

# The effects of different inoculation amounts of *Lactobacillus plantarum* on antioxidant capacity, chemical composition and sensory quality of *Rosa roxburghii* Tratt fruit pomace ensiled

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## Research Article

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# Abstract

In order to enable high-value disposal and proper preservation of *Rosa roxburghii* Tratt fruit pomace for waste valorization, ensiling was carried out to preserve *Rosa roxburghii* Tratt fruit pomace (RRTP) for 60 days at different inoculation amounts of *L. plantarum* ( $0$ ,  $5 \times 10^5$  CFU g<sup>-1</sup>,  $2.5 \times 10^6$  CFU g<sup>-1</sup> and  $1.25 \times 10^7$  CFU g<sup>-1</sup>, per gram of fresh RRTP, named the CK, Group 1, Group 2 and Group 3, respectively). Further, the effects of different inoculation amounts of *L. plantarum* on antioxidant status, chemical composition and sensory quality of RRTP were evaluated. The results showed that Group 2 and Group 3 increased antioxidant capacity and the proportion of total phenols ( $P < 0.01$ ) in RRTP silage compared with control. Moreover, yeasts molds were not found in Group 2 and Group 3. In addition, The two groups exhibited the best sensory quality. Overall, the right amount addition of The *L. plantarum* could offer a sustainable eco-friendly process to preserve RRTP and improve the quality of RRTP silage.

## 1. Introduction

Antioxidants have been widely used in feed additives in recent years because they can help to clear reactive oxygen, which prevent oxidative stress in animal production, thereby preventing the animals in a sub-healthy state [1–3]. The application of exogenous antioxidants such as phenolic compounds, flavonoids and vitamin C have been proved to be an effective strategy to mitigate oxidative stress [2, 4, 5]. But application of exogenous additives is limited by disadvantageous factors such as shortage of raw materials, low extraction quantity and high extraction cost [6]. Further, with the development of animal husbandry, a large silage is needed per annum. Thus, producing silage with antioxidant properties by using readily available raw materials is a useful and desired method to obtain rich exogenous additives with antioxidant properties [7].

*Rosa roxburghii* Tratt (RRT), a small deciduous horticultural shrub in the Rosaceae family, is characterized by rich antioxidants, such as phenolic compounds vitamin C, flavonoids, superoxide dismutase and is one of the most distinctive resources in China, with Guizhou Province having the highest planted area [8–10]. RRT is usually used as edible resource, and it is also an important traditional Chinese medicine because of its a various bioactivities, such as antioxidant, antimutagenic, and antiatherosclerotic [11]. Despite its remarkable nutritional and functional value, the main processing and utilization of RRT is to extract juice to make into fruit juice, fruit wine, and other products at present [12], resulting in yielding a large number of *Rosa roxburghii* Tratt fruit pomace (RRTP), a by-product of the extracted RRT. Moreover, the efficiency of juice extraction is however about 50%, indicating that RRTP still contain considerable antioxidants and other substances. If RRTP cannot be harmlessly treated in time, it will rot easily and produce malodorous smell, which will cause a huge waste of high quality resources and environmental pollution. Unfortunately, RRTP is mainly treated as agricultural waste, and discarded them by piling or burying burning at present.

Previous studies have shown that Lactic acid bacteria (LAB) and their fermentation metabolites have good antioxidant properties [13], especially some *Lactobacillus* species [3, 14, 15], which demonstrated a

positive effect on regulating oxidative stress of organisms or cells through generating superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) to scavenge separately the superoxide anion ( $O_2^-$ ), the excessive hydrogen peroxide ( $H_2O_2$ ) and hydroxyl radicals ( $\cdot OH$ ) in organisms [16]. Furthermore, many studies have previously studied *Lactobacillus* species as a microbial inoculant for Alfalfa and other other silages [3, 17]. But RRTP silage with *Lactobacillus* species has not been reported yet. Therefore, in this study, RRTP was ensiled at different inoculation amounts of *L. plantarum* and the effect on the antioxidant capacity, chemical composition and sensory quality of RRTP silage were investigated for proper preservation of RRTP and obtaining rich exogenous additives with antioxidant properties.

## 2. Materials and Methods

### 2.1. Materials

Fresh RRTP was collected from Sinopharm Group (Guizhou) Health Industry Development Co., Ltd, a subsidiary of China National Pharmaceutical Group. The commercial strain *L. plantarum* (Preservation number: ACCC11016) was purchud from China General Microbiological Culture Collection Center, Beijing, China.

### 2.2. Silage preparation

Fresh RRTP were mixed thoroughly and divided randomly into four piles. Completely randomized design was used to assign the prepared RRTP piles to one of the following treatments (Table 1): (1) without *L. plantarum*, named the CK; (2)Applications rate of *L. plantarum* was  $5\times10^5$  cfu  $g^{-1}$  fresh weight, named the Group 1; (3) Applications rate of *L. plantarum* was  $2.5\times10^6$  cfu  $g^{-1}$  fresh weight, named the Group 2; (4) Applications rate of *L. plantarum* was  $1.25\times10^7$  cfu  $g^{-1}$  fresh weight, named the Group 3. Additional Fresh RRTP from the four fields (about 250 g, respectively) was collected as fresh sample and frozen at -20 °C for further analysis. According to experimental design, the RRTP was mixed the inoculum (logarithmic growth phase of the colony). For the control group, the same amount of distilled water was used. After thorough mixing by hand, RRTP (800g) was packed into 200 mm × 300 mm polyethylene plastic bags; these bags were vacuumed and sealed using a vacuum extractor (Yongkang Jiuxian Technology Co., Ltd, Zhejiang, China) and stored at room temperature ( $25 \pm 2$  °C) for 60 d

Table 1  
Details of the treatments used in the study

| Item      | Addition amount of <i>L. plantarum</i> in RRTP |                            |                 |                  |
|-----------|------------------------------------------------|----------------------------|-----------------|------------------|
|           | CK group                                       | group 1                    | group 2         | group 3          |
| Treatment | 0                                              | $5\times10^5$ CFU $g^{-1}$ | $2.5\times10^6$ | $1.25\times10^7$ |

### 2.3. chemical analysis

The polyethylene plastic bags were opened at the end of the ensiling period and 100 gram sample of silage was frozen at -20 °C for further analysis. Twenty gram sample was collected from each bag and homogenized with distilled water (180 mL) for 30 seconds in a juicers in a juice (JYL-C3, Joyoung Co.Ltd., HangZhou, China) and filtered by four layers of cheesecloth [18]. The filtrate was divided into two parts and was frozen at -20 °C for later antioxidant and vitamin C analysis respectively. Water soluble carbohydrate (WSC), non-protein nitrogen (NPN), ammonia nitrogen(NH<sub>3</sub>-N), amino acid nitrogen(AA-N) were measured according to X. Zhang et al [19]. Crude protein was measured using a Kjeldahl nitrogen analyzer. Neutral detergent fiber(NDF) and acid detergent fiber(ADF) were performed according to the method of Van Soest et al [20].

## 2.4. Antioxidant capacity test of silage

The first filtrate previously stored at -20 °C was unfrozen and centrifuged at 4 °C, 10,000 × g for 10 min. The T-AOC, SOD, Glutathione peroxidase (GSH-PX), Superoxide anion (O<sub>2</sub><sup>-</sup>) and Hydroxyl free radical (OH•<sup>-</sup>) activities were determined from the supernatant using assay kits (Nanjing Jiancheng using commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, China) according to the manufacturer's instructions.

## 2.5. Analysis of total flavonoid and polyphenols

The total flavonoid concentration was determined by UV spectrophotometer (UV-1800, SHIMADZU) based on the method of Zhang et al. (2013) [19]. Total polyphenol conten was measured at 750 nm using the spectrophotometric method with the Folin-Ciocalteureagent [21].

## 2.6. Sensory quality of RRTP silage

Sensory quality was assessed after silage 60 d according to the sensory silage grade of the German Agricultural Association (DLG) [24].

## 2.7. Statistical analysis

The Origin software 2021 was used for the statistical analysis. One-way analysis of variance was used to evaluate the statistical significance of different groups. The means of different groups and standard deviations were calculated, and then compared for significance different ( $P < 0.05$ ).

# 3. Results and Discussion

## 3.1 Antioxidant status of RRTP ensiled

Considerable research have found that the *L. plantarum* have a positive antioxidant activity, which is related to production of antioxidant enzymes [22]. In this study, all groups inoculum *L. plantarum* had higher T-AOC activity than CK ( $P < 0.01$ ) (Table 2). This may be because the *L. plantarum* produces T-AOC. In addition, Group 3 ( $1.25 \times 10^7$  cfu g<sup>-1</sup>) had strong free radical scavenging activities against O<sub>2</sub><sup>-</sup>, and with high antioxidant enzyme activity (T-AOC, SOD, GSH) compared to control group and group1 ( $P <$

0.01). The possible reason was that *L. plantarum* secreted more T-AOC, SOD, GSH, as the inoculum amounts increased.

Table 2  
Antioxidant activities of RRTP ensiled without or with different addition amount of *L. plantarum* for 60 d.

| Variable <sup>1</sup>           | CK                | Group 1           | Group 2           | Group 3            | SEM <sup>2</sup> | <i>p</i> -value |
|---------------------------------|-------------------|-------------------|-------------------|--------------------|------------------|-----------------|
| GSH-Px(μmol/L)                  | 18.7 <sup>a</sup> | 19.7 <sup>a</sup> | 30.6 <sup>b</sup> | 32.8 <sup>b</sup>  | 2.29             | < 0.001         |
| O <sub>2</sub> <sup>-</sup> (%) | 84.2 <sup>a</sup> | 95.3 <sup>a</sup> | 97.9 <sup>a</sup> | 110.5 <sup>b</sup> | 4.2              | < 0.001         |
| OH• <sup>-</sup> (%)            | 60                | 58.5              | 61.6              | 58.4               | 1.68             | 0.202           |
| T-AOC (U/mL)                    | 74.8 <sup>a</sup> | 95.4 <sup>b</sup> | 91.4 <sup>b</sup> | 94.0 <sup>b</sup>  | 3.57             | < 0.001         |
| SOD(U/mL)                       | 50.8 <sup>a</sup> | 50.8 <sup>a</sup> | 62.2 <sup>b</sup> | 62.2 <sup>b</sup>  | 1.20             | < 0.001         |

<sup>1</sup> GSH-Px, glutathione peroxidase; O<sub>2</sub><sup>-</sup>, superoxide anion; OH•<sup>-</sup>, Hydroxyl Free Radical; T-AOC, total anti-oxidation competence; SOD, superoxide dismutase.

<sup>2</sup>SEM, standard error of the mean.

<sup>a-b</sup>Means of treatment within a row with different superscripts differ (*P* < 0.05).

## 3.2 Microbial population of RRTP silages ensiled

The count of LAB Yeasts Molds in RRTP ensiled for 60 d are shown in Tables 3. In the control group, *Lactobacillus plantarum* was not observed and molds was found, whereas Group 2 and Group 3 were contrary, showing *Lactobacillus plantarum* was observed and molds was not found probably due to high content *Lactobacillus plantarum* effectively inhibited the reproduction of harmful microorganisms.

Table 3  
Microbial population of RRTP silages ensiled for 60 d (n = 3).

| Item <sup>1</sup>                   | Fresh RRTP | CK                | Group 1           | Group 2           | Group 3           | SEM <sup>2</sup> | <i>P</i> value <sup>3</sup> |
|-------------------------------------|------------|-------------------|-------------------|-------------------|-------------------|------------------|-----------------------------|
| LAB (log <sub>10</sub> CFU/g FW)    | -          | ND                | 1.28 <sup>a</sup> | 4.28 <sup>b</sup> | 4.43 <sup>b</sup> | 0.21             | < 0.001                     |
| Yeasts (log <sub>10</sub> CFU/g FW) | -          | ND                | ND                | ND                | ND                | -                | -                           |
| Molds (log <sub>10</sub> CFU/g FW)  | -          | 3.06 <sup>b</sup> | 1.31 <sup>a</sup> | ND                | ND                | 0.15             | < 0.001                     |

<sup>1</sup> LAB, lactic acid bacteria; CFU, colony forming unit.

<sup>2</sup> SEM, standard error of the mean.

<sup>a-b</sup>Means of treatment within a row with different superscripts differ ( $P < 0.05$ ).

### 3.3 Sensory quality of RRTP ensiled

Sensory quality of RRTP after silage are shown in Table 4. The sensory scores of CK and G1 were significantly lower than those of G2 and G3 groups ( $P < 0.01$ ). After 60 days of silage, there was a bad smell in CK and G1 groups, but a sour aroma and well structured were observed in G2 and G3 groups. These results indicate that RRTP ensiled at high content *Lactobacillus plantarum* had better fermentation quality than that ensiled at low content *Lactobacillus plantarum*.

Table 4  
Sensory quality of RRTP after silage

| Items              | CK                                       | Group 1                                  | Group 2                                   | Group 3                                   | SEM <sup>1</sup> | <i>P</i> -value |
|--------------------|------------------------------------------|------------------------------------------|-------------------------------------------|-------------------------------------------|------------------|-----------------|
| Sensory evaluation | Brown,<br>Musty taste,<br>High viscosity | Brown,<br>Musty taste,<br>High viscosity | Yellow,<br>Sour aroma,<br>Well structured | Yellow,<br>Sour aroma,<br>Well structured | -                | -               |
| Score              | 8.7 <sup>a</sup>                         | 9.3 <sup>a</sup>                         | 15.5 <sup>b</sup>                         | 15.7 <sup>b</sup>                         | 0.51             | < 0.001         |

<sup>1</sup> SEM, standard error of the mean.

<sup>a-b</sup>Means of treatment within a row with different superscripts differ ( $P < 0.05$ ).

### 3.4 Chemical composition of RRTP ensiled

The concentrations of chemical composition of RRTP with different inoculation amounts of *L. plantarum* for 60 d are shown in Tables 5. Different inoculation amounts of *L. plantarum* had not effects on water soluble carbohydrate, crude protein, non-protein nitrogen, ammonia nitrogen, amino acid nitrogen, neutral detergent fiber, acid detergent fiber and Vc of RRTP. But application of *L. plantarum* increased the proportion of total phenols when compared to the control group ( $P < 0.01$ ). Phenolics are natural antioxidants found in vegetables and fruits and are characterized by scavenging free radicals, which could reduce oxidative stress levels<sup>10,23</sup>. Hence, the higher T-AOC activity observed at inoculation *L. plantarum* groups could be the result of the increase of total phenols.

Table 5

Chemical composition of RRTP ensiled with different addition amount of *L. plantarum* for 60 d (DM basis, dry matter).

| Items <sup>1</sup>                                    | CK                | Group 1           | Group 2           | Group 3           | SEM <sup>2</sup> | P-value |
|-------------------------------------------------------|-------------------|-------------------|-------------------|-------------------|------------------|---------|
| WSC, mg/kg                                            | 39.1              | 39.5              | 38.1              | 38.2              | 0.95             | 0.422   |
| CP, %                                                 | 7.2               | 7.3               | 7.2               | 7.3               | 0.046            | < 0.06  |
| NPN, g/kg                                             | 0.28              | 0.25              | 0.23              | 0.27              | 0.023            | 0.083   |
| NH <sub>3</sub> -N, g/kg                              | 9.72              | 9.57              | 9.35              | 9.35              | 0.166            | 0.146   |
| AA-N, mg/g                                            | 1.48              | 1.30              | 1.34              | 1.44              | 0.058            | 0.052   |
| NDF, %                                                | 41.3              | 40.8              | 40.4              | 40.1              | 0.440            | 0.155   |
| ADF, %                                                | 36.8              | 36.2              | 35.4              | 37.0              | 0.748            | 0.198   |
| Total flavonoid (mg of rutin equivalent/g of DM)      | 26.31             | 32.29             | 33.15             | 27.84             | 0.054            | 0.342   |
| Total phenols (mg of Gallic acide equivalent/g of DM) | 34.7 <sub>a</sub> | 48.8 <sup>b</sup> | 55.5 <sup>b</sup> | 53.8 <sup>b</sup> | 0.028            | < 0.001 |
| Vc(mg • 100g <sup>-1</sup> )                          | 6.6               | 7.1               | 7.3               | 7.1               | 0.39             | 0.39    |

<sup>1</sup> WSC, water soluble carbohydrates; CP, crude protein; NPN, non-protein nitrogen; NH<sub>3</sub>-N, ammonia nitrogen; AA-N, amino acid nitrogen; NDF, neutral detergent fiber; ADF, acid detergent fiber; Vc, vitamin C.

<sup>2</sup> SEM, standard error of the mean.

<sup>a-b</sup> Means of treatment within a row with different superscripts differ ( $P < 0.05$ ).

## 4. Conclusions

The current study has shown that the RRTP ensilage treatment with appropriate amount of *Lactobacillus plantarum* can have a better fresh-keeping effect and higher oxidative status relative to the control (without *Lactobacillus plantarum*) and the quality of silage is better with the increase of adding amount. In addition, higher content of phenolics was observed in RRTP ensiled at *Lactobacillus plantarum* inoculation compared to no *Lactobacillus plantarum*. Considering the low cost of RRTP and having good antioxidant activity and abundant resources in China, Silage RRTP by inoculating *Lactobacillus plantarum* could offer a sustainable cost-effective and eco-friendly process to develop potential exogenous additives for animal production and realize the long-term preservation of RRTP.

## Declarations

**The novelty statement** RRTP, a by-product of the extracted *Rosa roxburghii* Tratt fruit has high antioxidant activity and contains rich nutrients. Fresh RRTP was discarded by piling or burying burning at present because of its perishable properties, which cause a huge waste of high quality resources and environmental pollution. But, the research on the utilization of RRTP is rarely reported. Therefore, this study investigated the effects of different inoculation amounts of *Lactobacillus plantarum* on antioxidant capacity, chemical composition and sensory quality of RRTP ensiled. The results indicated that the right amount addition of the *L. plantarum* could offer a sustainable eco-friendly process to preserve RRTP and improve the quality of RRTP. Considering the low cost of RRTP and the abundant resources in China, RRTP silage is a useful and desired method to obtain rich exogenous additives with antioxidant properties.

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