APPLICATION OF LACTIC ACID IN PRESERVATION OF NINH THUAN GREEN GRAPES NH01-48

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ABSTRACT

Ninh Thuan green grapes NH01-48 is popularly cultured in Ninh Thuan province. However, due to the lack of preservation technology and/or facility, Ninh Thuan grapes are transported to and consumed in other areas of the country in a limited volume. This study aimed to assess application of lactic acid in treatment of Ninh Thuan grapes for preservation. Effect of lactic acid on grapes with or without challenging microorganisms was examined. Green grapes NH01-48 was washed with sterile water, and then dried on air. Grapes were challenged with a cocktail of microorganisms previously isolated from Ninh Thuan grapes, including four bacterial strains (Bacillus cereus, Pseudomonas oryzihabitans, Citrobacter sp., and Flavobacterium sp.), three yeast strains (Wickerhamomces anomalous, Hanseniaspora opuntiae, Debaryomyces nepalensis) and one fungus Penicillium sp. at 5 × 10⁴ CFU/ml. Then grapes were treated with 3 % lactic acid solution for 5 min (soaking) prior to be packed in plastic bags with punched hole of 3 mm diameter, distance between holes is 3 cm and preserved in refrigeration condition at 4 ± 0.5 °C. Grapes without challenging microorganisms/without acid pretreatment/without challenging microorganisms but treated with acid were used for comparison. Samples were taken every 5th day until 30 days. Color, hardness, total dry matter, titration acidity, and total microorganism count were measured. Results showed that when applied treatment of lactic acid on grape with and without challenging microorganisms, the change in grapes’ hardness, color, dry matter and titration acidity was minimized, demonstrating positive activity of lactic acid on grape physicochemical characteristics. Samples without lactic acid treatment were spoiled after 15-20 days of preservation, whereas the ones with acid treatment can be last for at least 30 days. Moreover, number of total microorganisms count after 30 days of preservation were acceptable in samples with lactic acid treatment (ca. 10³ CFU/g). Therefore, lactic acid can be potential for application in grapes preservation. The combined effect with other preservation techniques needs to be further studied.

Keywords: lactic acid, Ninh Thuan grapes, preservation.
1. INTRODUCTION

Grape (Vitis vinifera L.), which belongs to the Vitaceae family, favors a dry and sunny climate. In Vietnam, Ninh Thuan province has favorable natural conditions to grow and develop a variety of high quality grapes, including NH 01-48 green grape which has sweet taste and is preferred by customers. Grapes in general and Ninh Thuan NH01-48 grape in particular are susceptible to postharvest decay. The major causes are natural dehydration, physical damage in transport and handling, as well as microorganism activities.

In order to prolong the storage time of grapes, in addition to conventional cold storage, a number of technological solutions are combined to improve storage efficiency such as chemical treatment, biodegradable film wrapping, ozone solution, control atmosphere (CA) ... The use of organic acids in grape preservation is being studied by numerous scientists in the world and in Vietnam leading to promising results.

Organic acids associated with specific antimicrobial activity are short-chain acids (C1–C7) and are either simple monocarboxylic acids such as formic, acetic, propionic and butyric acids, or are carboxylic acids bearing an hydroxyl group (usually on the α carbon) such as lactic, malic, tartaric, and citric acids. Salts of some of these acids have also been shown to have performance benefits [1]. Lactic acid was found to inhibit grape spoilage microorganisms in previous studies in vitro [2, 3]. Result showed that at concentration of 3 % lactic acid could inhibit all tested spoilage microorganisms isolated from Ninh Thuan grape. Therefore, in this study 3 % lactic acid was assessed on grapes for potential use in pretreatment for further storage.

2. MATERIALS AND METHODS

2.1. Materials

NH 01-48 green grapes were sampled at the main harvest in April 2016 and 2017; harvesting maturity of sampling was from 115 to 120 days after cutting (pruning). Samples were taken in Thanh Son hamlet, Xuan Hai commune, Ninh Hai district, Ninh Thuan province where grape production is recognized to meet VietGAP standards. Collection and sampling were completed before 6 am of the day. Grapes were collected in the form of bunches which had relatively uniform fruit size (28 ± 2 mm long, 18 ± 2 mm wide). Defected fruits were then removed to obtain a mass of 550 - 650 grams per bunch. These grape bunches were then packed in PE pack, two bunches per bag, placed in styrofoam box covered by ice, transported by airplane route Nha Trang – Ha Noi to the laboratory of Food Technology department - School of Biology and Food Technology. Grape spoilage microorganisms used in this study were previously isolated from Ninh Thuan green grape (NH01-48) [4] including four bacteria (Bacillus cereus, Pseudomonas oryzihabitans, Citrobacter sp. and Flavobacterium sp.); three yeasts (Hanseniaspora opuntiae; Wickerhamomyces anomalus and Debaryomyces nepalensis); and one fungus (Penicillium corylophilum).

2.2. Methods

2.2.1. Sample preparation, treatment and storage condition

Fresh grape bunches were removed from unsatisfactory fruits, washed with sterile water and then drained for drying. Grapes after being washed were soaked in prepared microorganisms
solution (including four bacteria (*Bacillus cereus*, *Pseudomonas oryizabtans*, *Citrobacter* sp. and *Flavobacterium* sp.); three yeasts (*Hanseniaspora opuntiae*; *Wickerhamomyces anomalus* and *Debaryomyces nepalensis*); and one fungus (*Penicillium corylophilum*) a cocktail of $5 \times 10^4$ CFU/ml of each type of above mentioned spoilage microorganisms in saline water, experimented under safe conditions) for 5 minutes. Grapes after being drained were soaked in organic acid for 5 minutes. Untreated grapes, uncontaminated grapes treated with acid, and contaminated grapes without acid treatment were used for comparison.

The samples were named as follow: TN0.1: Unprocessed grape for preservation; TN0.2: grapes washed with sterile water and then preserved; TN0.3: grapes artificially contaminated with spoilage microorganisms then subjected to preservation; TN0.4: Grapes contaminated with microorganisms, then treated with 3 % lactic acid prior to preserve; TN0.5: Grapes treated with 3% lactic acid prior to preserve.

The samples were drained, packed with a 3 mm perforated hole PE bags, and stored in the refrigerator at temperature of $4 ^\circ C \pm 0.5$ with humidity of 90 – 95 %. Samples were analyzed every 5 days. Analytical criteria included: color, hardness, total soluble dry matter content, titration acidity and total microorganisms count.

2.2.2. Analytical methods

Total soluble dry matter was determined using refractometer. Total acid content (%) was assessed according to TCVN 5483 - 2007 for vegetables and fruits. The acid content was calculated in tartaric acid (g/100 g of product).

Fruit hardness was measured using texture analyzer TA.XT plus. The hardness of grapes was measured by squeezing the 5 kg piston into the fruits until it penetrated the fruit surface. Movement speed of piston was 2 mm/s. Each measurement proceeded for 10 samples, and then values were taken in average. Color of grapes was analysed Using Colorlite Sph860 machine. Each measurement proceeded for 10 samples for an average value.

Total microorganisms was counted by colony counting method on Plate Count Agar [5]. The shedding rate of fruit is the percentage of the number of fruit falling from the stalk of the grape bunch to the total mass of samples (%).

Experiment results were analyzed by ANOVA and LSD test (5 %) using SAS 610 statistical software.

3. RESULTS AND DISCUSSION

3.1. Effects of lactic acid treatment on grapes hardness

The results of studying the effect of lactic acid on the hardness of NH 01 - 48 grapes were shown in Fig. 1. Figure 1 showed that the grape hardness decreased during storage. All samples without lactic acid treatment had a faster rate of decline than the other samples. Two samples of TN0.1 and TN0.3 were spoiled after 15 days of storage and were discarded. Those samples had the lowest hardness (4.23 N and 4.25 N, respectively). This demonstrated that untreated samples with or without challenging microorganisms had shorter time of preservation in term of hardness. In the case of sample not treated with lactic acid, but only washed with distilled water to remove some of the microorganisms on the fruit surface (sample TN0.2), the hardness reduced at slower rate. However, the storage time was only five days more than those without
any treatment (TN0.1 and TN0.3). For two samples treated with 3% lactic acid, the hardness stayed the same and only reached the values of 4.42 N and 4.51 N after 30 days of storage, respectively.

![Graph](image1)

*Figure 1.* Effects of lactic acid treatment on grapes hardness by the time of preservation.

### 3.2. Effects of lactic acid treatment on grapes color

Effects of lactic acid treatment on the color of grapes were shown in Figure 2.

![Graph](image2)

*Figure 2.* Effects of lactic acid treatment on the color of grapes by the time of preservation.

The greater the ΔE, the greater the color of grapes changed. Samples without acid treatment (TN0.1, TN0.2 and TN0.3) possessed ΔE increasing sharply after 15 days of storage, while lactic acid treated samples had significantly slower ΔE increase (TN0.4 and TN0.5). This indicated that lactic acid was capable to retard the color change of the grape during storage. After 30 days, the color of grape bunches in TN0.4 and TN0.5 samples varied from green to bluish. While the remaining three samples were damaged very quickly and after 15 days brown spots appeared in the fruit. Effect of organic acids on the color change of fruits during preservation was also reported by a number of authors. Tran Thi Dinh et al. demonstrated positive effect of organic...
acids on the reduction of browning on the lychee and longan fruit during cold storage [6, 7]. In another study ascorbic acid was used to maintain the color of the pitaya fruit during storage [8].

3.3. Effects of lactic acid treatment on total soluble dry matter content of fruit

Effect of acid treatment on total dry matter content were shown in Fig. 3. For the first five days of preservation, total soluble dry matter content in all five studied samples tended to increase because of dehydration. Then the index reduced due to the fruit decomposition. This observation was in line with the aging process of the fruit.

![Figure 3](image)

*Figure 3. Effect of lactic acid treatment on total soluble dry matter content of grapes by the time of preservation.*

Two samples treated with lactic acid (TN0.4 and TN0.5) showed slower decline of the soluble dry matter. After 30 days of storage the values remained at 14.8 % and 14.6 %, respectively. This result indicated that lactic acid treatment was effective in maintaining the nutritional quality of grapes.

3.4. Effects of lactic acid treatment on the titration acidity of fruit

![Figure 4](image)

*Figure 4. Effect of lactic acid treatment on titration acidity content of NH01-48 grapes by the time of preservation.*
Figure 4 showed the effects of lactic acid treatment on total acidity in grape. Total acid content is one of the important indicators affecting the quality of grapes. This index decreases after harvesting means that grape quality is altered in a bad way. During preservation in this study the acid index decreased. However, samples treated with lactic acid (TN0.4 and TN0.5) demonstrated slower decreasing rates. This is significant in maintaining the quality of the grape. Sholberg et al. also studied grape preservation at 2 - 5 °C after immersing in acetic acid solution. Results showed that fruit quality indexes (Brix, titratable acidity, pH, and color) decreased slowly during the 6 weeks of storage [9].

3.5. Effects of lactic acid treatment on total microorganisms count of fruits

Total microorganisms count were shown in Figure 5. Total microorganisms count increased in all samples. The total microorganisms count of the initial grape specimen of NH01-48 was 1.81 log CFU/g.

![Figure 5. Effects of lactic acid treatment on total microorganisms count of grapes by the time of preservation.](image)

For the samples without acid treatment this value increased sharply and corrupted after 15 days. Total count of TN0.1 and TN0.3 samples increased to 5.08 and 4.95 log CFU/g after 15 days; sample TN0.2 reached to 5.04 log CFU/g after 20 days. This result indicated that these samples were damaged by microorganisms. Samples of TN0.4 and 0.5 reached values of 3.14 and 3.12 log CFU/g after 30 days. Thus, lactic acid concentration of 3 % inhibited spoilage microorganism on NH01-48 Ninh Thuan grape, therefore, prolonging the time of use for this grape variety while ensuring the criteria of total microorganism count for fresh fruit. Sholberg [10] also used acetic acid at a concentration of 8.0 mg/l to inhibit microorganisms on grapes such as Botrytis cinerea, Penicillium expansum, Monilinia spp., Rhizopus stolonifer. The study reduced rotting rates on the grape bunch and extended the storage to 74 days at 0 °C by MAP method. Venditti et al. also showed similar results when using this acid to preserve the ‘Taloppo’ grape [11, 12].

3.6. Effects of lactic acid treatment on rate of shedding fruits

The rate of shedding fruit is one of the important criteria for evaluating the quality of the grape bunch. The large number of shedding fruit indicates that the grape bunch has been
damaged during storage. The shedding rate of grape bunch in this study was determined and shown in Table 1.

Table 1. Effects of lactic acid treatment on rate of shedding grapes by the time of preservation (%).

<table>
<thead>
<tr>
<th>Time (day)</th>
<th>Rate of shedding (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>TN0.1</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>6.5</td>
</tr>
<tr>
<td>10</td>
<td>11.5</td>
</tr>
<tr>
<td>15</td>
<td>25.3</td>
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<td>20</td>
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<tr>
<td>25</td>
<td>-</td>
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<td>30</td>
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</table>

The table shows that TN0.1 and TN0.3 samples appeared shedding fruits after 5 days of preservation, which is 6.5 % and 5.4 %, respectively, and the rate reached 25.3 % and 21.5 % after 15 days of preservation. Meanwhile, the TN0.2 sample yielded shedding fruits later, but also reached 22.7 % on the 20th day of storage. On the other hand, 2 samples treated with 3 % lactic acid were not observed the shedding fruits during 15 days. By the 20th day, only 0.6 % of the fruit fell off and by the 30th day of storage, the shedding rate was only 4.5 % and 5.5 %. Thus, under the condition of freezing storage and lactic acid effect, it was reduced the rate of shedding fruit significantly comparing to the control samples. This is due to the effect of preservation on the activities of the cellulase and pectinesterase enzymes. Under low temperature and the effect of acid, the activities of these two enzymes were inhibited, increasing the binding force between the stalk and the grape, resulting in lower shedding rates comparing to other samples at the same storage conditions. This has also been shown in 2007 by Deng et al. [13].

4. CONCLUSION

In summary, the use of 3 % lactic acid was able to inhibit the activity of microorganisms which spoiled Ninh Thuan green grapes, slowing the variation in the nutritional quality of the green grape NH01-48. The treated samples could be preserved in minimum 15 days longer than untreated samples. This solution could be potential used in combination with other fruit preservation technique, for examples controlled atmosphere (CA), in order to extend shelf life of fresh fruits.

REFERENCES


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Tóm tắt

ỨNG DỤNG AXIT LACTIC TRONG BẢO QUẢN QUẢ NGO XANH NINH THUẬN NH 01-48

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Quả nho xanh NH 01-48 là một đặc sản nổi tiếng của tỉnh Ninh Thuận. Tuy nhiên dược công nghệ bảo quản còn hạn chế nên nho Ninh Thuận chỉ được vận chuyển và tiêu thụ ở một số địa phương trên cả nước với số lượng thấp. Mục đích của nghiên cứu này nhằm đánh giá việc ứng dụng axit lactic trong tiễn xử lí nho Ninh Thuận để bảo quản chúng. Hiệu quả của axit lactic trên quả nho nhiễm và không nhiễm vi sinh vật đã được kiểm chứng. Qua nho NH 01-48 được rưa bàng nước cắt rồi để khô trong không khí. Sau đó được những chú động vi sinh vật đã được phân lập trước đó, gồm 4 loại vi khuẩn (Citrobacter sp., Bacillus cereus, Pseudomonas oryizihabitas, Flavobacterium sp.), 3 loại nấm men (Wickerhamomces anomalous, Hansenaspora opuntiae, Debaryomyces nepalensis) và 1 chủng nấm mốc Penicillium ở mật độ 5.10⁴ CFU/ml. Sau đó nho được những trong dung dịch axit lactic 3% trong 5 phút trước khi bảo giở bằng tüi PE có đục lỗ với đường kính lỗ là 3 mm, khoảng cách giữa các lỗ là 3 cm và bảo quản lạnh ở nhiệt độ 4 ± 0,5 °C. Mẫu nho không những chủng vi sinh vật/không xỉn lố axit lactic được sử dụng làm màu đổi Chúng. Các mẫu được kiểm tra ở ngày thứ 5 đến ngày thứ 30 trong thời gian bảo quản. Các chỉ tiêu như: Mẫu sắc, độ cứng, chất khô hòa tan tổng số, axit tổng số và vi sinh vật tổng số được xác định. Kết quả cho thấy khi sử dụng axit lactic có và không nhiễm chủ động vi sinh vật, sự thay đổi của các chỉ tiêu về màu sắc, độ cứng, chất khô hòa tan tổng số, axit tổng số là không đáng kể, điều này đã chứng minh được tác động tích cực của axit lactic đến các đặc tính hóa lí của quả nho. Mẫu nho không được xử lí axit lactic bị thời hông sau 15-20 ngày bảo quản, trong khi mẫu được xử lí axit lactic có thể kéo dài đến 30 ngày. Hơn nữa, hàm lượng vi sinh vật tổng số sau 30 ngày bảo quản ở các mẫu xử lí axit lactic ở mức chấp nhận được (10⁴ CFU/g). Do đó, axit lactic có khả năng để ứng dụng trong bảo quản quả nho. Việc kết hợp hiệu quả của nó với các công nghệ bảo quản khác cần được tìm hiểu sâu hơn trong những nghiên cứu tiếp theo.

Từ khóa: axit lactic, nho Ninh Thuận, bảo quản.