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Fermentation of Date Pulp Residues Using *Saccharomyces cerevisiae* and *Pichia kudriavzevii*—Insights into Biological Activities, Phenolic and Volatile Compounds, Untargeted Metabolomics, and Carbohydrate Analysis Post In Vitro Digestion

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Abstract: Utilizing agricultural byproducts, such as date pulp residue (DPR), can contribute significantly towards achieving sustainable food production. This paper aimed to investigate the physicochemical properties of DPR and evaluate the health benefits of nonfermented and fermented DPR samples both pre- and post-digestion (bioaccessible portions). Additionally, it aimed to analyze the carbohydrates and untargeted metabolites in the bioaccessible portions. Fermentation of DPR by *Saccharomyces cerevisiae* and *Pichia kudriavzevii* resulted in an abundance of malic acid (over 2400 mg/100 g) and the identification of 42 volatiles, with different degrees of predominance observed in the samples. Twenty phenolics were determined by UPLC in fermented DPR, with (-)-epicatechin, tyrosol, and gallic acid being the most abundant. Bioaccessibility studies revealed that fermented DPR samples retained at least ~44% of α-glucosidase inhibition and exhibited improved α-amylase inhibition compared to nonfermented and undigested samples. In vitro cytotoxicity assays showed a more potent inhibitory effect of fermented DPR against MCF-7 and Caco2 cell lines (average inhibition of 55% and 74.4% for the two types of fermented samples) compared to nonfermented DPR. The untargeted metabolomics analysis identified C5-branched dibasic acid metabolism as the most prominent pathway, with four metabolites identified. Furthermore, the analysis of bioaccessible carbohydrate metabolites in the fermented DPR using LC-QTOF showed the presence of a group of phytochemicals, including three terpenoid metabolites.

Keywords: date pulp; antioxidant; amylase; glucosidase; ACE-inhibition; cytotoxicity

1. Introduction

The increasing amount of food waste has become a global concern due to its adverse effects, which are not limited to the environment but also extend to the economy and society [1]. According to the Food and Agriculture Organization of the United Nations (FAO) statistics, around 1.3 billion tons of food waste is generated yearly, which equals one-third of the overall food produced for human consumption being wasted or lost [2].

Recently, food waste has garnered the scientific community's attention because of its availability and composition [3]. This attention has led to the valorization of food waste through various approaches, where it can be used as a source of nutrient supplements, enzymes, biopolymers, food-grade pigments, and as an efficient substrate for producing novel value-added products [4–6].

The date palm (*Phoenix dactylifera* L.) has been extensively cultivated in arid and semi-arid regions of the world [7]. The top producers of date fruit globally in 2021, ranked in decreasing order, are Egypt, Saudi Arabia, Iran (Islamic Republic of), Algeria, Iraq, Pakistan, Sudan, Oman, United Arab Emirates, and Tunisia [8]. Production of date fruit was estimated at 9.6 million tons in 2021 [8]. Due to their high nutritional value, date fruits are now being produced, processed, and applied on a larger scale worldwide, corresponding to an increase in the levels of date fruit waste. Annually, around 20% of the date fruit production is lost as a result of over-ripening, inappropriate packaging and storage, contamination, and rough handling [9]. Typically, fresh dates are marketed, while low-quality and immature dates are utilized to produce food products such as syrup, jam, juice, chutney, and jelly [10,11]. The date processing industry generates a significant amount of date residues represented by date pulp residue (DPR), date seeds, and cull date fruits, which are mostly handled as waste [12]. Improper disposal of these residues can result in severe environmental pollution, health issues, and economic problems [13]. Viewed from another perspective, various scientific studies have reported that the high nutritional content of date processing waste makes it an efficient raw material for producing high-value-added bioproducts [14,15].

DPR is a solid byproduct of the date syrup/juice production process in the date processing industry [16]. About 17–28% of the DPR produced daily is generated by the date syrup industry, some of which is utilized as animal feed while the rest is disposed of in open lands [9]. As a raw material, DPR contains a significant amount of dietary fiber, carotenoids, sterols, minerals, phenolic compounds such as lignans, flavonoids, phenolic acids, and quinones [17,18].

The valorization of date fruit byproducts can remarkably contribute to sustainable agro-food waste usage and help mitigate environmental pollution [19]. Therefore, assessing the health-promoting attributes of agro-food byproducts is a critical initial step in transforming biowaste into marketable products. Recently, in vitro digestion has been used to obtain a more precise evaluation of the health-promoting benefits of fruit/vegetable byproduct, including ACE inhibition, antioxidant and antidiabetic activities, and antiproliferative effects [20,21]. Additionally, such bioaccessible portions have recently been analyzed using untargeted/targeted metabolomics via the UPLC-QTOF technique to explore metabolic pathways and identify metabolites that possess potential biological activities [22,23]. Analogously, an assessment of the date fruit byproduct is necessary.

Fermentation technology is a common and sustainable approach for developing high-value-added food products and preserving food [24]. The fermentation process relies on two key factors: the microorganisms employed and the substrate involved [25]. Microorganisms play a crucial role in initiating the fermentation process of food, which involves the conversion of primary metabolites into new secondary metabolites [25]. This transformation brings about organoleptic changes in the substrate and extends its shelf life [26]. Depending on the substrate being utilized, these microorganisms can be bacteria, fungi, or yeasts [27]. The fermentation mechanism varies based on the specific substrate, which can include dairy, meat, vegetables, fish, cereals, fruits, and even food waste [28]. Primary and secondary metabolites are produced during fermentation, such as antimicrobial peptides, vitamins, antibiotics, folates, carbon dioxide, organic acids, and alcohol. These compounds are referred to as bioactive compounds due to their therapeutic potential. Siriwardhana, Kalupahana [29] reported that bioactive compounds have the ability to promote health benefits by reducing excessive oxidative stress and alleviating inflammation and metabolic disorders. In this context, microbial bioconversion can be performed using the dietary

fiber and yeast-fermentable sugars present in DPR as a nutrient and carbon source for the targeted value-added products [30,31].

Consequently, the current work was implemented in the nonfermented and fermented DPR with the aim of evaluating the physicochemical characteristics, including volatile compounds, organic acids, and sugars, and quantifying phenolic compounds; assessing the health-promoting benefits (angiotensin-converting enzyme (ACE) inhibition, proteolytic activity, total phenolic compound (TPC), and activities of antidiabetic, antioxidant, and cytotoxicity) in the in vitro pre- and post-digestion (bioaccessible portions); and analyzing untargeted and carbohydrates metabolites by UPLC-QTOF.

2. Materials and Methods

2.1. Yeast Propagation, Proliferation, pH, and Titratable Acidity

Saccharomyces cerevisiae and *Pichia kudriavzevii*, with accession numbers OK441070 and OK441073, respectively, were previously isolated from traditional fermented food sources and identified as potential probiotic yeasts [32]. Both yeasts were subcultured in yeast extract–peptone–dextrose (YPD) broth and stored in glycerol stocks (50% v/v) at −80 °C. YPD broth (Himedia Laboratories Pvt ltd, Nashik, India) was used to culture *S. cerevisiae* and *P. kudriavzevii*, separately, before being used for fermentation. The yeast population in fermented DPR and unfermented DPR (control) was counted by serially diluting with 0.1% (w/v) peptone water and using the pour-plate technique. Triplicate plates were incubated aerobically at 25 °C for 6 days, and yeast populations were counted on YPD agar at 0, 3, and 6 days. The pH value of fermented DPR and control was measured using a calibrated digital Start-3100 pH meter (OHAUS Corporation, Parsippany, NJ, USA). Additionally, samples were titrated with 0.01 N NaOH to determine the titratable acidity (TA%), which was expressed as a percentage of lactic acid.

2.2. Fermentation of Date Pulp Residues

DPR was generated as a side stream of a date syrup produced through the combination of different date cultivars. DPR was sourced from the Emirates Dates Factory, a subsidiary of Al-Foah Company located in Alain, United Arab Emirates. To prepare for inoculated fermentation, DPR was mixed with distilled water at a 1:10 ratio. Subsequently, the diluted DPR was inoculated with a high concentration of *S. cerevisiae* and *P. kudriavzevii*, specifically 8.0 log CFU/mL (Figure S1). The mixture was then subjected to incubation at a temperature of 25 °C for a duration of 6 days until the pH level approached approximately 4.0. Throughout the fermentation process, DPR that remained uninoculated served as a control.

2.3. Physicochemical Properties

The physicochemical characteristics of fermented DPR and nonfermented DPR (control) were evaluated after six days of fermentation.

2.3.1. Sugars and Organic Acids

A chromatographic analysis of sugars was performed as detailed by Silva, Cruz [33], with minor modifications. Briefly, the analysis was carried out using SupelcoGel Ca+ column (300 × 7.8 mm; Supelco, Sigma-Aldrich, St Louis, MO, USA), at 85 °C, in isocratic mode using deionized water as the mobile phase at 0.5 mL min^{-1}. Detection was performed using PDA at 195 nm.

Organic acids were analyzed using high-performance liquid chromatography (HPLC) [34] and a gradient solvent delivery system (Thermo Scientific Dionex Ultimate 3000 Rapid Separation LC system with Chromeleon (c) Dionex version 7.2.4.8179 software, Bremen, Germany). The column used was a Supelcogel H 30 cm × 7.8 mm Cat # 59304-U at 25 °C. The mobile phase was 85% (v/v) phosphoric acid (0.04 M H_3PO_4), and the flow rate was 0.4 mL/min. The PDA 3000 Dionex Photo Diode Array Detector (190–800 nm) was used for detection. The sample solution was transferred into an HPLC autosampler

vial, and 20 µL of the sample extract was injected in duplicate. The concentration of organic acids in the sample was calculated from the calibration curve using external standards.

2.3.2. Volatile Compounds

Volatile compounds were analyzed using a procedure described by Liu, Aung [35], via a headspace (HS) solid-phase microextraction (SPME) method, combined with carboxen/poly (dimethylsiloxane) fiber (85 µm coating, Supelco, Sigma-Aldrich, Barcelona, Spain), a gas chromatography (GC)–mass spectrometer (MS), and a flame ionization detector (FID). Filtered DPR samples (1 mL) were mixed with a saturated sodium chloride solution (1 mL), and 2 mL of the mixed solution was extracted with HS-SPME at 60 °C for 50 min under a rotational speed of 250 rpm/min, in a 20 mL headspace vial. Volatile compounds were separated by a capillary column (60 m × 0.25 mm I.D., Agilent DB-FFAP, Santa Clara, CA, USA) coated with a 25 mm film thickness of polyethylene glycol modified with nitroterephthalic acid. Helium was used as the carrier gas, with a flow rate of 1.2 mL/min, and the temperature program increased from 50 °C (5 min) to 230 °C (30 min) at a rate of 5 °C/min. Volatiles were identified by comparing their mass spectra (MS) with NIST MS library, with a matching factor over 80.

2.3.3. Quantification of Phenolic Compounds

The quantification of phenolic compounds of fermented DPR and control samples was measured by using a Shimadzu U-HPLC system equipped with a reversed-phase analytical column of 2.1 × 50 mm, 1.7 µm particle size (Waters Acquity UPLC BEH C18, Waters Corporation, Milford, CT, USA). The column oven temperature was maintained at 45 °C and the flow rate was set at 0.4 µL/min throughout the experiment. Water and acetonitrile, each containing 5% formic acid, were used as mobile phases A and B, respectively. The injection volume was 2 µL, with a run time of 20 min. The gradient profile began at 5% and increased to 20% B for 6 min, then increased to 70% B for 15 min, followed by an increase to 100% B for 1 min before returning to the starting condition from 18–20 min. The effluents were measured at multiple wavelengths (260, 270, 280, 320, 340, and 370 nm). Phenolic compounds were identified by comparing their retention times and UV–Vis spectra with phenolic standards.

2.4. In Vitro Digestion by INFOGEST2.0 and Bioaccessible Portion

In vitro digestion was performed on fermented DPR and nonfermented DPR using the method described by Brodkorb et al. [36], and the bioaccessible portion was obtained by following the method of Ayyash et al. [37].

2.5. Health-Promoting Properties

The health-promoting attributes of fermented DPR and nonfermented DPR samples were assessed before and after a six-day fermentation period, considering both their undigested and in vitro-digested states (bioaccessible portions).

2.5.1. Inhibition of α-Amylase and α-Glucosidase

The α-amylase and α-glucosidase inhibition assays of the samples were carried out according to the method described by Ng et al. [38] for α-amylase and Ho et al. [39] for α-glucosidase. The following equation was used to calculate the inhibition percentage:

$$\text{Inhibition } \% = \left(1 - \frac{Abssample - Absblank}{Abscontrol}\right) \times 100 \quad (1)$$

2.5.2. Antioxidant Capacity

The DPRH (1,1-diphenyl-2-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$)) radical scavenging assays were conducted following the method

outlined by Ng and Kuppusamy [40]. The percentage of radical scavenging activity was determined using the following calculation:

$$\text{Scavening rate } \% = \left(\frac{Absblank - Abssample}{Absblank} \right) \times 100 \quad (2)$$

2.5.3. Cytotoxicity Activities

The samples of the bioaccessible portion were assayed against Caco-2 and MCF-7 carcinoma cell lines according to the method detailed by Ayyash et al. [41]. The cytotoxicity percentage was determined using the following equation:

$$\text{Antiproferative activity}(\%) = \left[1 - \frac{Rsample - R0}{Rctrl - R0} \right] \times 100 \quad (3)$$

2.5.4. OPA and TPC

The O-phthaldialdehyde (OPA) assay was conducted to estimate the total free amino acids as described in the method of [37]. The TPC of the samples was measured using the Folin–Ciocalteu reagent method [42].

2.5.5. ACE Inhibition

ACE inhibition activity was assessed according to the method described by Liu et al. [43]. The ACE inhibition activity (%) was calculated as

$$\text{ACE inhibition } \% = \left(1 - \frac{Ab - Aa}{Ab - Ac} \right) \times 100 \quad (4)$$

Ab refers to the absorbance without adding the sample solution (buffer solution added instead of the sample), and *Aa* refers to the absorbance in the presence of ACE and the sample solution. *Ac* refers to the absorbance of the blank (HCl was added before the addition of ACE).

2.6. Untargeted Metabolomics and Carbohydrate Metabolomics Analyses

2.6.1. Untargeted Metabolite Analysis

The UPLC-QTOF technique was employed to analyze the bioaccessible fractions of fermented DPR by *P. kudriavzevii* and control on the sixth day. The analytical system employed for separation and identification of metabolites consisted of a Waters UPLC I-Class Plus (Waters, USA) coupled with a Q Exactive high-resolution mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). A Waters ACQUITY UPLC BEH C18 column (1.7 µm, 2.1 mm × 100 mm, Waters, USA) maintained at a temperature of 45 °C was utilized for chromatographic separation. The mobile phase composition varied depending on the ionization mode: 0.1% formic acid (A) and acetonitrile (B) were used in positive mode, while 10 mM ammonium formate (A) and acetonitrile (B) were used in negative mode. For mass spectrometry, Q Exactive (Thermo Fisher Scientific, USA) was used for primary and secondary data acquisition. The equipment had a scanning range from 70 to 1050 m/z with a resolution of 70,000. During the MS acquisitions, the AGC target was set to 3×10^6, and the resulting data were analyzed using Compound Discoverer 3.3 (Thermo Fisher Scientific, USA) in combination with bmdb (BGI metabolome database), mzcloud database, and chemspider online database. The output data matrix contained details such as metabolite peak area and identification results, which were further subjected to analysis and processing. The software utilized for this purpose was Compound Discoverer, version v.3.3, with the following parameters: parent ion mass deviation: <5 ppm, mass deviation of fragment ions: <10 ppm, and retention time deviation: <0.2 min. Additional information can be found on the official website: https://mycompounddiscoverer.com (21 March 2023).

2.6.2. Carbohydrate Metabolites Analysis

To extract metabolites, 25 mg of bioaccessible samples were weighed and subjected to extraction using a precooled extraction reagent composed of methanol, acetonitrile, and water (2:2:1, *v*/*v*/*v*), with the addition of internal standards mix 1 (IS1) and internal standards mix 2 (IS2) to ensure sample preparation quality control.

The samples were homogenized for 10 min, sonicated, and incubated at −20 °C for 1 h before being centrifuged and the supernatant was then transferred for vacuum freeze drying. The metabolites were resuspended in 10% methanol and analyzed using LC-MS/MS with a 2D UPLC (waters, USA) and a tandem Q-Exactive mass spectrometer (Thermo Fisher Scientific, USA) with a heated electrospray ionization (HESI) source. The analysis was controlled by the Xcalibur 2.3 software program (Thermo Fisher Scientific, Waltham, MA, USA). The chromatographic separation and mass spectrometry were carried out as described in Section 2.6.1.

2.7. Data Processing

To perform a comprehensive analysis of the mass spectrometry data, the commercial software Progenesis QI (Waters, Manchester, UK) and an in-house metabolomics analysis process were utilized. Metabolite identification was conducted by referencing the HMDB (http://www.hmdb.ca/ (21 March 2023)) and KEGG (https://www.genome.jp/kegg/ (21 March 2023)) databases. Principal component analysis (PCA) and heatmap analysis were employed for further data exploration. For metabolite identification, the BMDB Library (BGI Metabolome Database), an internally developed database, was utilized. This database encompasses retention time (RT), MS1 spectrum (MS1), and MS2 spectrum (MS2) information for all standards. It covers key primary metabolites and metabolic intermediates involved in crucial metabolic pathways, such as carboxylic acids, amino acids, biogenic amines, polyamines, nucleotides, coenzymes and vitamins, monosaccharides and disaccharides, fatty acids, lipids, steroids, and hormones.

Furthermore, the HMDB database, known for its extensive coverage of human metabolites, was also employed in the analysis. It provides datasets across three categories: chemical data, clinical data, and analytical biology and biochemical data. Both water-soluble and fat-soluble metabolites are included in this resource. Additionally, the provided information includes links to other databases such as KEGG and PubChem, facilitating convenient access to further relevant information.

2.8. Statistical Analysis

All results were expressed as the mean of three independent determinations ± standard deviation (SD), unless otherwise mentioned. The statistical mean difference among the samples was determined by analysis of variance (ANOVA) with Tukey post-test [30]. PCA for variables and observations was conducted to estimate the structural correlation of the variables and to indicate the relationship between the fermented DPR and control. The statistical analyses were performed by XLSTAT software (Addinsoft, New York, NY, USA).

3. Result and Discussion

3.1. Yeast Proliferation, pH, and Titratable Acidity

The two yeast strains (*S. cerevisiae* and *P. kudriavzevii*) maintained high proliferation, more than 7.0 log CFU/mL, throughout 6 days of fermentation (Figure S1A). In contrast, yeast growth in the nonfermented DPR (control) remained low, at around one log CFU/mL over 6 days, indicating that the two yeast strains were suitable for the DPR environment. Figure S1B shows that the pH of the fermented DPR decreased from an average of 5.26 to below 4.1 over 6 days ($p < 0.05$), while the pH of the nonfermented DPR remained relatively unchanged at an average of 5.4 during the six days. The decreasing pH in the sample fermented by *P. kudriazevii* was slightly faster than that in *S. cerevisiae* ($p > 0.05$). In line with the pH value, an increase in titratable acidity (Figure S1C) was observed in the fermented samples during the fermentation period ($p > 0.05$). The alterations in pH and titratable

acidity could be explained by the increased amounts of organic acids synthesized during yeast fermentation.

3.2. Physicochemical Properties

3.2.1. Evaluation of Sugars and Organic Acids

Chromatographic analysis of sugars in the fermented DPR showed that sucrose, glucose, fructose, and arabinose were not present in significant amounts, likely due to their consumption by *S. cerevisiae* and *P. kudriavzevii* during fermentation. Glucose is metabolized into organic acids via the glyoxylate and tricarboxylic acid (TCA) cycles [34].

In this study, six organic acids were studied (Figure 1) in nonfermented DPR (control) and fermented DPR by two yeast strains after 6 days of fermentation. These organic acids included oxalic acid, citric acid, malic acid, lactic acid, acetic acid, and propionic acid. Compared to the control, all screened organic acids showed an increase in concentration, except for oxalic acid which decreased in two samples of fermented DPR. Additionally, propionic acid was newly produced in fermented DPR by *S. cerevisiae*. The levels of lactic acid and malic acid in fermented DPR by *S. cerevisiae* (748.3, 4359.1 mg/100 g) and *P. kudriavzevii* (1698.6, 2463.5 mg/100 g) were significantly higher than their corresponding controls (475, 234.5 mg/100 g), respectively, indicating that yeast fermentation significantly promoted the synthesis of lactic and malic acids. The sample fermented by *P. kudriavzevii* had slightly higher amounts of citric and acetic acids (244.8, 185.5 mg/100 g) than the sample fermented by *S. cerevisiae* (195.8, 154.8 mg/100 g), respectively.

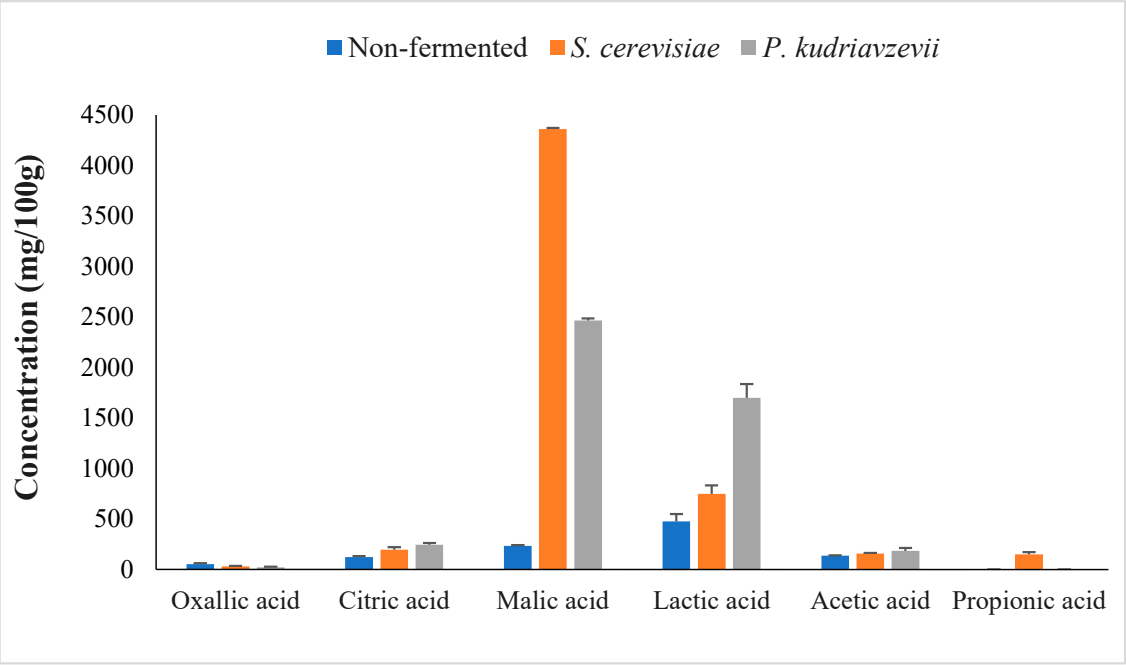

Figure 1. Concentrations of organic acids (mg/100 g) in nonfermented (control) and fermented date pulp residues by *S. cerevisiae* and *P. kudriavzevii*. Values are the mean values (n = 3). Error bars express standard deviations.

Differences in organic acid content are influential factors that contribute to variations in flavor among different varieties of date fruits [44]. In this aspect, Ghnimi et al. [45] and Elshibli and Korpelainen [46] described that each variety of date fruit has its own pattern of organic acid content. However, malic acid has been identified as a common acid with varying levels in date fruits [47,48].

In the present work, the high concentration of lactic acid and acetic acid in fermented DPR compared to the control was related to the sugar-metabolizing pathway of two yeast strains (*S. cerevisiae* and *P. kudriavzevii*). These yeast strains enabled the conversion of

sugars in DPR into lactic acid, ethanol, acetic acid, and CO_2. The increase in malic acid and citric acid may have resulted from the accumulation of these acids as byproducts of yeast fermentation through the oxidative pathway of the tricarboxylic acid (TCA) cycle [49,50]. As shown in Figure 1, malic acid was the predominant organic acid in the fermented samples, associated with the tart, mellow, smooth, and sour taste, as well as giving the date its distinguished fruity taste [48,51]. In line with our results, Alahyane et al. [52] and Cherif et al. [53] reported an abundance of malic acid in date palm fruits.

3.2.2. Evaluation of Volatile Compounds

Although the influence of yeast fermentation on the volatile profiles has been extensively studied in different food products, especially wine [54–56], there is still a need to understand the effect of selective yeast fermentation on the volatiles of date pulp residue (DPR). Figure 2 shows the chemical categories of volatile compounds that were detected in both fermented and nonfermented DPR. In total, 42 volatile compounds were identified, including 10 volatile acids, 8 esters, 6 alcohols, 4 phenols, 3 ketones, 4 aldehydes, and 10 compounds belonging to various other chemical groups (Table S1). Figure 2 highlights that when *S. cerevisiae* and *P. kudriavzevii* were used to ferment DPR, volatile compounds belonging to the alcohol group were synthesized in higher amounts (~41% and 28% RPA, respectively) compared to the control (~11% RPA). On the other hand, the RPA% of esters decreased by 18% in the samples fermented with *S. cerevisiae* compared to the control (~30.4%).

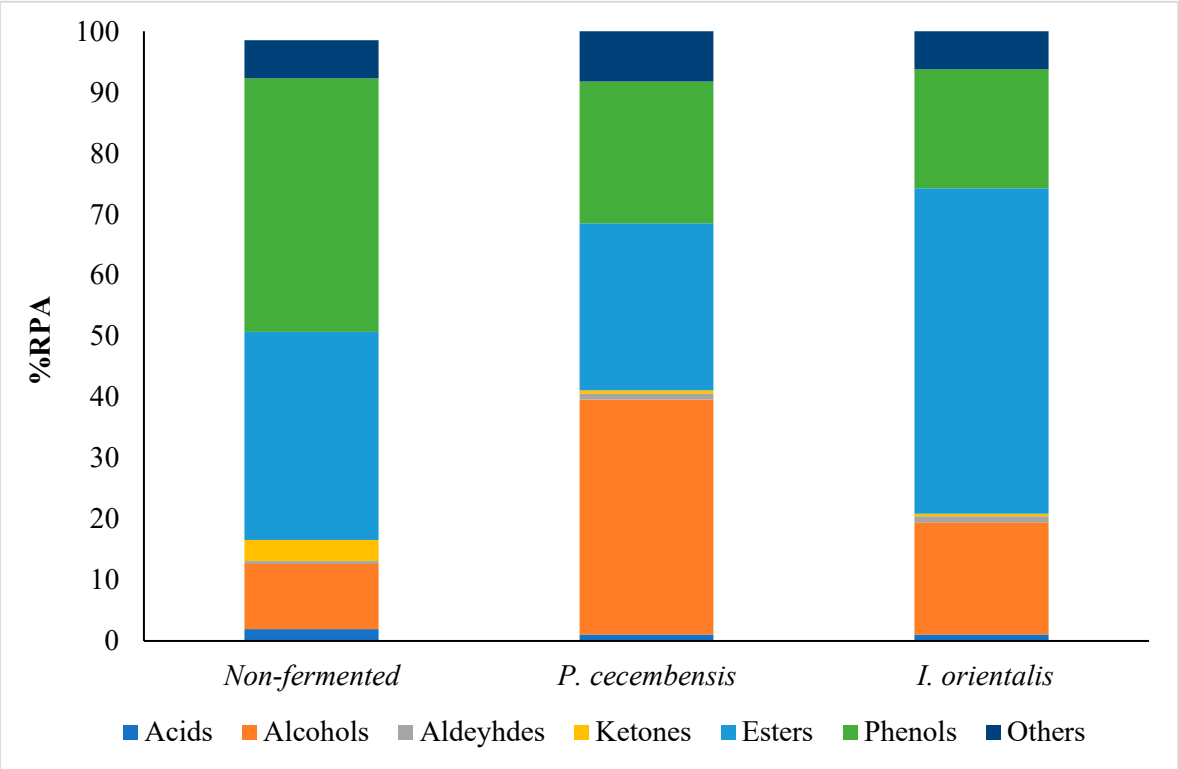

Figure 2. Chemical classes of volatile compounds identified in nonfermented and fermented date pulp residues, represented in % relative peak area (RPA).

Among the volatile acids, acetic acid had a comparably higher level, with greater amounts found in the *P. kudriavzevii* sample, which is compatible with the results of our organic acids analysis (Figure 1).

Varied patterns were noted for the formation of volatile alcohols (Table S1). Isobutanol was newly synthesized in two fermented samples, with the highest level observed in the *P. kudriavzevii* sample. The production of this alcohol can be attributed to the degrada-

tion of valine in the yeast cytosol via the Ehrlich pathway [57]. The concentrations of 2-phenylethanol and isoamyl alcohol were significantly increased after fermentation, with the highest level found in the sample fermented by *S. cerevisiae*. The increasing levels of these alcohols (2-phenylethanol, isoamyl alcohol) could be derived from the essential amino acids phenylalanine and leucine [58,59], respectively, which might be present in the fermented DPR.

An increasing trend was noted in the class of esters after fermentation (Table S1). Among all esters, ethyl acetate was the prevalent volatile ester in all samples, which may impart a solvent-like aroma if in excess. It has been documented that the yeast generation of volatile acetates, such as isoamyl, ethyl, and 2-phenethyl acetate, occurs through alcohol acetyltransferases that use acetyl-CoA to acetylate various alcohols [60,61]. Verstrepen et al. [62] and Saerens et al. [63] reported that the formation of esters in fermented foods by yeast depends on the yeast strain used, which may clarify the variations in ester production between the two yeast strains utilized in the present study (*S. cerevisiae* and *P. kudriavzevii*). Among all the volatile phenols, 2,4-di-*tert*-butylphenol was the most abundant in the fermented samples.

Previous studies, such as those conducted by Siddeeg et al. [64], Saafi et al. [65], and Flowers et al. [44], have described the volatile compounds present in date palm fruits. However, what sets our work apart is its focus on the evaluation of the volatile compounds generated in date pulp residues after yeast fermentation. This key difference may explain some of the discrepancies between our findings and those of previous studies.

3.2.3. Phenolic Compounds Profile

Phenolics derived from food have been linked to several health benefits [66]. Fermentation has the ability to change the phenolic composition of plant-based foods by releasing specific phenolic monomers from their complex structures [67]. Yeast enzymes, such as pectinases and β-glucosidase (β-glucoside glucohydrolase, EC 3.2.1.21), play a role in facilitating the release of these phenolic monomers [68].

In our work, a total of ~70, 194, and 204 mg/kg of 20 phenols were quantified by the UPLC in nonfermented DPR (control), and fermented DPR by *P. kudriavzevii* and *S. cerevisiae*, respectively (Figure 3). In general, yeast fermentation significantly increased the level of phenolic compounds from the byproduct, especially gallic acid, tyrosol, syringic acid, and (-)-epicatechin. Following fermentation, two new flavonoids were detected, namely, apigenin in small quantities and (-)-epicatechin in dominant quantities. UPLC analysis suggests that these two flavonoids might have been synthesized by the yeast strains used in fermenting the date pulp residues.

In the control sample, the proportions of flavonoids, phenolic acids, and other phenols (phenylethanoid and phenolic aldehyde) were approximately 15%, 71%, and 14% of total phenols, respectively. In the sample fermented by *P. kudriavzevii*, the proportion changed to ~47%, 36%, and 17%, while in the sample fermented by *S. cerevisiae*, it was ~42%, 44%, and 14%, respectively, of total phenols. It is evident that fermentation significantly improved the contribution of flavonoids to the total phenols at the expense of the proportion of phenolic acids. (-)-Epicatechin, a flavan-3-ol compound, had the highest concentration among all phenols in the fermented DPR samples, with levels of 75 and 67 mg/kg, respectively, whereas it was not detected in the control sample. This increase in the level of (-)-epicatechin by fermentation may be attributed to its release from oligomeric and polymeric proanthocyanidins and melanin [69,70]. Interestingly, the production of gallic acid, a hydroxybenzoic acid derivative, significantly increased from 10.7 mg/kg to 46.0 mg/kg in the sample fermented by *S. cerevisiae*, and was enhanced to 15.0 mg/kg in the sample fermented by *P. kudriavzevii*. The phenylethanoid compound, tyrosol, underwent considerable development after fermentation, increasing from 8.4 mg/kg to 30.3 and 26.4 mg/kg in samples fermented by *P. kudriavzevii* and *S. cerevisiae*, respectively. Tassoult et al. [71] determined the presence of tyrosol in various Algerian date fruit varieties and date pastes (ranging from 8.17–16.37 mg/kg and 16.12–16.57 mg/kg, respectively); however, the quan-

tity of tyrosol in our study was notably higher. This disparity may be mainly ascribed to yeast metabolites secreted during fermentation, as several studies have described the production of tyrosol, during alcoholic fermentation by different yeast species [72,73] via the Ehrlich pathway.

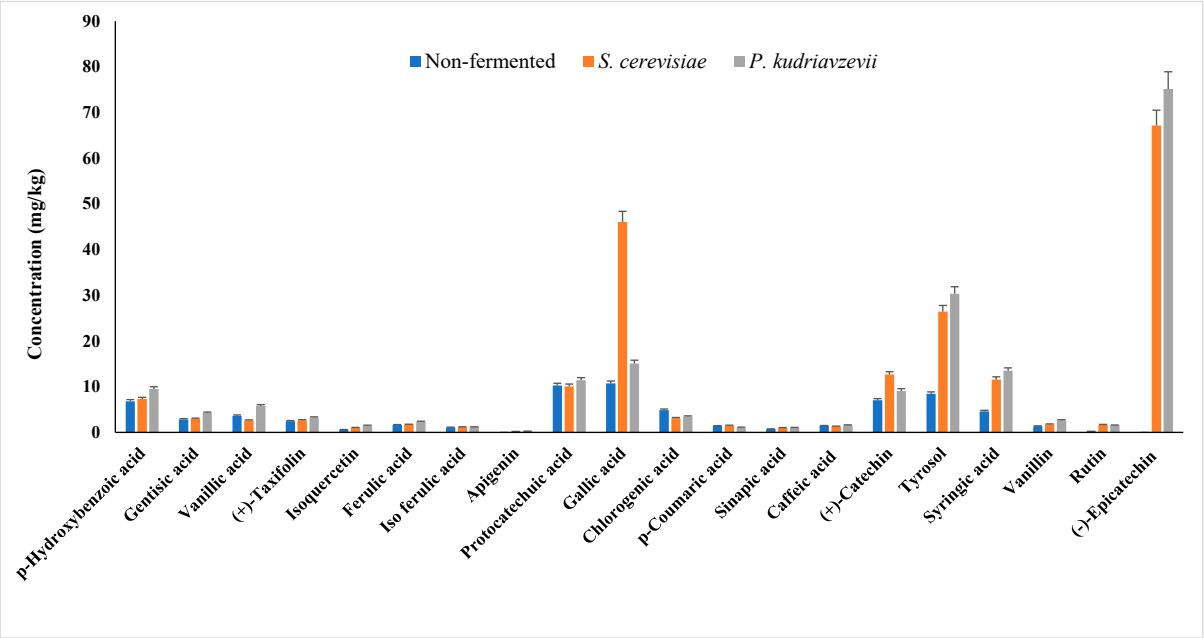

Figure 3. Concentrations of phenolic compounds (mg/kg) of nonfermented and fermented date pulp residues measured by U-HPLC. Values are the mean values (n = 3). Error bars express standard deviations.

Consistent with our results, previous studies reported by Alshwyeh [74], Amira et al. [75], and Zihad et al. [76] have identified most of these phenols in date palm fruits in varying quantities. However, differences between our findings and others in terms of the quantity and type of phenols, organic acids, and volatile compounds may be attributed to the effects of yeast metabolism, variations among date varieties, and date product matrices (date fruits and date pulp residues), as well as external factors such as geographic origin, environmental conditions (e.g., sunlight, fertilizers), and extraction methods.

3.3. Health-Promoting Attributes

Selective fermentation is one of the proposed solutions for reducing food waste, achieving sustainable food production, and creating novel food products, all within an integrated circular economy. In this regard, the current paper evaluates the health benefits of fermented DP in a bioaccessible portion. As illustrated in Figure S2A, in the bioaccessible portions, the TPC for all samples (*S. cerevisiae*, *P. kudriavzevii*, control) averaged 9.3 mg GAE/mL. Figure S2A describes that the TPC in the bioaccessible portions had a significant ($p < 0.05$) decline compared to undigested samples and a slight decline in the fermented DPR compared to the control. In general, the DPR fermented by two yeast strains had a higher OPA than the control (Figure S2B). For the *S. cerevisiae*, *P. kudriavzevii*, and control samples, the OPA absorbances measured before digestion were 0.51, 0.65, and 0.40. These absorbances decreased sharply in the bioaccessible portions to 0.34, 0.40, and 0.23, respectively.

Figure 4A–D exhibits the antidiabetic activities inhibitions (A), antioxidant capacities (B), ACE inhibition (C), and cytotoxicity capability (D) of nonfermented and fermented DPR for undigested samples and bioaccessible portions. Our data revealed that the health-promoting properties showed different trends in the results of in vitro post-digestion (bioaccessible portions) compared to predigestion. In the bioaccessible portion, α-amylase

inhibition and cytotoxicity activities showed improved levels. However, antioxidant capacities, TPC, and OPA exhibited a decline, whereas ACE inhibition had comparatively constant levels in the bioaccessible portion.

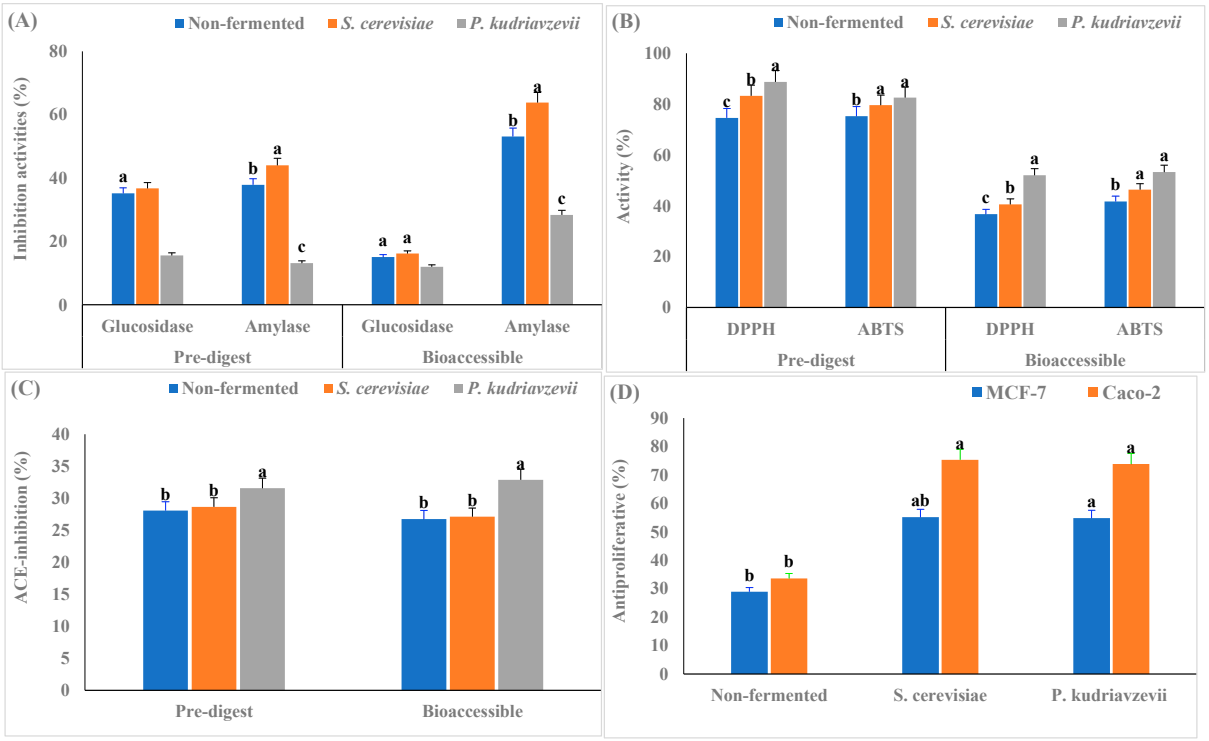

Figure 4. Assays of health-promoting benefits of nonfermented and fermented date pulp residues for undigested samples and bioaccessible portions: Antidiabetic activities inhibitions (**A**), antioxidant capacities (**B**), ACE inhibition (**C**), and cytotoxicity capability (**D**). Values are the mean values (n = 3). Error bars express standard deviations. a–c: mean values with different lowercase letters different significantly at $p < 0.05$.

The unchanged and upward trends of ACE inhibition and α-amylase/cytotoxicity inhibition activities in the bioaccessible portions of fermented DPR propose a quantitative conservation and/or development in the bioactive compounds responsible for these features throughout the digestion process. The strain *S. cerevisiae* had a significantly higher α-amylase inhibition than *P. kudriavzevii*. The relatively high inhibitory effect of α-amylase in our study is in accordance with that found by Barros et al. [77] for a bioaccessible portion of fermented soursop residue by multiple bacterial probiotics.

In contrast, downward trends in the examined nutraceutical properties (OPA, TPC, α-glucosidase inhibition, and antioxidant activities) were observed at various levels in the bioaccessible portion. These reductions could be mainly attributed to exposure to digestive conditions with a changed pH and digestive enzymes, resulting in the degradation of bioactive compounds [78]. Moreover, during digestion, phenolic compounds are generally sensitive to interactions with specific dietary constituents such as minerals, dietary fiber, and macromolecules [79,80]. Thus, the physicochemical and structural properties of nutrients can be altered through these interactions, affecting their bioaccessibility [81]. In this context, the antioxidant capacities may decrease in the bioaccessible portion due to the conversion of antioxidant phenolic compounds into different compounds with new chemical properties [82]. Another potential explanation is that during digestion, the polyphenols that are linked to health-promoting benefits usually bind to the food matrix [83]. Consequently, the high amount of insoluble phenolic compounds present in the bound fiber of date pulp residues might not be fully digested, as was observed in this trial.

Likewise, in line with our results, Andrade Barreto et al. [84] reported that the antioxidant capacities of fermented orange juice by *S. cerevisiae* were preserved in reasonable percentages in the bioaccessible portion. On the contrary, the results obtained by Djaoudene, Mansinhos [85] showed a significant enhancement in TPC and antioxidant capacities for Algerian date extracts after being subjected to in vitro digestion. However, the differences between our findings and others could be ascribed to the differences in the metabolic activities during microbial fermentation [86], which definitely varies according to the microorganism used. In addition, the specificity of the screened food matrix plays a role in obtaining different results in terms of its content of bioactive compounds and the structural alterations that take place on those compounds during digestion [80].

3.4. Untargeted and Carbohydrates Metabolomics Analyses

3.4.1. Untargeted Metabolite Analysis

The untargeted metabolomics methodology permits the investigation and classification of a wide spectrum of metabolites in a sample, irrespective of prior knowledge of their identities. This approach offers valuable knowledge concerning the chemical composition of fermented and unfermented DPR and their possible impacts on human health.

Due to the relatively superior antioxidant capacities of the *P. kudriavzevii* sample compared to the *S. cerevisiae* sample, it was selected, along with the nonfermented DPR (control), to conduct this part of the study. Figure 5A,B exhibit the outcomes of PCA and heatmap analyses, respectively, of metabolites differentially present in fermented DPR by *P. kudriavzevii* and the control in the bioaccessible portions of the sixth day of fermentation. The PCA analysis (Figure 5A) displays the observed dissimilarities between the nonfermented DPR (control) and *P. kudriavzevii* samples, indicating the differences in metabolites between these two groups. PC1 and PC2 represented 91.78% and 5.38% of the total variations, respectively. Furthermore, the unsupervised hierarchical clustering method shows that the three replicates of each experimental treatment formed a separate cluster, with the *P. kudriavzevii* sample forming a cluster distinct from the control sample (Figure 5B). Figure 5B illustrates the bioaccessible metabolites grouped into two significant clusters, G1 and G2, reflecting the differences in metabolite concentrations between these groups. Notably, metabolites in G1 and G2 showed a significant variation in *P. kudriavzevii* sample compared to the control. Additionally, the volcano plot (Figure S3) depicts the differential metabolites classified as upregulated, downregulated, and nonsignificant. According to Figure S3, in the fermented DPR by *P. kudriavzevii*, 165 metabolites were upregulated, 244 were downregulated, and 918 showed no significant differences compared to the control.

In our study, the results of the PCA (Figure 5A) reveal significant differences in the metabolite profiles of the *P. kudriavzevii*-fermented sample compared to the control group. Furthermore, the heatmap in Figure 5B provides further support for this observation by highlighting disparities in both the types and levels of metabolites between the two samples. These results suggest that during DPR fermentation, the biological actions of *P. kudriavzevii* may have led to the synthesis of novel bioactive compounds or the enhancement of present ones. Specifically, the *P. kudriavzevii* sample showed an increase in the concentrations of metabolites in the G2 group, while those in the G1 group decreased compared to the control. Therefore, it is likely that *P. kudriavzevii* metabolized some of the compounds in the G1 group while simultaneously increasing the production of those in the G2 group during DPR fermentation.

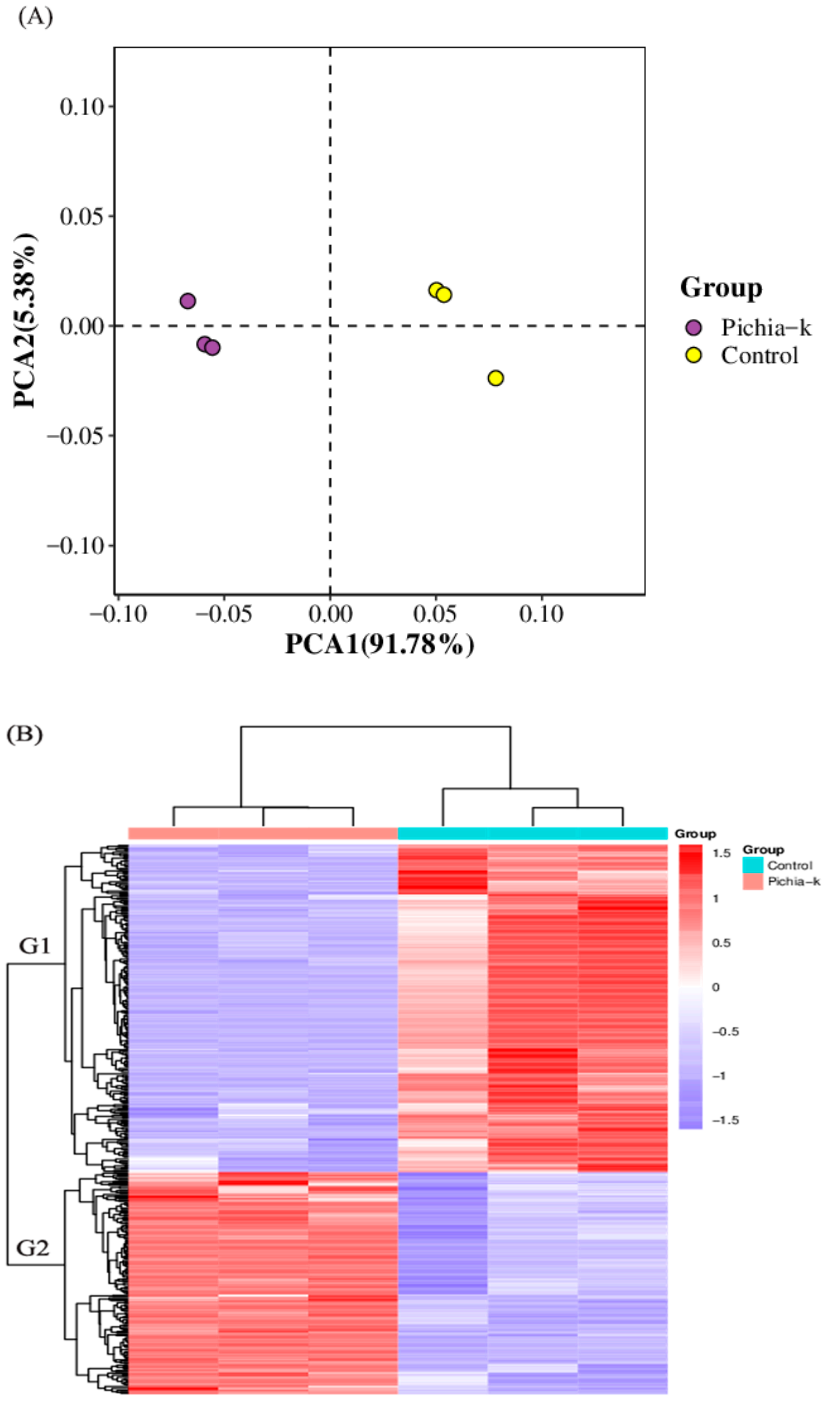

Figure 5. PCA scores (**A**) compare *P. kudriavzevii* with control, and heatmap (**B**) illustrates the metabolite concentration. The colors on the heatmap range from violet to red, and their specific color references are explained in the online version of the article. The cluster analysis in panel (**B**) presents the expression patterns of the differential metabolites in the two sample groups. Each row in the heatmap denotes a differential metabolite, whereas each column indicates a sample. The color scale spans from violet to red, where violet represents a low expression level, and red reflects a high expression level.

3.4.2. Metabolic Pathway Analysis

By performing metabolic pathway enrichment analysis on differential metabolites using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database, significant al-

terations in metabolic pathways can be identified, contributing to the interpretation of biological phenotypes.

In our paper, metabolic pathways with a $p < 0.05$ were regarded as highly enriched with different metabolites. To showcase the top 10 enriched metabolic pathways, the study employed bubble charts, as seen in Figure 6, which revealed the smallest *p*-values. It is noteworthy that the C5-branched dibasic acid metabolizing pathway had a significant increase in differential metabolites, with four metabolites identified in this pathway, including itaconate, L-glutamic acid, oxoglutaric acid, and (+/−)-2-hydroxyglutaric acid (Table S2). Bambouskova et al. [87] and Mills et al. [88] described the anti-immune and anti-inflammatory mechanisms of itaconate. L-glutamic acid, a nonessential amino acid, exhibits antioxidant activity [89], while oxoglutaric acid has the ability to boost the production of collagen and may have an impact on processes related to aging [90]. Seven metabolites, such as L-tryptophan, indole-3-acetic acid, and skatole, were detected in the tryptophan metabolizing pathway. Generally, the results indicated that fermentation had a significant impact on the amino acids.

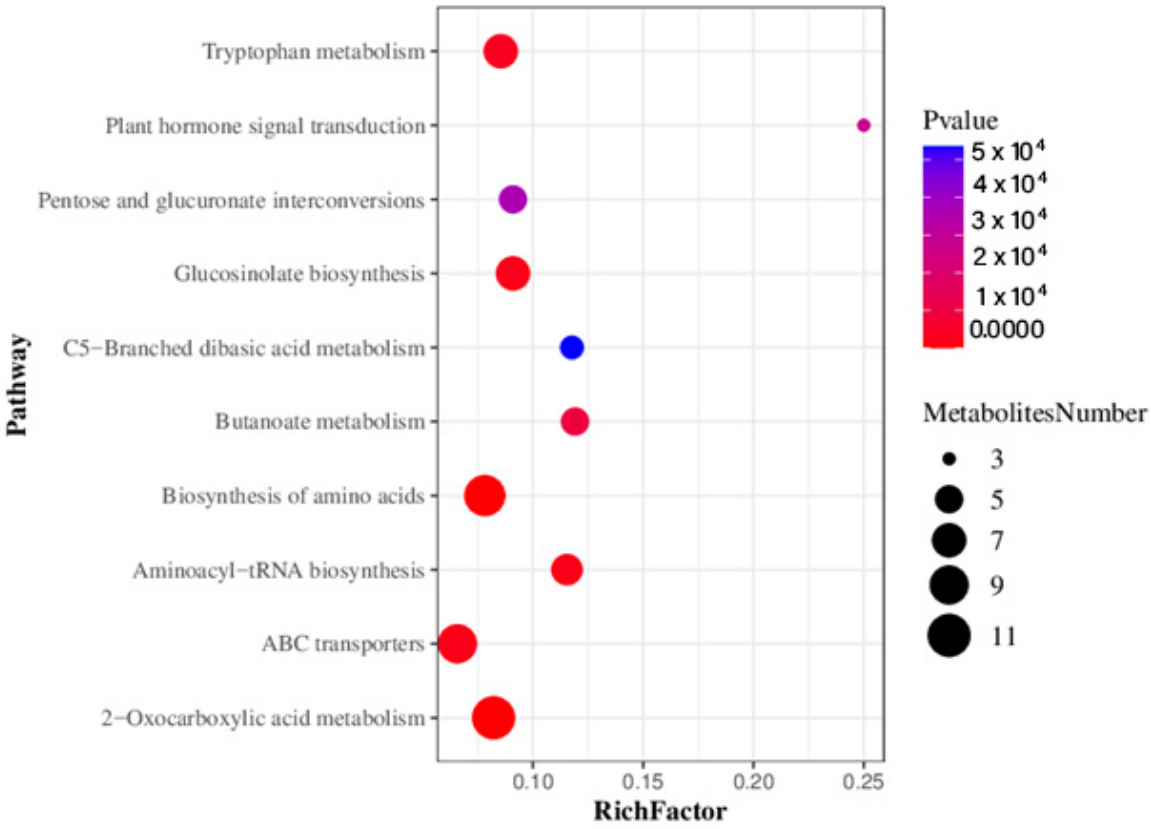

Figure 6. The *x*-axis shows the RichFactor, representing the proportion of differential metabolites in a pathway relative to all annotated metabolites in the same pathway. The dot size reflects the number of differential metabolites annotated within the pathway. The *y*-axis represents the name of the KEGG metabolic pathway. A higher RichFactor value indicates a higher ratio of annotated differential metabolites in the pathway. The size and color of the dots reflect the number and level of annotated differential metabolites within the pathway.

3.4.3. Carbohydrate Metabolites Analysis

Figure 7 presents the analysis of carbohydrate metabolism (Figure 7A) and the identification of pathways correlated with differentially regulated metabolites using the KEGG database (Figure 7B). In Figure 7A, the differentially regulated metabolites between the *P. kudriavzevii* sample and the control were classified into lipids, compounds with biological roles, phytochemical compounds, and others that remained uncategorized. The most signifi-

cant categories of differential metabolites were lipids (8), followed by amino acids, peptides, and analogues (7), and terpenoids (3). Figure 7B displays the major metabolic pathways of carbohydrate identified through the KEGG database analysis, which include global and overview maps (12), amino acid metabolism (5), lipid metabolism (5), biosynthesis of other secondary metabolites (3), and membrane transport (3).

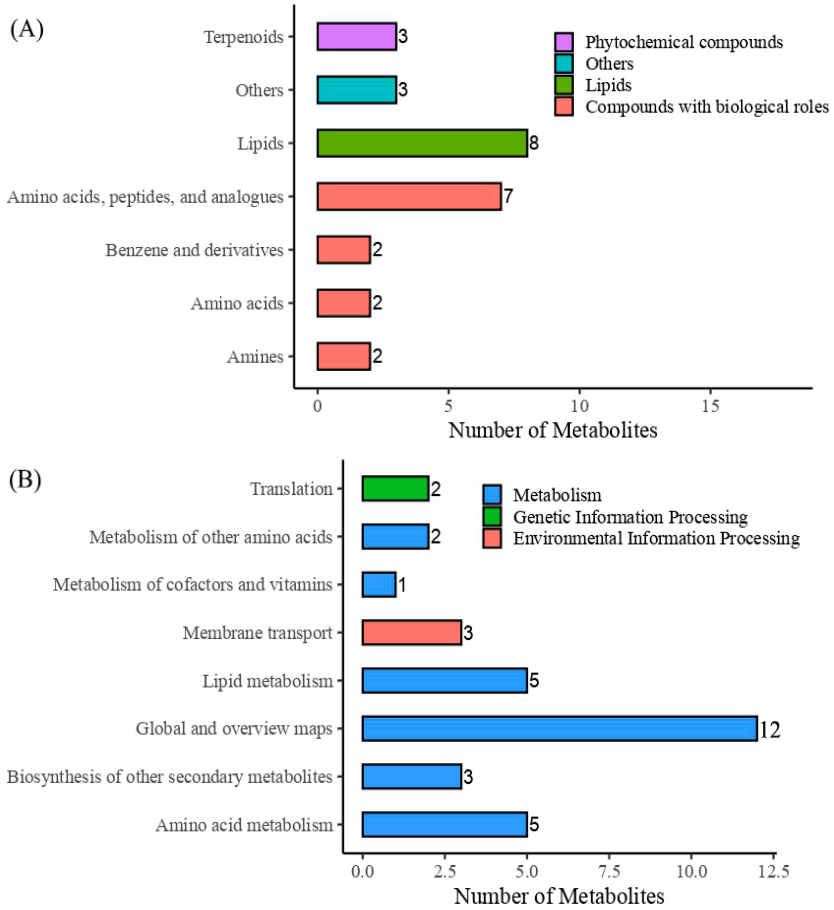

Figure 7. Identification of differential carbohydrate metabolites (**A**) and analysis of their KEGG pathway (**B**) in the bioaccessible portions of the *P. kudriavzevii* sample and the control.

The findings obtained from Figure 7A indicate that the bioaccessible portions contained a diverse range of compounds, including amino acids, benzene and derivatives, amines, and peptides, all of which possess biological activities. Furthermore, the LC-QTOF analysis of bioaccessible portions revealed the presence of three metabolites, including tutinolide, triterpenoids, and monoterpenoids, that belong to the terpenoid class of phytochemical compounds. Terpenoids have been extensively studied and found to have significant effects on human health [91]. For example, monoterpenoids exhibit antibacterial properties that can disrupt microbial multiplication and development, as well as interfere with their physiological and metabolic processes [92]. On the other hand, triterpenoids have been shown to possess a variety of biological properties such as antiulcer, anti-inflammatory, antibacterial, hepatoprotective, antiviral, antiatherosclerotic, immunomodulatory, and cholesterol-lowering effects [93,94]. Overall, the majority of metabolomics findings suggested that fermentation significantly enhanced the functions of DPR.

4. Conclusions

Fermentation of date pulp residue (DPR) with yeast isolates enhanced its health-promoting properties by improving its chemical composition and maximizing its potential effects. The yeast strains used in the fermentation process efficiently converted the sugars

in DPR, resulting in elevated levels of lactic acid and acetic acid in the fermented DPR. *S. cerevisiae* produced more alcoholic compounds, while *P. kudriavzevii* resulted in higher levels of ester compounds, highlighting the importance of selecting the appropriate fermentation agent to manipulate the chemical composition of volatile compounds in DPR. The content of phenols increased in DPR samples following fermentation, especially gallic acid, tyrosol, syringic acid, and (-)-epicatechin. This increase may have positive impacts on the potential health-promoting attributes of FDPR compared to nonfermented DPR. Evaluating the bioaccessibility of fermented DP is crucial for ensuring its usability in metabolism, and assessing the health-promoting properties of the bioaccessible portion is necessary to fully understand the potential benefits of DPR. Fermentation of DPR using yeast species can enhance the health benefits of DPR, such as amylase inhibition and cytotoxicity. Moreover, the FDPR (undigested samples and bioaccessible portions) exhibited higher antioxidant activities compared to the control, providing more opportunities to utilize date byproducts. The findings of the untargeted metabolomics analysis suggest that fermentation has a significant impact on the C5-branched dibasic acid metabolizing pathway, while the analysis of carbohydrate metabolites revealed the presence of three compounds belonging to the terpenoid class of phytochemicals: tutinolide, triterpenoids, and monoterpenoids. These findings indicate that fermentation can enhance the functions of DPR.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation9060561/s1. Figure S1. Yeast strains proliferation (A), pH (B) and titratable acidity (C) of the non-fermented and fermented date pomace by *S. cerevisiae* and *P. kudriavzevii* during at 25 °C for 6 days. Figure S2. Total phenolic compounds (A) and OPA absorbances (B) of undigested samples and bioaccessible portion of non-fermented (control) and fermented date pomace by *S. cerevisiae* and *P. kudriavzevii*. Figure S3. A volcano map displays the differential metabolites between non-fermented and fermented date palm pomace by *P. kudriavzevii.* Table S1. Relative peak area (RPA%) and peak areas ($\times 10^5$) of main volatile compounds in non-fermented (control) and fermented date pomace by *S. cerevisiae* and *P. kudriavzevii*. Table S2. Top 10 enriched metabolic pathways with identified metabolites.

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