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Research article

Valorisation of fresh waste grape through fermentation with different exogenous probiotic inoculants

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ABSTRACT

The disposal of fresh waste grape berries restraining the sustainable development of vineyards. The aims of this study were to evaluate the effects of different exogenous probiotic inoculants on the fermentation of fresh waste grape berries. In the fermentation process, the variations of pH and EC value, chemical characteristics of the fermentation products, as well as the microbial communities' composition were simultaneously observed. In addition, the feasibility of using the fermentation products as chemical fertilizer substitute in agricultural production also has been verified in this study. The results indicated that the different probiotic inoculants has shown clear impacts on the variation trends of pH and EC value in the grape waste fermentation. *Lactobacillus casei* and *Zygosaccharomyces rouxii* are ideal probiotics for the fermentation of waste grape, which enhanced the contents of free Aa and other nutrients in fermentation products. Compared with Fn treatment (without exogenous inoculants), the total free Aa contents in Fs (inoculation with *Z. rouxii*) and Fm (inoculation with *L. casei* and *Z. rouxii* mixture) treatments have improved by 199.1% and 325.5%, respectively. The microbial communities' composition during the fermentation process also been greatly influenced by the different inoculants. At the genus level, *Lactobacillus* and *Pseudomonas* were the dominant bacteria, while *Saccharomyces* and *Candida* were the dominant fungi in the fermentation. Using the fermentation products as chemical fertilizer substitute has enhanced the quality of *Kyoho* grape. Compared with traditional chemical fertilization treatment (T1), application with fermented grape waste (T2) has significantly improved VC and soluble solid contents in grape berries by 16.89% and 20.12%, respectively. In conclusion, fermentation with suitable probiotics was an efficient approach for the disposal and recycling of fresh waste grape in vineyards.

1. Introduction

Fruit and vegetable waste (FVW) is routinely generated in large quantities in the processes of cultivation, harvesting,

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transportation, storage, marketing and processing. It is reported that China has generated approximately 1.3 million tons FVW annually, but less than 20% of such wastes were properly treated or reused [1]. Most of these wastes is usually discarded randomly, used as animal feedstock [2], transported to landfill site and incineration plant, or disposed of in the ocean [3]. In these processes, a series of environmental issues have been caused, including foul and toxic gas, contaminations to water, soil and air bodies, threats to food safety and human health [4]. Therefore, the scientific disposal and recycle of FVW has attracted increasing interest in recent decades. FVW is generally rich in fermentable sugars, cellulose, hemicellulose and abundant nutrient elements, suggesting that FVW is an excellent recyclable resources [5]. The reutilization of FVW could promote a set of benefits by reducing waste discharge and increasing the economic gains.

Compared with vegetable wastes, fruits wastes are usually contains higher saccharides and suitable to develop many value-added products. For example, fruit waste was an ideal raw material for the production of 2,3-butanediol [6]. Pectinases could be produced from fruit wastes under low temperature condition by *Saccharomyces cerevisiae* [7]. Banana waste has great potential to produce ethanol with a low-cost and sustainable production method [8]. Grape waste, including grape pomace from wine making process and rotten fruit from vineyards are both highly valuable recyclable resources [9], which led to a global research drive on grape waste to extract valuable components from them or turned these wastes into useful resources [10]. For example, grape pomace is an ideal raw material for the isolation of bio-based succinic acid [11]. Grape pomace is also used for the production of biochar, which could further adopt for the removal or absorption of heavy metal elements and pesticides [12,13]. In addition, the mix fermentation of grape and apple wastes with fungal was an effective way for the mobilization of phenolic antioxidants [14].

In the fermentation process of grape waste, the exogenously added probiotics play a vital role in determining the substances composition of the products, releasing of available nutrients, increasing of target products' yield, as well as shorten the fermentation period [15]. Previous researches indicated that the co-inoculation of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* reduced the fermentation time and improves the bacterial diversity in the fermentation of grape pomace [16]. Grape pomace fermentation with *Ulocladium botrytis* reduced the phytotoxic monoaromatic compounds in the fermented products, thereby enhanced their potential use as organic amendments in agricultural soils [17]. Therefore, the selection of probiotics in the fermentation process is also very important for the recycling of grape waste.

Grape is the major fruit cultivated in Shanghai suburb with planting area of 5267 ha, which accounted for approximately 25% of the total fruit trees planting area in Shanghai [18]. Rotten grape is generally generated in the production and marketing process through multiple sources. Some berry will be abandoned before sale because fail to meet the marketing standards. Dehiscent fruit usually appeared due to the influences of rainy weather condition, or pests and diseases. In addition, fruit thinning will be performed in the green-berry stage and veraison stage to ensure good berry quality and maturity [19]. Based on our investigation, the amount of waste grape can reach up to 30% of the total grape production in some vineyards in Shanghai suburb. In consequence, seeking a feasible approach for the recycling of theses waste grape is an urgent issue for the clean and efficient production of grape in Shanghai. If these waste grapes are directly discarded in the vineyard, it will not only the waste of agricultural resources, but also easily breed diseases and insect pests, as well as other environmental issues.

However, few studies have reported the fermentation and recycling of the waste grape generated in vineyards. The variation of exogenously probiotics' community composition during the fermentation process still remains unclear at present. Hence, the objective of the present study was to: 1) evaluate the effects of different inoculated probiotics on the fermentation of waste grape; 2) analysis the probiotics' variations and chemical composition of the fermented products; 3) explore the application value of fermented products in agricultural soils. This study tried to provides a feasible way for the recycling of waste grape from vineyards.

2. Materials and methods

2.1. Grape raw materials

The waste grape used in this study was provide by the Shanghai Malu Grape Theme Park, which located in the Jiading District (31°38′ N, 121°32′ E), the main grape production area in Shanghai suburb. Before fermentation experiments, 50 kg waste grape was collected from Shanghai Malu Grape Theme Park. The waste grape was washed with water and cleaned up the residue branches, sundries and other components that difficult to decomposition. Then, the cleaned waste grape crushed by hand to separate the skin and pulp to make sure it could more easily be decomposed during fermentation process.

2.2. Microorganisms and culture conditions

In this study, three probiotic agents, including an Enzymes Bacteria (EB), a *Lactobacillus casei* (BNCC134415) and a *Zygosaccharomyces rouxii* (CGMCC16260) were used for the fermentation of waste grape. The EB is a commercial agent and purchased from the Jiangsu New-bio Biotechnology Corporation (Jiangsu, China). According to the products description, the EB is a powder mixture of *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus paracei*, which is usually used for the fermentation of fresh fruits and vegetables for drinkable products. The L. *casei* strain was purchased in lyophilized from the Beina Biotechnology Institute (Beijing, China). The *Z. rouxii* strain was isolated from the stacking site of waste grape in the Malu Grape Theme Park. In the experiments, the EB agent was added directly into the waste grape according to the instructions and dosage for fermentation. The lyophilized cells of *L. casei* and *Z. rouxii* were inoculated into 100 mL MRS broth and YPD broth, respectively, and incubated at 37 °C for 18 h in an anaerobic incubator. Then, the cultures were subcultured again at 37 °C for 18 h under anaerobic conditions prior to use.

2.3. Experimental design and fermentation process

Four treatments with three duplicates were designed for the fermentation experiments of waste grape, including: 1) natural fermentation without exogenously inoculum (Fn); 2) fermentation with *Z. rouxii* inoculum (Fs); 3) fermentation with EB agent inoculum (Fe); 4) fermentation with a mixture of *L. casei* and *Z. rouxii* inoculums (Fm). The fermentation experiments were conducted in a 6 L plastic drum. The lid of the plastic drum is equipped with a one-way exhaust valve to ensure that the gas produced during the fermentation process can be discharged smoothly without bursting. First, put 1.5 kg waste grape into the plastic drum. Then 0.5 kg of brown sugar was added as the carbon source of microorganisms. Third, add water into the plastic drum to 5 L and adjusted pH to 4.50. Finally, exogenously probiotics are inoculated into the drum and then stirred evenly. The EB agent was added into the drum directly based on the recommended dose. Fs treatment was inoculated with 200 mL cultures of *Z. rouxii*. Fm treatment was inoculated with a mixture of 100 mL *Z. rouxii* and 100 mL L. *casei* cultures. During the first week, the lid of the drum will be opened every day and stirred evenly to promote venting. After that, open the lid and stir once a week until the fermentation finished.

2.4. Chemical analysis of fermentation products

The pH and electrical conductivity (EC) value was measured every week during the whole fermentation period. The pH value was measured with the pH meter (METTLER TOLEDO) and EC value was measured with the EC meter (METTLER TOLEDO). The contents of free amino acids (Aa) in the fermented products were determined by the High Speed Amino Acid Analyzer (Hitachi, Japan) [20]. The contents of main ions in the fermentation products were determined by the Ion Chromatograph (Thermo Fisher, Germany) [21]. The content of available N in the fermentation products was determined by Alkaline Hydrolysis Diffusion method [22]. The content of total phenol was determined by Folin-Ciocalteu colorimetric method [23] and the content of total acids were determined by Acid-base Titration method.

2.5. Illumina sequencing and data processing

During the waste grape fermentation process, 20 mL of fermentation products for each drum were sampled in the 1st, 3rd, 5th and 10th weeks for microorganisms' analysis. The microbial DNA was extracted from these samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.) according to manufacturer's protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV–vis spectrophotometer (Thermo Scientific, Wilmington, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3–V4 region of the bacteria 16S rRNA gene was amplified with primers 338F (5′-ACTCCTACGG-GAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) and the ITS1 region of fungus was amplified with primers ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) by thermocycler PCR system (GeneAmp 9700, ABI, USA).

Purified amplicons were pooled in equimolar and paired-end sequenced (2×300) on an Illumina MiSeq platform (Illumina, San Diego,USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China). Raw fastq files were demultiplexed, quality-filtered by Trimmomatic and merged by FLASH with the following criteria: i) The reads were truncated at any site receiving an average quality score <20 over a 50 bp sliding window. ii) Primers were exactly matched allowing 2 nucleotide mismatching, and reads containing ambiguous bases were removed. iii) Sequences whose overlap longer than 10 bp were merged according to their overlap sequence. Operational taxonomic units (OTUs) were clustered with 97% similarity cutoff using UPARSE (version 7.1 http://drive5.com/uparse/) and chimeric sequences were identified and removed using UCHIME. The taxonomy of 16S rRNA gene sequences and ITS gene sequences were analyzed by RDP Classifier algorithm (http://rdp.cme.msu.edu/) against the Silva 16S rRNA database (v138) and ITS_fungi database (unite 8.0), respectively, using confidence threshold of 70%.

2.6. Fertilization experiments

In order to validate the fertilization effect of fermented product in agricultural production, a field experiment was conducted in a typical vineyard (*Kyoho*) of Shanghai suburb in the next grape season. Two treatments were designed: T1) managed with the traditional fertilization method in local vineyard; T2) reduce the traditional chemical fertilizer rate by 25%, then applied with fermented product of waste grape. The fermented product of Fm treatment was selected for the fertilization experiment. For the using of the fermentation products, the solid residue of waste grape was buried into the soil to 20 cm depth as basal fertilizer in March (applied as organic fertilizer). The fermentation broth was applied twice a month from April to August. For each application, 250 mL of the original fermentation broth was diluted 20 times to 5 L and then applied around the roots of grapevines with a radius of 1.0 m. Meanwhile, 100 mL of the original fermentation broth was diluted 100 times to 10 L and then used as foliar applications with a power spraye. During the whole grape season, each grapevine received 10 roots' applications and 10 foliar applications, which means each grapevine received 3.5 L original fermentation broth. After harvested, the yield and quality of *Kyoho* grape were evaluated.

2.7. Data analysis and graphic work

Data analysis was performed with Microsoft Excel 2010 (Microsoft Corporation, USA). Graphic work was conducted with Origin 9.0 (Origin Lab Corporation, USA). The significant difference tests among treatments were conducted with one-way ANOVA at a significance level of 0.05 (IBM SPSS Statistics, USA).

3. Results and discussion

3.1. pH and EC dynamics during fermentation process

The initial pH was artificially adjusted to 4.50 before fermentation. In the first week, the pH was dropped sharply to 3.60–3.73 for all treatments (Fig. 1a). Then, the pH of Fn that without microbial inoculum stabilized in approximately 3.75 until the 10th week. The pH of Fs and Fm showed slightly increasing and observed with 3.95 and 3.88, respectively, in the 10th week. In contrast, the pH of Fe was continuously decreased after the 1st week and maintained in 3.40 in the 10th week. The variations of pH during the fermentation process of waste grape showed different patterns across different microbial inoculants. Previous studies have demonstrated that pH is a vital factor that affecting the fermentation of FVW [1,24]. In order to eliminate the influence of pH on the fermentation process, the initial pH for all treatments were adjusted to a same level. However, the pH variations displayed different patterns with different microbial inoculants. The fermentation of FVW is a typical acidification process. The great drop of pH in the first week may be caused by the decomposition of organic acids into small molecular acids under the high activities of the inoculated microorganisms. This pH variation pattern also supported by previous studies, which have reported the great drop of pH in the first week of FVW fermentation [25]. In addition, the pH also has been validated to be a crucial factor in maintaining metabolic pathways and microbial communities in an optimal domain for the production of the target substance [26].

The variation pattern of EC value during the fermentation process was different from the pH dynamics. In the first two weeks, the EC value of all increased linearly, then decreased sharply to a stable level in the third week and then remind stable until the 10th week (Fig. 1b). Across the treatments with different inoculants, the highest EC value was observed in Fm and varied from 4.78 to 3.49 with a peak of 5.43, while the lowest EC value was appeared in Fn and varied from 2.88 to 3.63 with a peak of 4.44. The changes of EC value in the fermentation process of FVW may related to many factors, such as ion concentrations, reducing sugars, pH and amino acids concentrations [27]. However, the deeply mechanisms of EC variations during FVW fermentation needs further study.

3.2. Free amino acids contents in fermentation products

The richness of soluble substances in the raw materials of FVW can be metabolized by probiotics to free amino acids [15]. As shown in Fig. 2, 16 kinds of free Aa profiles were measured after fermentation. The results indicated that the different inoculated microorganisms showed clear impacts on free Aa contents in the fermentation products of waste grape. For example, the Fs and Fm treatments significantly increased the contents of Lys, g-ABA, Phe, Tyr, Leu, Ile, Val, Ala, Gly, Thr and Asp, compared with Fn treatment ($P < 0.05$). The Fe treatment also clearly increased the free Aa contents of Orn, Phe, Tyr, Leu and Glu ($P < 0.05$), compared with Fn treatment. In general, the total free Aa contents in Fe, Fs and Fm treatments have significantly increased by 49.1%, 199.1% and 325.5%, respectively, compared with Fn treatment. Furthermore, Ala and Asp were the richest free Aa among the all detected free Aa and showed greatly variations on the contents across different treatments. In contrast, Cys was the poorest free Aa on the contents but showed no obvious differences across the different inoculations.

The results indicated that inoculation of suitable microorganisms, such as *L. casei* and *Z. rouxii,* was beneficial to improve the free Aa contents in the fermentation products of waste grape [28]. has reported that the probiotics equipped with enzyme systems for using and transferring Aa during their metabolic activities and changed the types and contents of free Aa during the fermentation process. The increasing of free Aa contents in the fermentation products of waste grape also greatly enhanced its' application value in agricultural production. For a long time in the past, it was generally believed that amino acids could not directly absorbed by plants and needs to be converted into simple inorganic nitrogen through mineralization. However, more and more experiments showed that the absorption of amino acids as a nutrient nitrogen source by plants accounts for a considerable proportion of the total nitrogen source utilization in most soil systems, and amino acids play an important role in the soil system with relatively scarce nitrogen sources [29–31]. With subsequent research, it has been found that amino acids not only be used as organic nitrogen sources for plants, but also

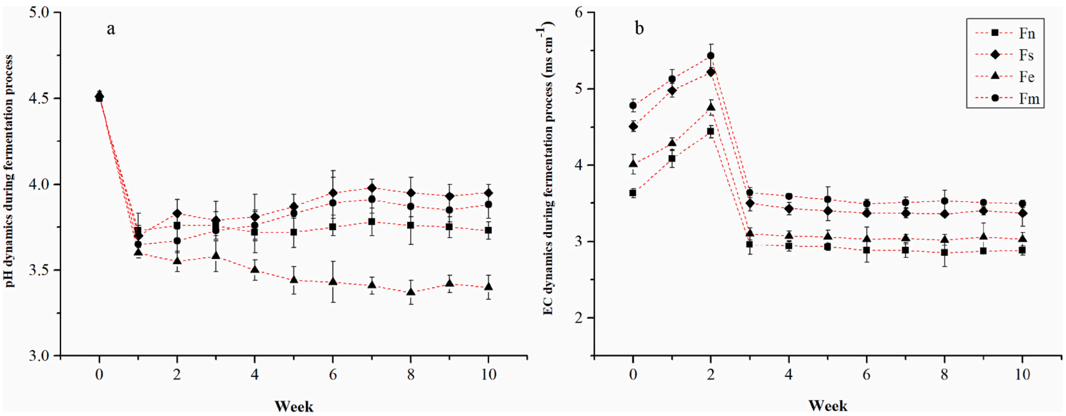

Fig. 1. pH (a) and EC (b) dynamics during waste grape fermentation process with different microbial inoculants.

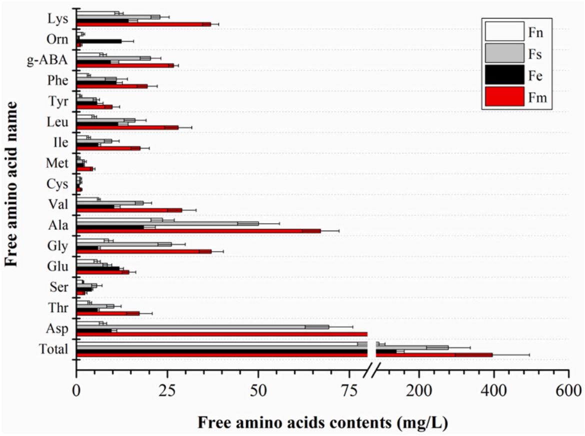

Fig. 2. Free amino acids contents in waste grape fermentation products with different microbial inoculants.

have multiple functions such as participating in protein synthesis, regulating and promoting plant growth.

3.3. Ions content in fermentation products

The ions contents in fermentation products are important sources of nutrients when use the fermentation products of waste grape as fertilizer. The kation and anion profile with different inoculations were showed in Fig. 3a and b, respectively. Under different inoculation conditions, some ions contents varied greatly across the treatments. The contents of NH_4^+, Na^+ and F^- were significantly increased under inoculation conditions when compared with Fn ($P < 0.05$). However, no clear differences were observed for the contents of Ca^{2+}, Mg^{2+}, K^+, PO_4^{3-}, NO_3^-, SO_4^{2-} and Cl^- across the treatments. The NH_4^+ was the lowest abundant and K^+ was the highest abundant among the 10 detected ions in the fermentation products. In the sum of all ions, the Fs, Fe and Fm treatments has improved by 7.33%, 10.95% and 13.50% compared with Fn treatment. The results of ion profile indicated that exogenous inoculations have improved the total ions contents in the fermentation products of waste grape, which may probably enhanced the application value of fermented waste grape. Inorganic nitrogen, such as NH_4^+ and NO_3^- were important nitrogen source for plant growth [32]. K^+ and Mg^{2+} have been proved to play important roles in the formation of fruit flavor and enhancement of fruit qualities [33,34].

3.4. Nitrogen, total phenol and total acids contents

After fermentation, the total available nitrogen (AN) content showed no significant differences across the four treatments (Fig. 4), even though the lowest content of 96.25 mg/L was detected in Fe treatment. This result was consistent with that of ions profile results, which displayed close NO_3^- content across the different treatments. The AN was an important component in the fermented products of waste grape, which was more readily absorbed by plants than organic N [35]. High contents of total phenol (424.92–509.17 mg/L) were detected in the fermented products of waste grape. Previous study has pointed out that the exogenous probiotics, such as *Lactobacillus plantarum*, could enhance the content of total phenol in the fermentation of fruits [36]. However, no significant

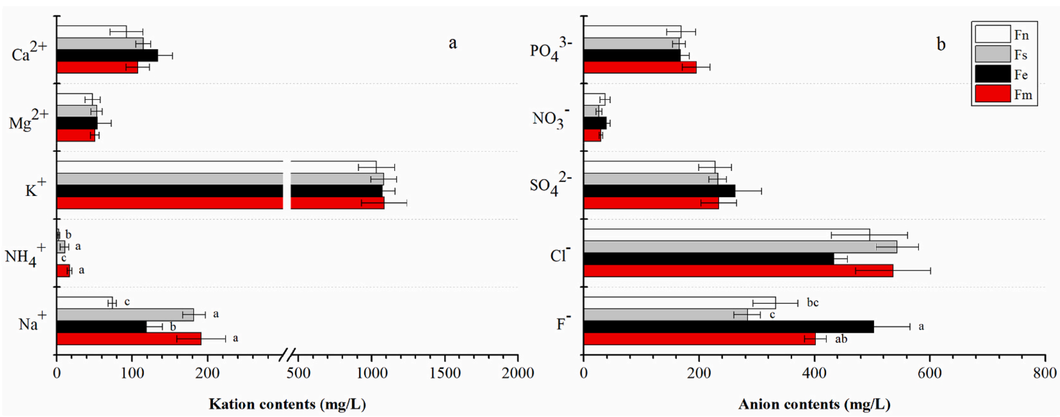

Fig. 3. Kation (a) and anion (b) contents in waste grape fermentation products with different microbial inoculants (different letters indicates significant differences at $P < 0.05$ level).

differences were observed in this study among the different exogenous probiotics. In addition, the total acids were measured after fermentation of waste grape. The highest total acids of 189.27 mg/L was detected in Fe treatment, and showed significant higher than the other treatments ($P < 0.05$). The relatively higher total acids content in Fe treatment was agree with the lower pH variations during the fermentation process. In general, the different exogenous inoculums showed no significant effects on the contents of AN and TP in the fermented products of waste grape, but the addition of EB was significant increased the total acids content after fermentation.

3.5. Microbial variations and the impacts on fermentation process

The dynamic variations of microbial communities during the fermentation process were analyzed with high-throughput sequencing technology. During the whole fermentation period, we selected four time points (T1-the 1st week, T2-the 3rd week, T3-the 5th week and T4-the 10th week) to investigate the effects of different inoculums on bacteria and fungi communities' composition in the waste grape leavening. After removing low-quality sequences and chimeras, the total effective sequences of 789626 reads for bacteria and 536652 reads for fungi were obtained, respectively.

As shown in Fig. 5a, the bacteria community composition has greatly changed by exogenous inoculation of probiotics. For Fn treatment without exogenous inoculum, *Lactobacillus* was the most abundant genus and increased from 25.99% at T1 stage to 82.15% at T4 stage. When *Saccharomyces* was inoculated in Fs treatment, the proportion of *Lactobacillus* was depressed (6.23%) at T1 stage, however, it increased sharply at the following 3 stages and becoming the most abundant genus (58.75%–85.94%). These results indicated that the inoculation of fungi, such as *Saccharomyces*, has clear effects on the composition of bacterial communities. Perhaps the competition between *Saccharomyces* and *Lactobacillus* for fermentation substrate inhibited the activity of *Lactobacillus* at the T1 stage. In the Fm treatment with mixture inoculation (*Lactobacillus* and *Saccharomyces*), the abundance of *Lactobacillus* was improved to 79.19% at T1 stage. The mixed inoculation may effectively enhance the activity of *Lactobacillus* at the beginning of fermentation, thus promoting the decomposition of waste grape. Meanwhile, *Lactobacillus* continually maintained the most abundant genus from T1 to T3 stage (69.72%–79.19%), and then rapidly decreased to 9.74% at T4 stage. It is interesting to note that the bacteria communities' composition of Fm at T4 stage showed the highest diversity across the all treatments and fermentation period. The higher *Lactobacillus* abundance and bacteria community diversity in Fm treatment may contribute to the higher free Aa contents in the fermentation products of waste grape. In contrast, the bacteria communities' composition in Fe treatment during the fermentation process was quite different with other 3 treatments. The *Fructobacillus* (64.33%) was the dominant genus at T1 stage, and the *Pseudomonas* (67.94%–95.08%) was the dominant genus at T2 and T3 stages. The *Lactobacillus* plays the most abundant genus only at T4 stage (57.48%). This great difference of bacteria communities' composition may lead to the differentiation of substances composition in fermented product of Fe treatment. In general, *Lactobacillus* was the dominant genera during the fermentation process of waste grape, excepting for that in Fe treatment. This result was consistent with previous research that fermented with FVW [37]. The benefits of mixed inoculation in the maintaining of high activity of *Lactobacillus* during the fermentation process also have been validated by these researches. Previous publication also reported the advantage of Co-inoculation in the reduction of fermentation time [16]. In summary, the exogenous probiotics and the suitable inoculum play a very important role in the disposal and recycling of FVW.

The dynamic variation of fungi during the fermentation process was shown in Fig. 5b. Only in Fs treatment that with *Saccharomyces* inoculation was fungi detected at all 4 stages. *Saccharomyces* and *Candida* were the dominant genera in Fs treatment, and when *Saccharomyces* decreased from 87.96% to 6.65% at T1 to T4 stage, the abundance of *Candida* increased from 7.61% to 88.61%. The Competition among microorganisms may cause changes in microbial community. In Fe treatment, fungi were detected only at T1 stage. It is possible that the inoculation of EB agent inhibited the activity of the native fungi in the later fermentation stages. In Fn treatment, the T3 stage was detected the highest diversity of fungi communities across all treatments. This means that even without the

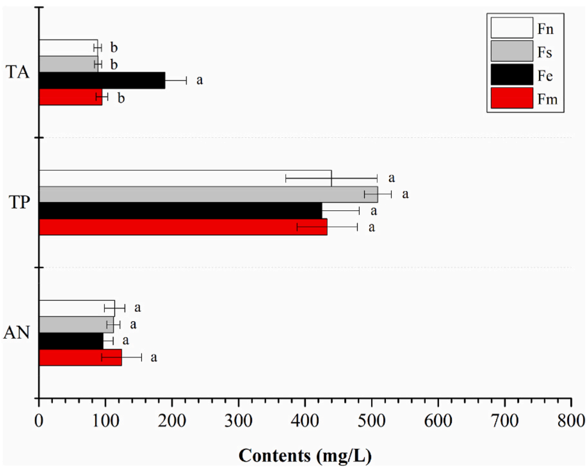

Fig. 4. Available nitrogen (AN), total phenols (TP) and total acids (TA) contents in waste grape fermentation products with different microbial inoculants (different letters indicates significant differences at $P < 0.05$ level).

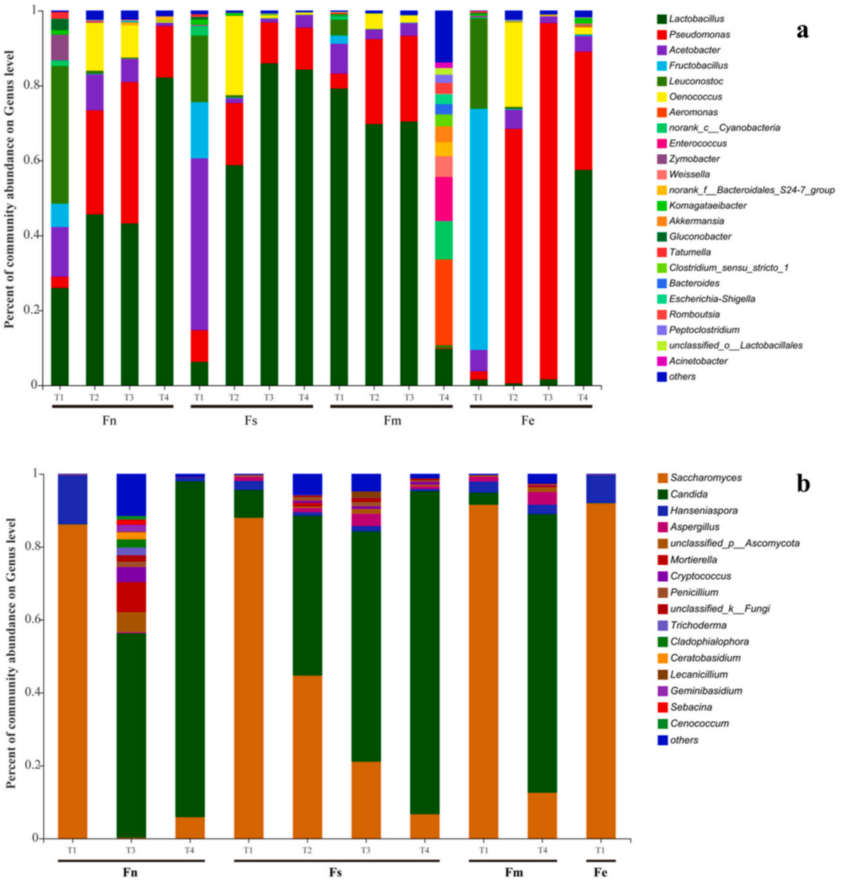

Fig. 5. Bacteria (a) and fungi (b) community composition on genus level with different inoculants in different fermentation periods.

inoculation of exogenous microorganisms, the native microorganisms can also contribute to the fermentation of waste grape. In the early stage of fermentation, *Saccharomyces* reproduces rapidly for all treatments due to the rich nutrients and suitable external environment. With the fermentation progressed and the competition among different fungi communities, the dominant fungi changed to *Candida* at T4 stage. Along with the drastic changes in the fermentation environment, some fungi are difficult to survive, but spores may still exist. This might explain the detected fungi communities in Fn and Fm treatments at T4 stage, but not detected in T2 stage. The fermentation of fruits or vegetables was generally involved complex communities of microorganisms [38]. In this study, the different exogenous inoculants showed distinct impacts on the variation of microbial communities during fermentation, which are similar to those of other reports [37]. Their results also indicated that the dominant genera tended to be the same after fermentation, even with different exogenous inoculants. The specific inoculant and dominant genera have been reported closely related to the yield, flavor and chemical composition of fermentation products [15,39]. However, the relationship and the underlying mechanisms of different inoculants and dominant genera with the chemical composition of fermentation products are still unclear in this study and require further research.

3.6. Fertilization effects of fermented waste grape

The fermentation product of Fm treatment was selected to applied in a vineyard to evaluate its' fertilization effect. The results displayed that the contents of vitamin C (173 mg kg^{-1}) and soluble solid (19.7%) of *Kyoho* grape in T2 treatments has significantly enhanced compared with T1 treatment (vitamin C 148 mg kg^{-1}, soluble solid 16.4%). The contents of VC and soluble solid in grape berries of T2 has significantly improved by 16.89% and 20.12%, respectively, compared with T1. However, no significant difference was observed on grape yields (8450 kg ha^{-1} in T1 vs. 8785 kg ha^{-1} in T2). This fertilization experiment indicated that the fermented waste grape could utilized as nutrients supplements in vineyard to maintaining grape yields while enhancing fruit qualities, even under a reduction of chemical fertilization rate by 25% condition. This beneficial effect of fermented waste grape could be attributed to the abundant nutrients and active substances in the fermentation products [40,41]. assessed the feasibility of using the composting products of grape waste from wine industry as organic fertilizer in agricultural production. Their results demonstrated that the by-products of grape industrialization are qualified raw material with adequate levels of organic matter and essential elements important to the plants, therefore, affirming the composting products of grape waste are suitable for the production of agricultural supplies of high quality, while achieving high environmental benefits. However, there is few study focus on the recycling and utilization of fresh waste grape berries in vineyards so far. Our study provides a new insight to solve this problem.

The use of agrochemicals such as fertilizers and pesticides enhance the grape production, however, in long term it degrades the soil quality and also causes environmental pollution issues. Meanwhile, the use of agrochemicals also leads to the gathering of heavy metals in plant tissues thus reducing the fruit nutritional value and edibility [42]. Therefore, the reducing of agrochemicals plays significant role in the sustainable development of modern agricultural production. In this study, the fresh waste grape berries were recycled by fermentation approach with exogenous probiotics. The fermentation products are rich in living probiotics and nutrients, and have been verified to be an ideal substitute for chemical fertilizer using in vineyards. The active microorganisms in fermentation products have capability to convert important nutritionally elements from unavailable to available form through biological processes, thereby, enhancing grape yields and fruits' qualities [43]. The application of fermented waste grape has multiple benefits, including cheap source of nutrients, excellent suppliers of micro and macronutrients, supplier of organic matter, as well as counteracting negative impact of agrochemicals. This study provides an effective approach for the disposal of fresh waste grape berries in vineyards, which simultaneously achieved the recycling of waste grape, reducing the chemical inputs, as well as enhancing the environmental benefits in vineyards.

4. Conclusion

Fresh waste grape berries from vineyards were fermented with different exogenous probiotics inoculants. The fermentation dynamics, chemical composition of the fermentation products and the microbial community compositions were simultaneously measured during the fermentation process. The results indicated that the different microbial inoculants caused different pH and EC dynamics trends in the four treatments. The *Z. rouxii* inoculant (Fs) and a mixture inoculants of *Z. rouxii* and *L. casei* (Fm) were ideal exogenous probiotics for the recycling of waste grape, which have significantly improved the contents of free Aa, NH_4^+ and Na^+ in the fermentation products, compared with other two treatments that without exogenous inoculant and with EB agent inoculant. The microbial communities' composition varied greatly across different treatments and fermentation periods. On genus level, the *Lactobacillus* and *Pseudomonas* were the dominant bacteria, while *Saccharomyces* and *Candida* were the dominant fungal in the fermentation process. The fermentation products of waste grape could utilized as fertilizer substitute in vineyard, which significantly improved *Kyoho* VC and soluble solid contents by 16.89% and 20.12%, respectively, even under fertilization rate reduced by 25%. In general, fermentation with exogenous probiotics is a feasible way for the disposal of fresh waste grape berries in vineyard. The fermentation products provide an alternative to fertilizer in agricultural production, which can not only improve crop quality but also reduce the amount of fertilizer applied. To achieve this goal, a suitable inoculant is the key. However, the chemical analysis of fermentation product in this study was insufficient. Some valuable active substances warrant further analysis. Moreover, the fertilization effect of fermentation product in other agricultural systems also needs to be further verified.

Author contribution statement

Zheng Zhao: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
Lina Sun: Analyzed and interpreted the data; Wrote the paper.
Zhimin Sha: Deping Zhou: Contributed reagents, materials, analysis tools or data.
Changbin Chu: Qingfeng Wang: Performed the experiments.
Shuhang Wu: Conceived and designed the experiments.

Data availability statement

Data will be made available on request.

Declaration of competing interest

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

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