [11], β -glucosidases [28] that depolymerized macromolecular phytochemicals (isoflavone β -glycosides) into simpler ones (aglycones). The discrepancies in the phytochemical levels could be attributed to the varying adaptability and ability of the strains (LAB and AAB) to produce more hydrolytic enzymes to initiate depolymerization. The TPC of the fermented jujube purees ranged from 397.54 to 493.41 mg gallic acid equivalent per 100 g for bi- and tri-cultures of LABs co-cultivated with AAB. Lactic-acetic acid co-fermentation yielded an increasing trend for all phytochemical contents and a corresponding increase in antioxidant activity (Table 2). The ability of LAB to produce β -glucosidase enzymes from accessible nutrients in the well-adjusted jujube puree may explain why the fermented samples had a significantly higher TPC ($p < 0.05$) than the control JCON (Table 2). From the results, it could be inferred that the addition of *Lactobacillus helveticus*, Lh-43 or *Lactobacillus plantarum*, Lp-28 to bicultural form of *Lactobacillus casei*, Lc-122 and *Acetobacter pasteurianus*, Ap-As.1.41 could significantly improve the TPC of jujube puree (408.28 ± 0.38 or 493.41 ± 1.23 versus 397.54 ± 1.07 mg GAE/100 g, FW respectively). Substantial decrease and marginal increase ($p < 0.05$) respectively in TPC of purees produced from

Table 2
Phytochemical and antioxidant properties of lactic-acetic acid fermented jujube puree.

| Parameters Phytochemicals | Lactic-acetic acid co-fermented jujube purees | | | | | | |
|------------------------------|---|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|
| | JLcAp | JLhAp | JLpAp | JLcLhAp | JLcLpAp | JLhLpAp | JCON |
| TPC (mg GAE/100g, FW) | 397.54 \pm 1.07 ^e | 443.07 \pm 2.40 ^b | 414.93 \pm 0.38 ^c | 408.28 \pm 0.38 ^d | 493.41 \pm 1.23 ^a | 445.01 \pm 0.72 ^b | 389.59 \pm 0.70 ^f |
| TFC (mg RE/100g, FW) | 54.62 \pm 1.23 ^d | 58.23 \pm 0.16 ^b | 59.04 \pm 0.42 ^b | 56.50 \pm 0.00 ^c | 87.08 \pm 0.17 ^a | 48.48 \pm 0.82 ^c | 20.77 \pm 0.33 ^f |
| TFLC (mg QE/100g, FW) | 21.96 \pm 0.01 ^b | 22.02 \pm 0.01 ^b | 21.37 \pm 0.02 ^c | 20.59 \pm 0.02 ^c | 22.77 \pm 0.00 ^a | 21.04 \pm 0.05 ^d | 14.23 \pm 0.03 ^f |
| Antioxidants | | | | | | | |
| % ABTS ^{•+} -SA | 91.18 \pm 0.49 ^b | 93.43 \pm 0.27 ^a | 94.35 \pm 0.15 ^a | 93.38 \pm 0.17 ^a | 93.51 \pm 0.02 ^a | 94.03 \pm 0.28 ^a | 84.42 \pm 0.89 ^c |
| % DPPH [•] -SA | 86.73 \pm 0.12 ^c | 86.90 \pm 0.09 ^c | 88.64 \pm 0.10 ^a | 87.45 \pm 0.14 ^b | 88.37 \pm 0.14 ^a | 88.38 \pm 0.11 ^a | 73.79 \pm 0.26 ^d |
| FRAP (mM Fe ²⁺) | 4.56 \pm 0.01 ^c | 4.58 \pm 0.01 ^c | 6.00 \pm 0.03 ^a | 4.76 \pm 0.02 ^b | 4.63 \pm 0.04 ^c | 4.45 \pm 0.03 ^d | 4.32 \pm 0.04 ^e |

Data expressed as mean \pm 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/100g, fresh weight; TFC- Total flavonoid content in milligram rutin equivalent/100g, fresh weight; TFLC- Total flavonol content in milligram quercetin/100g, fresh weight; % ABTS^{•+}-SA – 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid percentage scavenging activities; % DPPH[•]-SA – 2,2-diphenyl-1-picrylhydrazyl percentage scavenging activities; FRAP (mM Fe²⁺) – Ferric reducing antioxidant properties in millimolar of iron (II) tetraoxosulphate (VI) (FeSO₄); JLcAp- *Lactobacillus casei*- *Acetobacter pasteurianus* puree; JLhAp- *Lactobacillus helveticus*- *Acetobacter pasteurianus* puree; JLpAp- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; JLcLhAp- *Lactobacillus casei*-*Lactobacillus helveticus*- *Acetobacter pasteurianus* puree; JLcLpAp- *Lactobacillus casei*- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; JLhLpAp- *Lactobacillus helveticus*- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; (JCON)- puree with no bacteria inoculants (unfermented).

Lactobacillus helveticus, Lh-43 (443.07 ± 2.40 mg GAE/100 g, FW) in combination with either *Lactobacillus casei*, Lc-122 (408.28 ± 0.38 mg GAE/100 g, FW) or *Lactobacillus plantarum*, Lp-28 (445.01 ± 0.72 mg GAE/100 g, FW) in the presence of *Acetobacter pasteurianus*, Ap-As.1.41 could probably be expected based on previously published studies of Xia et al. [20], who emphasized that *Lactobacillus helveticus* and *Acetobacter pasteurianus* are frequently isolated fermenters in vinegar especially, cereals. Nevertheless, *Lactobacillus plantarum*, Lp-28 when tri-cultured with *Lactobacillus helveticus*, Lh-43 and *Acetobacter pasteurianus*, Ap-As.1.41 significantly improved the TPC of the fermented purees compared to bicultural form of *Lactobacillus plantarum*, Lp-28 and *Acetobacter pasteurianus*, Ap-As.1.41 (445.01 ± 0.72 versus 414.93 ± 0.38 mg GAE/100 g, FW respectively). The uniquely higher TPC content could be that *Lactobacillus helveticus*, Lh-43 might have positively influenced the activity of *Lactobacillus plantarum*, Lp-28 and *Acetobacter pasteurianus*, Ap-As.1.41 in a synergistic way.

The uniquely highest TPC observed in tri-cultured puree, JLcLpAp (493.41 ± 1.23 mg GAE/100 g, FW), suggested a very high mutual cohabitation of LABs (*Lactobacillus casei* Lc-122, *Lactobacillus plantarum* Lp-28) to deglycosylate complex phenolics in the cellular compartments of the jujube puree during fermentation into more soluble conjugated phenolic compounds. These TPC quantities may have been exacerbated by the acetic acid bacteria, *Acetobacter pasteurianus* Ap-As.1.41, which is known to produce acetic acid that can penetrate cell membranes and cause alterations in normal fundamental physiological functions [29].

TFC ranged from 20.77 ± 0.33 to 87.08 ± 0.17 mg RE/100g, FW for both the control, JCON, and fermented jujube purees (Table 2). TFC increased significantly in the fermented purees, with the highest content observed in JLcLpAp, displaying a significantly ($p < 0.05$) highest value of 87.08 ± 0.17 mg RE/100 g FW compared to the control, JCON. Kwaw et al. [11] suggested that TFC levels may increase due to the enzymatic degradation of complex polyphenols into simpler flavonol molecules (conjugated subgroups of flavonoids) during fermentation. Similarly, a significant amount of flavonols was quantified in the fermented samples compared to the control; the highest was observed in JLcLpAp (22.77 mg QE/100 g FW) and showed significant ($p < 0.05$) correlations with TPC ($r = 0.585$) and TFC ($r = 0.866$) concentrations (Table 5). The higher correlation between the flavonol and flavonoid contents could align with the findings of Rauf et al. [30], and their combined concentrations provide a better commensuration with the reports of Ali et al. [27] on flavonoid concentrations in commercial red date vinegar. Flavonoids are crucial antecedents of numerous physiological processes, such as anticancer activity, anti-inflammatory properties, and suppression of carcinogenesis [13,21]. Triculture of *Lactobacillus plantarum* Lp-28, *Lactobacillus casei* Lc-122, and *Acetobacter pasteurianus*, Ap-As.1.41 possessed the most improved total phenolic contents. Nevertheless, based on these findings, *Lactobacillus plantarum* Lp-28, *Lactobacillus casei* Lc-122, and *Lactobacillus helveticus* Lh-43 in bi- or tri-cultivation with *Acetobacter pasteurianus*, Ap-As.1.41 could address issues pertaining to the functional nature of fresh unfermented jujube purees.

3.1.1. Antioxidative activities of the lactic-acetic fermented jujube purees

Food's antioxidative activities define the food's potential health in humans [21]. Dynamic changes in DPPH[•]-SA, ABTS^{•+}-SA, and FRAP antioxidant activities are shown in Table 2. The increase in DPPH[•]-SA inhibition (which varied from 73.79 ± 0.26 to 88.64 ± 0.10 % for JCON and lactic-acetic acid fermented samples, respectively) and the significantly favorable effects of lactic-acetic acid fermentation on DPPH radical scavenging activities were demonstrated by the data. In terms of how LABs and AAB affected DPPH[•]-SA, significant variations ($p < 0.05$) were observed. Interestingly, fermented puree with *Lactobacillus plantarum*, Lp-28, and *Acetobacter pasteurianus*, Ap-As.1.41 showed a high value (88.64 ± 0.10 %) in their potency to scavenge DPPH radicals. This could be explained by the findings of Kwaw et al. [11], who alluded credit to the high hydrolyzing ability of *Lactobacillus plantarum* to form micro-phenolic fractions from macro ones during the fermentation of mulberry juice. This unique functionality of *Lactobacillus plantarum*, Lp-28 might have led to undistinguished ($p < 0.05$) DPPH radical scavenging activity when in triculture-cohabitation with *Lactobacillus casei*, Lc-122 or *Lactobacillus helveticus*, Lh-43 and *Acetobacter pasteurianus*, Ap-As.1.41. Furthermore, samples of *Lactobacillus casei*, Lc-122 or *Lactobacillus helveticus*, Lh-43 bi-cultured with *Acetobacter pasteurianus*, Ap-As.1.41 did not show significant differences ($p < 0.05$) (Table 2), however their tricultures (JLcLhAp) were significantly different with improved DPPH[•]-SA radical scavenging activity (87.45 ± 0.14 %) compared to the control, JCON (73.79 ± 0.26 %). From our findings, it could be said that the lactic-acetic acid fermentation with bi- or tri-cultures of *Lactobacillus plantarum* Lp-28, *Lactobacillus casei*, Lc-122 or *Lactobacillus helveticus*, Lh-43 and *Acetobacter pasteurianus*, Ap-As.1.41 may have improved the availability of polyphenol compounds with proton-donating abilities exhibited by hydrogen atom transfer from an antioxidant during DPPH[•]-SA, with a reduction in the anomalous electron of the nitrogen atom in DPPH, and consequent formation of hydrazine [16].

The ABTS^{•+}-SA test was conducted to evaluate the capacity of the fermented samples to scavenge ABTS radicals by electron transfer, as well as the influence of lactic-acetic acid fermentation on the ABTS^{•+}-SA of jujube puree. The ABTS^{•+}-SA activity of JLAP was significantly greater than JCON's (Table 2). This demonstrates how lactic-acetic acid fermentation could impact the components of the jujube puree that allowed the transfer of electrons to scavenge ABTS free radicals. For the control, JCON, and fermented samples, the ABTS^{•+}-SA inhibition ranged from 84.42 ± 0.89 to 94.35 ± 0.15 %. There were no significant differences ($p < 0.05$) in ABTS^{•+}-SA between or among the following lactic-acetic acid fermented purees: JLhAp, JLpAp, JLcLhAp, JLcLpAp and JLhLpAp (93.43 ± 0.27, 94.35 ± 0.15, 93.38 ± 0.17, 93.51 ± 0.02, 94.03 ± 0.28 % respectively). Nonetheless, ABTS^{•+}-SA of JLcAp was different ($p < 0.05$) and lower (91.18 ± 0.49 %) than that of the aforementioned fermented purees, yet, with highly enhanced ABTS^{•+}-SA compared to the control, JCON (84.42 ± 0.89 %). The variance in ABTS^{•+}-SA across JLAP compared to the reference, JCON, might be attributable to the magnitude of the impact of LABs and AAB on the polyphenolic fractions of the puree during fermentation (Table 2). While the findings of Kwaw et al. [11] reported a close association between ABTS^{•+}-SA and the total flavonoid content (high number of acidic and phenolic hydroxyl groups), it is also strongly argued that differences in ABTS^{•+}-SA could be a result of the chemical composition of the food matrix. This corroborates the strong favorable association between ABTS^{•+}-SA and TFC ($r = 0.753$) and TFLC ($r = 0.902$) (Table 5).

FRAP is an important indicator that can be used to assess phenolic-reducing capacity [16]. According to Ekumah et al. [21], the FRAP assay utilizes an electron-donating reductant (antioxidant)-mediated redox reaction of reduced Fe^{2+} -TPTZ under fixed conditions. According to our results (Table 2), lactic-acetic acid fermentation had a favorable impact on the FRAP of jujube purees. The FRAP of JLAP ranged from 4.45 ± 0.03 to 6.00 ± 0.03 mM FeSO_4 compared to the control, JCON (4.32 ± 0.04 mM FeSO_4) with notable differences ($p < 0.05$). The highest FRAP, 6.00 ± 0.03 mM FeSO_4 , was observed in the sample, JLpAp (*Lactobacillus plantarum* Lp-28 bi-cultured with *Acetobacter pasteurianus*, Ap-As.1.4). The PCA plot (Fig. 2), which displayed the FRAP assay in propinquity with JLcAp, could validate the high deglycosylation tendency of *Lactobacillus plantarum* Lp-28 [11], and *Acetobacter pasteurianus*, Ap-As.1.41, on the macromolecular polyphenolic fraction of the jujube puree into simpler flavonol molecules. Moreover, there seemed to be a somewhat significant ($p < 0.05$) positive correlation between $\text{ABTS}^{\bullet+}$ -SA and FRAP ($r = 0.444$) (Table 5). Based on literature [11,16] on $\text{ABTS}^{\bullet+}$ -SA, it is possible to assert that changes in FRAP may be caused by LAB and AAB's effects on the phenolic contents of the fermented samples, particularly flavonoids, although this fraction did not exhibit any significant associations with FRAP (Table 5).

3.1.2. Lactic-acetic bacteria co-fermentation's impact on color attributes of jujube puree

The color attributes (L^* , a^* , and b^*) and the overall color difference (ΔE) were used to analyze the impact of lactic-acetic acid co-fermentation on the color qualities of JLAP. According to the findings (Table 3), the L^* and b^* values of the fermented samples were higher than those of the control samples, whereas their a^* values were lower, which agrees with the results of Liu et al. [31]. From the results, *Lactobacillus casei*, Lc-122, and *Lactobacillus helveticus*, Lh-43 enhanced the lightness, L^* , decreased the redness, a^* , and modified the yellowness, b^* , in almost the same magnitude as *Lactobacillus casei*, Lc-122, and *Lactobacillus plantarum*, Lp-28 in tri-cultures with *Acetobacter pasteurianus*, Ap-As.1.41 compared to their corresponding bicultural forms of *Lactobacillus casei*, Lc-122 with *Acetobacter pasteurianus*, Ap-As.1.41. The significant increase ($p < 0.05$) trend in the L^* and b^* values of the fermented samples compared to the control suggests that the darker color [16] of the jujube purees faded and became lighter, whereas the yellowness as a result of enzymatic and non-enzymatic browning during drying [32] was modified into amber, clear, and transparent liquids [1] during fermentation. An opposite trend was observed for *Lactobacillus helveticus*, Lh-43 in tri-cultures with *Lactobacillus casei*, Lc-122, *Lactobacillus plantarum* Lp-28, *Acetobacter pasteurianus*, Ap-As.1.41. Perhaps this inconsistency could be explained respectively by reports of Chai et al. [33] and Chelladhurai et al. [34] on co-culture inhibitory effects and temperature abnormalities of thermophilic *Lactobacillus helveticus*, Lh-43 in tri-cultures with *Lactobacillus casei*, Lc-122 or *Lactobacillus plantarum* Lp-28 and *Acetobacter pasteurianus*, Ap-As.1.41. Moreover, this inversion could be explained by the findings of Kwaw et al. [11], who suggested that high L^* and b^* with low a^* could result from low monomeric anthocyanin concentrations [18]. However, the anthocyanin content of jujubes is reported to decrease right from the ripening of the fruit [35], and this decrease is intensified by certain post-management practices such as drying [36]. It could be deduced that *Lactobacillus helveticus* Lh-43, with little inhibitory effect from *Acetobacter pasteurianus* Ap-As.1.41, performed excellently in modifying the darker color of the raw jujube puree in its bicultural form. Interestingly, bi- and tri-cultures of the bacterial strains used in our study showed unique metabolism, resulting in a slightly noticeable differential chromatic range ($5.0 < \Delta E < 10.0$) [11]. Therefore, improving jujube color during lactic-acetic acid fermentation can enhance biotechnological functionality and organoleptic quality [21].

3.1.3. Influence of lactic-acetic acid bacteria on the chemical structure of fermented jujube puree

In the present study, FTIR spectra of the fermented samples were compared with those of the control JCON. Typical FTIR spectra of the fermented and control samples ranged from 774 cm^{-1} to 3313 cm^{-1} . Spectral data served as metabolic fingerprints of fermented jujube purees, providing useful information regarding the biochemical changes that occur during lactic-acetic acid fermentation. FTIR spectroscopy reveals the molecular structure and environment through specific vibrational modes, allowing molecules to spin or vibrate [37]. According to our data, the variations in peak positions (Fig. 1) demonstrated by their wavenumbers (Table 4) indicated that lactic-acetic acid fermentation impacted the chemical structure of the Junzao jujube puree. In Fig. 1, the fingerprint region ($500\text{--}1500 \text{ cm}^{-1}$) highlights variations in the peak positions and demonstrates the adverse impact of the β -glycosidic bonds between

Table 3
Colorimetric assessment of lactic-acetic acid fermented jujube purees, JLAP.

| Properties | JLAP samples | | | | | | |
|------------|-----------------------|------------------------|------------------------|------------------------|------------------------|-----------------------|--------------------|
| | JLcAp | JLhAp | JLpAp | JLcLhAp | JLcLpAp | JLhLpAp | JCON |
| L^* | 29.27 ± 0.13^d | 32.15 ± 0.03^a | 27.42 ± 0.37^f | 30.57 ± 0.15^c | 31.36 ± 0.10^b | 30.27 ± 0.12^c | 28.13 ± 0.10^e |
| a^* | $8.56 \pm 0.73^{b,c}$ | 5.39 ± 0.70^d | $11.36 \pm 0.50^{a,b}$ | $7.08 \pm 0.18^{c,d}$ | $6.15 \pm 0.07^{c,d}$ | $6.91 \pm 0.28^{c,d}$ | 12.91 ± 2.44^a |
| b^* | 7.82 ± 0.16^d | 10.76 ± 0.62^a | 5.24 ± 0.49^f | $9.54 \pm 0.18^{b,c}$ | $10.33 \pm 0.09^{a,b}$ | 9.13 ± 0.15^c | 6.32 ± 0.17^e |
| H° | 42.54 ± 2.62^c | 63.49 ± 1.63^a | 24.81 ± 3.06^d | 53.45 ± 1.29^b | $59.23 \pm 0.51^{a,b}$ | 52.84 ± 1.07^b | 26.61 ± 4.24^d |
| C | 11.66 ± 0.55^b | $12.04 \pm 0.87^{a,b}$ | $12.52 \pm 0.26^{a,b}$ | $11.88 \pm 0.04^{a,b}$ | $12.02 \pm 0.06^{a,b}$ | 11.45 ± 0.23^b | 14.40 ± 2.23^a |
| ΔE | $4.99 \pm 2.41^{a,b}$ | 9.66 ± 1.69^a | 3.07 ± 0.62^b | $7.23 \pm 1.56^{a,b}$ | 8.60 ± 1.86^a | $7.06 \pm 1.72^{a,b}$ | – |

Data expressed as mean \pm standard deviation. Means in the same row with different superscript letters are significantly different ($p < 0.05$).

Note.

L^* - lightness-darkness; a^* - redness-greenness; b^* - yellowness-blueness; C- chroma; H° -hue angle in degrees; ΔE -the overall difference in color. JLcAp- *Lactobacillus casei*- *Acetobacter pasteurianus* puree; JLhAp- *Lactobacillus helveticus*- *Acetobacter pasteurianus* puree; JLpAp- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; JLcLhAp- *Lactobacillus casei*-*Lactobacillus helveticus*- *Acetobacter pasteurianus* puree; JLcLpAp- *Lactobacillus casei*-*Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; JLhLpAp- *Lactobacillus helveticus*- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; (JCON)- puree with no bacteria inoculants (unfermented).

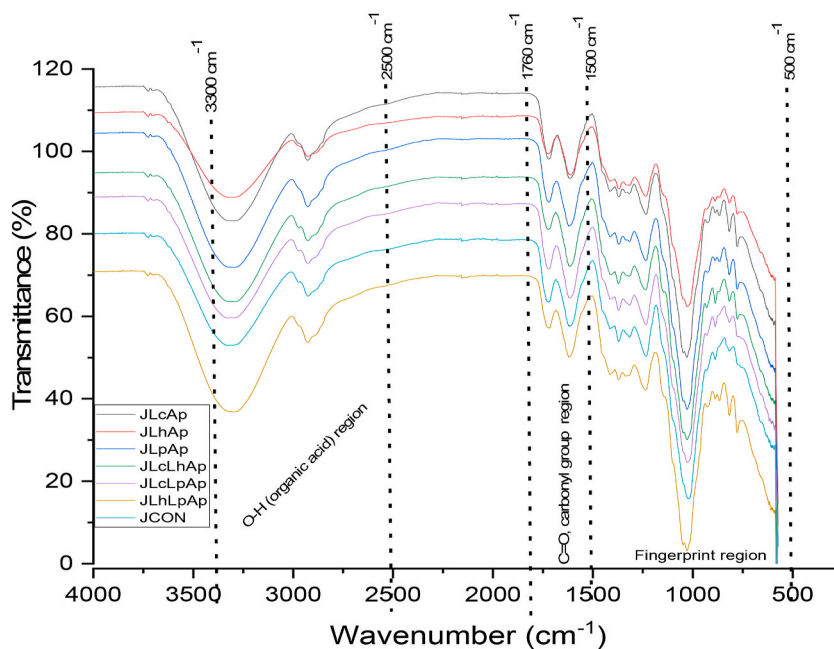


Fig. 1. FTIR spectra of fermented and unfermented jujube purees (wavenumber range: 500–4000 cm^{-1}). Note.

JLcAp- *Lactobacillus casei*- *Acetobacter pasteurianus* puree; JLhAp- *Lactobacillus helveticus*- *Acetobacter pasteurianus* puree; JLPpAp- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; JLCpAp- *Lactobacillus casei*-*Lactobacillus helveticus*- *Acetobacter pasteurianus* puree; JLCpAp- *Lactobacillus casei*- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; JLhLpAp- *Lactobacillus helveticus*- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; (JCON)- puree with no bacteria inoculants (unfermented).

hemicellulose, cellulose, and glucose in the polysaccharides [10] of the jujube puree during lactic-acetic acid fermentation. Perhaps this effect could objectively correspond to the peak numbers in the wavenumber range of 500–1500 cm^{-1} and the degree of impaction projected as the breakdown of starch, cell walls, and breakage of polysaccharide linkages, releasing more organic acids that have the propensity to deglycosylate complex phenolics in the cellular compartments of the jujube puree during fermentation into more soluble conjugated phenolic compounds [11]. In addition, it was unsurprising to see fewer peaks within the 500–1000 cm^{-1} wavenumber range in the unfermented sample, JCON. In addition, wavenumbers between 1000 and 1500 cm^{-1} generally suggest complex C–O–C ether stretching [10]. The changes in wavenumbers at approximately 1030 cm^{-1} for the unfermented sample (control) and those of the fermented samples (JLcAp, JLhAp, JLCpAp, JLCpAp, and JLhLpAp, respectively): 1026, 1028, 1027, 1026, 1032, and 1031 cm^{-1} to 1419 cm^{-1} (control) and fermented samples: 1416, 1417, 1417, 1414, 1415, and 1415 cm^{-1} could suggest C–O–C ether vibration bonds.

Based on the comprehensive reports by Ahmad & Ayub [37] and Pavli et al. [38], wavenumbers above 1500 cm^{-1} provide information about the functional groups (chemical structure) of the fermented samples and control. Given this apprehension, the wavenumber of the lactic-acetic acid-fermented jujube purees from FTIR could be grouped into two categories. First, the range of 1500–1760 cm^{-1} signifies a C=O acetyl group due to the carbonyl group in the ester linkages of fat molecules or hemicellulose [10, 38]. The fermented samples and control showed narrow differences in wavenumbers in the carbonyl functional group range. Although the control, JCON, showed similar wavenumbers (1717 cm^{-1}) as the fermented samples (JLcAp and JLhLpAp), the highest wavenumber (1724 cm^{-1}) was achieved by JLhAp, followed by JLCpAp (1723 cm^{-1}), JLPpAp (1718 cm^{-1}), and JLCpAp (1716 cm^{-1}).

The broadband region (2500–3300 cm^{-1}) is characterized by O–H organic acids [37]. The similar broad range (2925–3314 cm^{-1}) of the fermented samples compared to the control, JCON (2922–3307 cm^{-1}), signified the improved formation of organic acids in the fermented jujube purees by the LAB and AAB strains. These discrepancies might be attributed to the varying adaptability and capacity of the strains [11] to create more hydrolytic enzymes to initiate cellular depolymerization and production of organic acids [17]. Nevertheless, the anticipated functional compounds in the fermented samples were similar and were projected to be predominantly carbonyl groups and organic acids (Fig. 1). The Fourier transform infrared spectroscopy results showed changes in the positions of the peaks (characteristic of structural changes) in the fermented samples, which supported the efficacy of lactic-acetic acid fermentation of Junzao jujube puree with high yield and improved antioxidant activity.

3.2. Multivariate analysis of the phytochemical contents, antioxidative properties, and color attributes of lactic-acetic acid fermented jujube puree

To emphasize the important characteristics of each of the JLAP, the phytochemical content (TPC, TFC, TFLC), antioxidative activities (ABTS^{•+}-SA, DPPH[•]-SA, FRAP), and color parameters (L*, a*, b*, H^o, C, ΔE) were subjected to principal component analysis

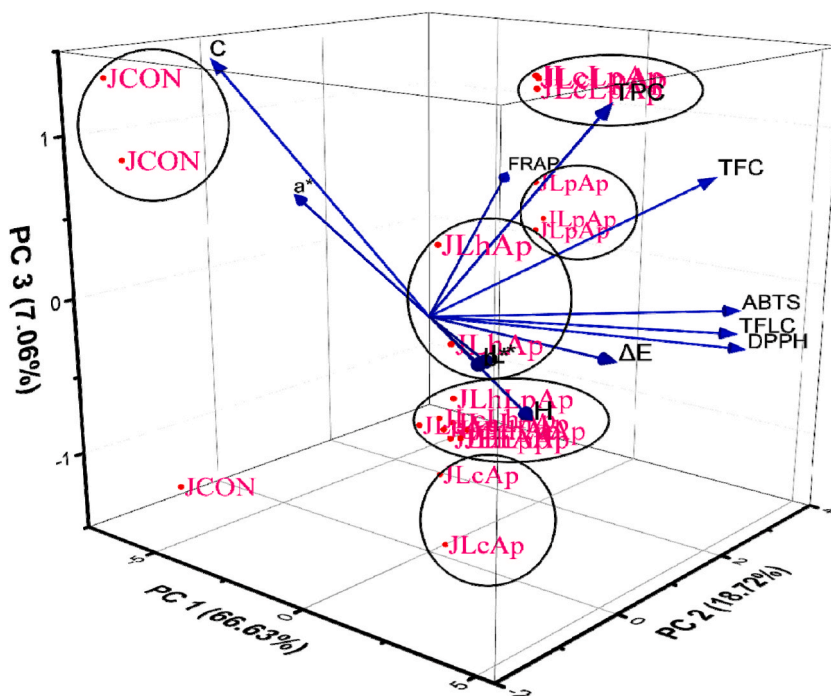


Fig. 2. 3D principal component analysis of phytochemical contents, antioxidant activities and color of JLAP.

Note TPC- total phenolic content; TFC- total flavonoid content; TFLC- total flavonoid content; ABTS-SA – 2,2-azino-bis-3- ethylbenzothiazoline-6-sulfonic acid percentage scavenging activities; DPPH-SA– 2,2-diphenyl-1-picrylhydrazyl percentage scavenging activities; FRAP – ferric reducing antioxidant properties in millimolar of FeSO_4 ; L^* - lightness-darkness; a^* - redness-greenness; b^* - yellowness-blueness; H° -hue angle in degrees; C- chroma; ΔE -the overall difference in color. JLcAp- *Lactobacillus casei*- *Acetobacter pasteurianus* puree; JLhAp- *Lactobacillus helveticus*- *Acetobacter pasteurianus* puree; JLpAp- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; JLcLhAp- *Lactobacillus casei*-*Lactobacillus helveticus*- *Acetobacter pasteurianus* puree; JLcLpAp- *Lactobacillus casei*- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; JLhLpAp- *Lactobacillus helveticus*- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; (JCON)- puree with no bacteria inoculants (unfermented).

Table 4

Specific wavenumbers from FTIR of the lactic-acetic acid co-fermented jujube purees.

| Number | Wavenumber (cm^{-1}) of lactic-acetic acid fermented purees | | | | | | |
|--------|--|-------|-------|---------|---------|---------|------|
| | JLcAp | JLhAp | JLpAp | JLcLhAp | JLcLpAp | JLhLpAp | JCON |
| 1 | 3307 | 3314 | 3306 | 3306 | 3312 | 3308 | 3307 |
| 2 | – | – | – | 2974 | 2972 | – | – |
| 3 | 2927 | 2929 | 2925 | 2928 | 2924 | 2925 | 2922 |
| 4 | 1716 | 1724 | 1718 | 1723 | 1716 | 1716 | 1717 |
| 5 | 1613 | 1611 | 1614 | 1613 | 1613 | 1616 | 1613 |
| 6 | 1416 | 1417 | 1417 | 1414 | 1415 | 1415 | 1419 |
| 7 | 1369 | 1369 | 1370 | 1366 | 1368 | 1373 | 1376 |
| 8 | 1314 | 1313 | 1318 | 1315 | 1313 | 1321 | 1311 |
| 9 | 1235 | 1230 | 1231 | 1241 | 1236 | 1232 | 1235 |
| 10 | 1026 | 1028 | 1027 | 1026 | 1032 | 1031 | 1030 |
| 11 | 927 | – | 921 | 929 | 931 | 918 | – |
| 12 | – | 885 | 888 | 886 | 887 | – | 886 |
| 13 | 865 | – | 864 | – | – | 863 | – |
| 14 | 818 | 813 | 814 | 819 | 817 | 815 | 815 |
| 15 | 774 | 775 | 781 | 776 | 778 | 777 | – |

Note.

JLcAp- *Lactobacillus casei*- *Acetobacter pasteurianus* puree; JLhAp- *Lactobacillus helveticus*- *Acetobacter pasteurianus* puree; JLpAp- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; JLcLhAp- *Lactobacillus casei*-*Lactobacillus helveticus*- *Acetobacter pasteurianus* puree; JLcLpAp- *Lactobacillus casei*-*Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; JLhLpAp- *Lactobacillus helveticus*- *Lactobacillus plantarum*- *Acetobacter pasteurianus* puree; (JCON)- puree with no bacteria inoculants (unfermented).

Table 5

Pearson's correlation coefficients matrix of phytochemical contents, antioxidant activities, and color properties of lactic-acetic acid bacteria co-cultured jujube purees (JLAP).

| | TPC | TFC | TFLC | ABTS ^{•+} -SA | DPPH [•] -SA | FRAP | L* | a* | b* | C | H | ΔE |
|------------------------|---------|---------|---------|------------------------|-----------------------|---------|---------|---------|--------|---------|--------|----|
| TPC | 1 | | | | | | | | | | | |
| TFC | 0.781* | 1 | | | | | | | | | | |
| TFLC | 0.585* | 0.866* | 1 | | | | | | | | | |
| ABTS ^{•+} -SA | 0.541* | 0.753* | 0.902* | 1 | | | | | | | | |
| DPPH [•] -SA | 0.515* | 0.794* | 0.956* | 0.974* | 1 | | | | | | | |
| FRAP | -0.077 | 0.265 | 0.286 | 0.444* | 0.402 | 1 | | | | | | |
| L* | 0.655* | 0.517* | 0.507* | 0.419 | 0.401 | -0.495* | 1 | | | | | |
| a* | -0.643* | -0.629* | -0.708* | -0.618* | -0.646* | 0.291 | -0.893* | 1 | | | | |
| b* | 0.645* | 0.512* | 0.494* | 0.412 | 0.401 | -0.533* | 0.988* | -0.879* | 1 | | | |
| C | -0.323 | -0.499* | -0.691* | -0.607* | -0.704* | -0.044 | -0.405 | 0.769* | -0.390 | 1 | | |
| H | 0.664* | 0.565* | 0.592* | 0.506* | 0.509* | -0.454* | 0.979* | -0.959* | 0.974* | -0.568* | 1 | |
| ΔE | 0.654* | 0.661* | 0.726* | 0.693* | 0.668* | -0.174 | 0.842* | -0.874* | 0.841* | -0.557* | 0.876* | 1 |

Correlation matrix coefficients with asterisk * denote significant correlation (p<0.05).

Note. TPC- total phenolic contents in milligram gallic acid equivalents/100 g fresh weight; TFC- total flavonoid content in milligram rutin equivalents/100 g fresh weight, TFLC- total flavonol content in milligram quercetin equivalents/100 g fresh weight; ABTS^{•+}-SA – 2,2-azino-bis-3- ethylbenzothiazoline-6-sulfonic acid percentage scavenging activities; DPPH[•]-SA – 2,2-diphenyl-1-picrylhydrazyl percentage scavenging activities; FRAP – ferric reducing antioxidant properties in millimolar of FeSO₄; L*- lightness-darkness; a*- redness-greenness; b*- yellowness-blueness; H^o-hue angle in degrees; C- chroma; ΔE-the overall difference in color.

(PCA) and segregated into three main groups. The output is shown in Fig. 2. The first two principal components (PC), PC1 (66.63 %) and PC2 (18.72 %) explained the majority (85.35 %) of the variation in the samples while the addition of a third principal component, PC3 (7.06 %) increased the variation in the samples to 92.41 %. Therefore, relying on the strength of the association of the measured parameters with the principal components suggests that a cluster of variables could appropriately describe each lactic-acetic acid fermented sample. The first group (predominantly JLPaP and JLPaP) was on the negative side of PC1 and positive side of PC2, and they were highlighted by distally high a*, marginally and proximally close to FRAP. The second group (JCON) was observed in the negative sides of PC1 and PC2 with no measured outcome. It is unsurprising as this sample could be a measure and an indication of the inherent properties of ordinary jujube puree. The third group (made up of samples: JLPaP, JLPaP, JLPaP and JLPaP) was situated on the positive side of PC1 and negative side of PC2 and commonly shared almost all the attributes (high: L*, b*, H, C, ΔE, TPC, TFC, TFLC, ABTS^{•+}-SA, DPPH[•]-SA) measured. Interestingly, upon these improved characteristics of the samples in group three, sample JLPaP appeared to be closest to these shared attributes.

4. Conclusions

The LAB and AAB strains used impacted the constituents of the Junzao jujube puree. The fermentation process significantly enhanced the color and phytochemical concentrations of the jujube puree's phenolic, flavonoid, and flavonols. There was a potent increase in the free radical scavenging activities of ABTS^{•+}-SA, DPPH[•]-SA, and FRAP of the jujube puree after fermentation of lactic-acetic acid bacteria. However, fermentation using triculture of *Lactobacillus casei*, *Lactobacillus plantarum*, and *Acetobacter pasteurianus* showed the highest total phenolic contents and antioxidant activities with improved chromatic attributes. Given this, lactic-acetic acid fermentation will help position jujube puree as a premium beverage with various pharmacological properties, such as anti-aging, antimicrobial, anticancer, and analgesic properties. The chemical structure was affected in the measure of the interactions from the cohabitation of the various cultures as elucidated by the Fourier-transform infrared (FTIR) spectroscopy. With FTIR showing major functional groups in the fermented samples, elucidating the volatiles and non-volatiles will be key to identifying certain aromas or flavor precursors in this novel beverage. Despite these interesting findings, developing a thoroughly categorized metabolomic profile that represents this beverage's biochemical and molecular characterization is necessary.

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Data and material availability

The authors have adequately explained the supporting information in the article, and no data is available.

CRedit authorship contribution statement

Turkson Antwi Boasiako: Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Conceptualization. **John-Nelson Ekumah:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis. **Sanabil Yaqoob:** Writing – review & editing, Writing – original draft. **Afusat Yinka Aregbe:** Writing – review & editing. **Yanshu Li:** Methodology. **Kwami Ashiagbor:** Writing – review & editing. **Wang Lu:** Writing – review & editing, Methodology. **Isaac Duah Boateng:** Writing – review & editing, Visualization, Formal analysis. **Yongkun Ma:** Visualization, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

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.

References

- [1] A.K. Rashwan, N. Karim, M.R.I. Shishir, T. Bao, Y. Lu, W. Chen, Jujube fruit: a potential nutritious fruit for the development of functional food products, *J. Funct. Foods* 75 (866) (2020) 104205, <https://doi.org/10.1016/j.jff.2020.104205>.
- [2] B. Wang, et al., Changes in phenolic compounds and their antioxidant capacities in jujube (*Ziziphus jujuba* Miller) during three edible maturity stages, *Lwt* 66 (2016) 56–62, <https://doi.org/10.1016/j.lwt.2015.10.005>.
- [3] C. Feng, et al., Quality characteristics and antioxidant activities of goat milk yogurt with added jujube pulp, *Food Chem.* 277 (620) (2019) 238–245, <https://doi.org/10.1016/j.foodchem.2018.10.104>.
- [4] D. Li, D. Yue, D. Liu, L. Zhang, S. Song, Phytochemical and chemotaxonomic study on *Ziziphus jujuba* Mill. (Rhamnaceae), *Biochem. Systemat. Ecol.* 91 (2020) 104058, <https://doi.org/10.1016/j.bse.2020.104058>, April.
- [5] A.S. Eweys, Y.S. Zhao, O.M. Darwesh, Improving the antioxidant and anticancer potential of *Cinnamomum cassia* via fermentation with *Lactobacillus plantarum*, *Biotechnol. Reports* 36 (October) (2022) e00768, <https://doi.org/10.1016/j.btre.2022.e00768>.
- [6] V. Gowd, N. Karim, L. Xie, M.R.I. Shishir, Y. Xu, W. Chen, In vitro study of bioaccessibility, antioxidant, and α-glucosidase inhibitory effect of pelargonidin-3-O-glucoside after interacting with beta-lactoglobulin and chitosan/pectin, *Int. J. Biol. Macromol.* 154 (2020) 380–389, <https://doi.org/10.1016/j.jbiomac.2020.03.126>.

- [7] Y. Pu, et al., Effect of harvest, drying and storage on the bitterness, moisture, sugars, free amino acids and phenolic compounds of jujube fruit (*Zizyphus jujuba* cv. Junzao), *Sci. Food Agric.* 98 (2) (2017) 628–634, <https://doi.org/10.1002/jsfa.8507>.
- [8] X. Pan, S. Zhang, X. Xu, F. Lao, J. Wu, Volatile and non-volatile profiles in jujube pulp co-fermented with lactic acid bacteria, *LWT* 154 (2022) 112772, <https://doi.org/10.1016/j.lwt.2021.112772>.
- [9] R. Denkova-Kostova, et al., Investigation of probiotic properties of *Lactobacillus helveticus* 2/20 isolated from rose blossom of *Rosa damascena* Mill, *BIO Web Conf* 58 (2023) 02002, <https://doi.org/10.1051/bioconf/20235802002>.
- [10] N.D.K. Akpabli-Tsigbe, J. Osabutey, B.K. Mintah, K. Tano-Debrah, Y. Ma, Cleavage of macromolecule (protein/polysaccharide)-phenolic bond in soybean cell wall through *Lactobacillus casei* and *Lactobacillus helveticus* mixed culture solid-state fermentation for chlorogenic acid extraction, *Food Biosci.* 55 (June, 2023), <https://doi.org/10.1016/j.foodchem.2023.102903>.
- [11] E. Kwaw, et al., Effect of lactobacillus strains on phenolic profile, color attributes and antioxidant activities of lactic-acid-fermented mulberry juice, *Food Chem.* 250 (2018) 148–154, <https://doi.org/10.1016/j.foodchem.2018.01.009>. December 2017.
- [12] Q. Li, et al., *Lactobacillus fermentum* as a new inhibitor to control advanced glycation end-product formation during vinegar fermentation, *Food Sci. Hum. Wellness* 11 (5) (2022) 1409–1418, <https://doi.org/10.1016/j.fshw.2022.04.031>.
- [13] C. Wu, T. Li, J. Qi, T. Jiang, H. Xu, H. Lei, Effects of lactic acid fermentation-based biotransformation on phenolic profiles, antioxidant capacity and flavor volatiles of apple juice, *Lwt* 122 (November 2019) 109064, <https://doi.org/10.1016/j.lwt.2020.109064>, 2020.
- [14] S. Yaqoob, et al., The effect of lactic acid bacteria and co-culture on structural, rheological, and textural profile of corn dough, *Food Sci. Nutr.* 10 (1) (2022) 264–271, <https://doi.org/10.1002/fsn3.2666>.
- [15] A.A. Atallah, E.A. Ismail, H.M. Yehia, M.F. Elkhadragey, A.S. Aloufi, D.G. Gemiel, Physicochemical, microbiological and microstructural characteristics of Sucrose-free probiotic-frozen yogurt during storage, *Foods* 11 (8) (2022), <https://doi.org/10.3390/foods11081099>.
- [16] J. Li, et al., Improvement of antioxidant properties of jujube puree by biotransformation of polyphenols via *Streptococcus thermophilus* fermentation, *Food Chem. X* 13 (December 2021) 100214, <https://doi.org/10.1016/j.fochx.2022.100214>, 2022.
- [17] T. Li, T. Jiang, N. Liu, C. Wu, H. Xu, H. Lei, Biotransformation of phenolic profiles and improvement of antioxidant capacities in jujube juice by select lactic acid bacteria, *Food Chem.* 339 (July 2020) 127859, <https://doi.org/10.1016/j.foodchem.2020.127859>, 2021.
- [18] G. Liu, et al., The effect of different pretreatment methods on jujube juice and lactic acid bacteria-fermented jujube juice, *Lwt* 181 (November 2022) 114692, <https://doi.org/10.1016/j.lwt.2023.114692>, 2023.
- [19] M.S. Safari, M. Keyhanfar, R. Shafiei, Investigating the antibacterial effects of some *Lactobacillus*, *Bifidobacterium* and *acetobacter* strains killed by different methods on *Streptococcus mutans* and *Escherichia coli*, *Mol. Biol. Res. Commun.* 8 (3) (2019) 103–111, <https://doi.org/10.22099/mbrc.2019.33582.1399>.
- [20] M. Xia, et al., Interaction of acetic acid bacteria and lactic acid bacteria in multispecies solid-state fermentation of traditional Chinese cereal vinegar, *Front. Microbiol.* 13 (September) (2022) 1–14, <https://doi.org/10.3389/fmicb.2022.964855>.
- [21] J.N. Ekumah, et al., Effect of selenium supplementation on yeast growth, fermentation efficiency, phytochemical and antioxidant activities of mulberry wine, *Lwt* 146 (2021) 111425, <https://doi.org/10.1016/j.lwt.2021.111425>. March.
- [22] W. Guo, et al., Effects of *Lactocaseibacillus casei* fermentation on the bioactive compounds, volatile and non-volatile compounds, and physiological properties of barley beverage, *Food Biosci.* 53 (April) (2023) 102695, <https://doi.org/10.1016/j.foodchem.2023.102695>.
- [23] A. Kumaran, R.J. Karunakaran, *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India, *LWT - Food Sci. Technol.* 40 (2) (2007) 344–352.
- [24] I.D. Boateng, W. Zhang, Y.Y. Li, F.K. Saalia, X.M. Yang, Non-thermal pretreatment affects *Ginkgo biloba* L. seed's product qualities, sensory, and physicochemical properties, *J. Food Sci.* 87 (1) (2022) 94–111, <https://doi.org/10.1111/1750-3841.15999>.
- [25] B. Kafle, et al., Fourier-transform infrared spectroscopy for characterization of liquid protein solutions: a comparison of two sampling techniques, *Vib. Spectrosc.* 124 (2022) 2023, <https://doi.org/10.1016/j.vibspec.2022.103490>. December.
- [26] A. Khadivi, F. Beigi, Morphological and chemical characterizations of jujube (*Zizyphus jujuba* Mill.) to select superior accessions, *Food Sci. Nutr.* 10 (7) (2022) 2213–2223, <https://doi.org/10.1002/fsn3.2831>.
- [27] Z. Ali, H. Ma, M.T. Rashid, A. Wali, S. Younas, Preliminary study to evaluate the phytochemicals and physicochemical properties in red and black date's vinegar, *Food Sci. Nutr.* 7 (6) (2019) 1976–1985, <https://doi.org/10.1002/fsn3.1009>.
- [28] A. Krakowska-Sieprawska, K. Rafinska, J. Walczak-Skierska, B. Buszewski, The influence of plant material enzymatic hydrolysis and extraction conditions on the polyphenolic profiles and antioxidant activity of extracts: a green and efficient approach, *Molecules* 25 (9) (2020) 1–17, <https://doi.org/10.3390/molecules25092074>.
- [29] J. Song, et al., Improving the acetic acid fermentation of *acetobacter pasteurianus* by enhancing the Energy metabolism, *Front. Bioeng. Biotechnol.* 10 (2022) 1–12, <https://doi.org/10.3389/fbioe.2022.815614>. March.
- [30] A. Rauf, et al., Proanthocyanidins: a comprehensive review, *Biomed. Pharmacother.* 116 (2019), <https://doi.org/10.1016/j.biopha.2019.108999>. February.
- [31] G. Liu, et al., The effect of different pretreatment methods on jujube juice and lactic acid bacteria-fermented jujube juice, *Lwt* 181 (2023) 114692, <https://doi.org/10.1016/j.lwt.2023.114692>.
- [32] Y. Cao, et al., Colour change and kinetics of winter Jujube slices during pulsed air-jet impingement drying, *Czech J. Food Sci.* 36 (4) (2018) 302–308, <https://doi.org/10.17221/259/2017-CJFS>.
- [33] L.J. Chai, et al., Modulating microbiota metabolism via bioaugmentation with *Lactobacillus casei* and *Acetobacter pasteurianus* to enhance acetoin accumulation during cereal vinegar fermentation, *Food Res. Int.* 138 (2020) 109737, <https://doi.org/10.1016/j.foodres.2020.109737>.
- [34] K. Chelladhurai, M. Ayyash, M.S. Turner, A. Kamal-Eldin, *Lactobacillus helveticus*: health effects, current applications, and future trends in dairy fermentation, *Trends Food Sci. Technol.* 136 (2023) 159–168, <https://doi.org/10.1016/j.tifs.2023.04.013>. February.
- [35] C. Sen Wu, Q.H. Gao, X.D. Guo, J.G. Yu, M. Wang, Effect of ripening stage on physicochemical properties and antioxidant profiles of a promising table fruit 'pear-jujube' (*Zizyphus jujuba* Mill.), *Sci. Hort.* (Amsterdam) 148 (2012) 177–184, <https://doi.org/10.1016/j.scienta.2012.09.026>.
- [36] K. Chen, D. Fan, B. Fu, J. Zhou, H. Li, Comparison of physical and chemical composition of three Chinese jujube (*Zizyphus jujuba* Mill.) cultivars cultivated in four districts of Xinjiang region in China, *Food Sci. Technol.* 39 (2019) 912–921, <https://doi.org/10.1590/fst.11118>.
- [37] A. Ahmad, H. Ayub, Fourier Transform Infrared Spectroscopy (FTIR) Technique for Food Analysis and Authentication, 2022, https://doi.org/10.1007/978-981-19-5422-1_6.
- [38] F.G. Pavli, A.A. Argyri, N.G. Chorianopoulos, G.J.E. Nychas, C.C. Tassou, Effect of *Lactobacillus plantarum* L125 strain with probiotic potential on physicochemical, microbiological and sensorial characteristics of dry-fermented sausages, *Lwt* 118 (May 2019) 108810, <https://doi.org/10.1016/j.lwt.2019.108810>, 2020.