



Article

Vacuum Packaging Controlled Crown Rot of Organically-Grown Balangon (*Musa acuminata* AAA Group) Banana

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Abstract: Balangon bananas take about 23 to 28 days from harvest to reach Japan since the fruit have to be assembled from small and scattered farms, hence the problems of premature ripening and crown rot. The effectiveness of vacuum packaging in retarding ripening and in controlling crown rot has not been documented for organically-grown Balangon bananas. Balangon bananas harvested from farms in Don Severino Benedicto, Negros Occidental, Phillipines, were washed three times in tap water, then packed (wet packing) in a 13-kg capacity corrugated fibreboard carton lined with 0.05 mm thick low density polyethylene (LDPE) bag, and vacuum-packed using an ordinary vacuum cleaner. Bananas treated with 1% sodium bicarbonate were also subjected to vacuum packing. Packaged bananas were then loaded in refrigerated vans (13.0–13.5 °C), transported to Manila and then to the UPLB-PHTRC laboratory for simulated domestic and international shipments which took about 25 days from harvest until the bananas reached Japan. Bananas were then taken out of the sealed LDPE, allowed to equilibrate at 18 °C, treated with 2500 µL/L ethephon, and held at 23 °C for ripening. During the 25-day holding at 13.0–13.5 °C, bananas that were vacuum-packaged remained green. In the control (not vacuum-packaged), a few fingers in each hand started to ripen. The most significant effect of vacuum packaging in combination with 13 °C storage was the control of crown rot, particularly when bananas started to ripen. With vacuum packaging, the incidence of crown rot at the ripe stage was 2.8% compared with 55.7% in the control. Sodium bicarbonate did not control crown rot alone, nor contribute to the reduction caused by packaging and vacuum associated with the control of decay was the high visual quality rating of the fruit. Extended storage under vacuum-packed conditions did not significantly affect the physico-chemical and sensory attributes of bananas at the ripe stage.

Keywords: organic banana; crown rot; vacuum packaging; sodium bicarbonate

1. Introduction

Banana is one of the major sources of income and foreign exchange earnings which accounts for about 9% of total agricultural exports of the Philippines. The major export variety is Cavendish, and is mainly handled by multinational corporations. The other variety that is exported and has found a niche market in Japan as a “non-chemical” banana is the Bungulan (*Musa acuminata*, AAA group), popularly known as Balangon [1]. Balangon accounts for about 47% of the export volume of small banana holdings. Balangon bananas for export are grown by clusters of farmers who follow a protocol for product quality and safety. However, even with this quality system, problems still occur when

the fruit reaches Japan. It was reported that losses of about 6%–15% of the fruit occur due to crown rot and anthracnose (Alter Trade Japan, 2012 personal communication) and mechanical damage. Since Balangon bananas are grown organically, and fruit come from different production areas where pre- and postharvest management practices differ, it is expected that quality problems will occur. Moreover, the long interval from harvest to final distribution to market cooperatives in Japan, which takes about 21–28 days, ultimately results in quality deterioration and decay. In Balangon banana, Alvindia et al. [2] reported that the most active crown rot pathogens were *Colletotrichum musae*, *Fusarium verticillioides*, *Lasiodiplodia theobromae*, and *Thielaviopsis paradoxa*. These fungi infect the crown through fresh wounds created during dehanding and trimming.

Modified atmosphere packaging (MAP) is an ideal preservation technique and is known to have great potential in extending the postharvest life of fruits and vegetables [3]. Vacuum packaging which is an active form of MAP has been shown to retard ripening of bananas even with extended holding [4,5]. Vacuum packaging is currently being practiced in conventionally-grown Cavendish bananas exported to the Middle East wherein sea shipment takes about 18–21 days from the port of Davao, Philippines. In the case of these conventionally-grown bananas, pretreatment with fungicide after harvest is the supplementary treatment for crown rot control. For organic Balangon bananas, synthetic fungicides are not allowed, hence the need for alternative supplementary treatments to MAP that are compatible with organic standards. Alvindia [6] recommended supplementary treatments of generally regarded as safe (GRAS) compounds like sodium bicarbonate for the control of crown rot of bananas. His earlier studies showed a 92% reduction in decay using sodium bicarbonate in combination with a biological control agent [7]. This study was conducted with the following objectives: (a) to control the incidence and severity of crown rot of Balangon bananas during 25-day storage under MAP at 13.0–13.5 °C and during poststorage at 23 °C; (b) to determine the physico-chemical changes at the ripe stage of Balangon bananas subjected to MAP; and (c) to assess the sensory acceptability at the ripe stage of previously-stored bananas under MAP.

2. Experimental Section

Green but commercially mature, organically-grown Balangon bananas were harvested from farms in Don Salvador Benedicto, Negros Occidental, Philippines, and transported to the central packinghouse facility in Bacolod City. Dehanded bananas were washed three (3) times (in wash tanks) with running water. During the first washing, the crown was trimmed and the damaged fingers were removed. Hands of bananas were then divided into clusters consisting of 2–3 clusters per hand.

2.1. Vacuum Packaging Treatment

Vacuum packaging as an active form of MAP was employed in the study. The banana corrugated fibreboard carton (13-kg capacity) was lined with non-perforated 0.05 mm thick, low density polyethylene (LDPE) bag prior to packing of bananas that were still wet (wet packing as practiced commercially). To prevent abrasion, polystyrene liners were inserted in between the layer and clusters of bananas. Each carton weighing about 13.0 kg contained 18 clusters of bananas. Vacuum was applied for about 10 s using an ordinary vacuum cleaner to evacuate the air inside the LDPE bag. The bags were sealed by making a knot (through hand twisting) and tied with rubber bands. For the combination treatment of vacuum packaging and 1% sodium bicarbonate, baking soda (BS) which was composed mainly of sodium bicarbonate was used. The banana clusters were dipped for one (1) min in a freshly-prepared 1% solution of BS, then packed wet in LDPE bags and subjected to vacuum packing. To determine the efficacy of sodium bicarbonate in controlling crown rot when used as a single treatment, clusters of bananas dipped in 1% BS were packed wet in boxes without LDPE liner. The control consisted of banana clusters washed only in water (as currently practiced by the company) and packed wet in cartons without LDPE liner.

2.2. Inter-Island Domestic Transport and Simulated Export Shipment to Japan

Packed cartons of bananas were temporarily kept under ambient condition in the company packinghouse for about 12 h and were then loaded in a reefer van (13.5 °C) together with other bananas for export and transported to Manila. Holding in the domestic reefer vans lasted for 14 days rather than the normal 7 days since the bananas could not be loaded in the international reefer van due to port congestion in Manila. A simulated sea shipment export to Japan was conducted at the PHTRC-UPLB laboratory wherein experimental cartons of bananas were stored at 13.0 °C for 11 days representing the 5-day sea shipment and 6-d temporary storage upon arrival in Japan.

2.3. Induction of Ripening after MAP

On the 26th day from harvest, bananas were removed from vacuum packaging and exposed to air at 18 °C for induction of ripening. Once the pulp temperature reached 18 °C, bananas were dipped for 30 s in 2500 µL/L ethephon (2-chloroethyl phosphonic acid, 46% a.i.) as ethylene source insuring that the crown and the pedicel were not dipped in the solution. The temperature of the ripening room was set at 23 °C to simulate the condition during retail distribution in Japan.

2.4. Data Gathered

The levels of oxygen (O₂), carbon dioxide (CO₂) and ethylene (C₂H₄) in boxes of bananas subjected to vacuum packaging were determined at different points during the domestic inter-island and export sea shipment to Japan as follows:

- DAH 7: 7 days after MA packaging, arrival of bananas at Alter Trade Corp. warehouse
- DAH 8: 8 days after MA packaging, arrival at PHTRC-UPLB laboratory
- DAH 14: 14 days after MA packaging, shipment departure for Japan
- DAH 19: 19 days after MA packaging; arrival of bananas in Japan
- DAH 26: 26 days after MA packaging, opening of the MA-packed bananas for initiation of ripening

Strips of electrical tape were placed onto each LDPE bag, and headspace samples were taken through the tape using a 1.0 mL disposable syringe. The O₂ and CO₂ that accumulated in the headspace were determined using a gas chromatograph (Shimadzu 8A GC) fitted with a thermal conductivity detector (TCD), and for C₂H₄, a flame ionization detector (FID, Shimadzu 12A GC). Upon opening of the vacuum-packaged bananas and during ripening, the incidence and severity of crown rot were monitored in each box following a rating of 0 to 7 [6] as follows (Table 1):

Table 1. Crown rot index used in assessing the severity of infection.

Crown Rot Index	Description
0	no discoloration or mycelial growth on the crown
1	discoloration or mycelial growth limited on the surface of the crown
2	discoloration or mycelial growth less than 10% of crown area
3	11%–40% discoloration or mycelial growth on crown area
4	41%–70% discoloration or mycelial growth on crown area
5	71%–100% discoloration or mycelial growth on crown area
6	Discoloration or mycelial growth advanced to finger stalks
7	Finger stalk rot occurs causing the fingers to drop when handled

The change in the visual quality of the fruit was monitored daily at 23 °C using the following rating: 8, 9 (excellent, field fresh), 6, 7 (very good, defects fair), 4, 5 (good, defects moderate), 3 (limit of marketability), and 1, 2 (poor, limit of edibility). Defects attributed to deteriorative changes after harvest were considered to include discoloration, crown rot, anthracnose, and shriveling. Firmness was determined on two paired sides at the middle portion of the finger using a fruit pressure tester (digital force gauge SX Series Model 2256, Aikoh Engineering Co., Ltd., Osaka, Japan) and the value

was expressed as kg-force. Total soluble solids (TSS) content was determined after homogenizing 10 g pulp in 20 mL water and drops of clear extract was placed in an Atago digital refractometer. Titratable acidity (TA) was determined from the extract titrated with 0.1 N NaOH to a faint pink color with phenolphthalein as indicator. To determine if the different treatments affected the internal quality of the fruit, bananas were subjected to sensory evaluation at the ripe stage with 10 sensory panelists. A 9-point Hedonic rating scale was used where 9 represents the most favorable response.

2.5. Experimental Design

The experiment was laid out in a Completely Randomized Design (CRD) consisting of three replicates per treatment with each box representing a replicate. Each box consisted of 18 clusters of bananas. Analysis of Variance (ANOVA) was done using the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA), and means were compared using the Least Significance Difference (LSD) at 5% level.

3. Results and Discussion

3.1. Gas Levels during Storage

Vacuum packaging resulted in a decrease in O₂ and an increase in CO₂ levels (Figure 1A,B). Pesis et al. [4] reported that after vacuum packaging, O₂ levels retained in the bag ranged from 0.3% to 3% depending on the permeability of the plastic film. Oxygen levels in vacuum packs decreased to 4%–7% while that of the MAP+BS packs ranged from 4%–5% which was lower than MAP alone although differences were not significant (Figure 1A). In the case of CO₂, very low levels (1.08%–2.67%) accumulated in the vacuum packs indicative of dramatic retardation of respiration rate brought about by the modification of the atmosphere. One of the responses of crops to low O₂ is the reduction in the rate of respiration [4,8]; hence CO₂, which is a product of respiration, did not accumulate in the MA packs. Ethylene did not accumulate to levels that would induce ripening of bananas kept under MAP. Throughout the 25-day storage of bananas under vacuum packaging, C₂H₄ concentrations remained low at 0.08–0.17 µL/L.

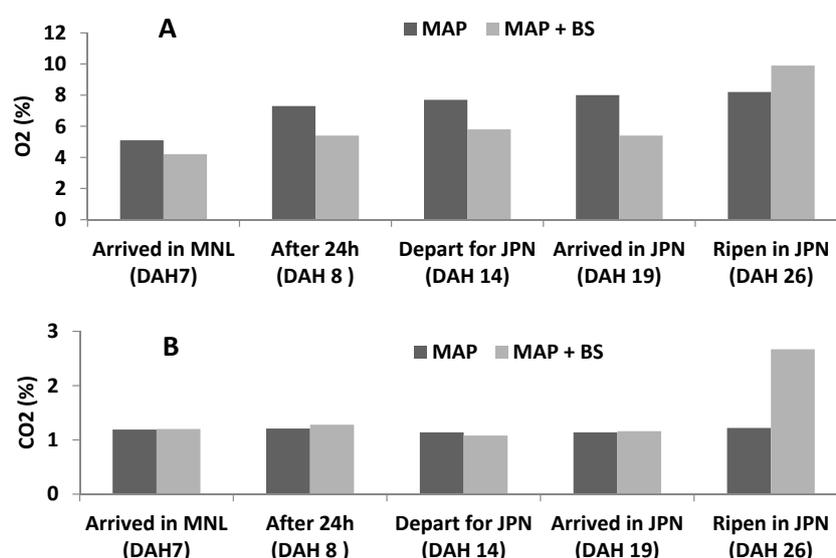


Figure 1. Oxygen (A) and carbon dioxide (B) levels in MA packs of bananas measured at different handling steps from harvest (start of MAP) until bananas reached Japan (simulated sea shipment). Each mean was obtained from three (3) cartons containing 18 clusters of bananas. Differences between treatments were not significant at 5% level, LSD.

3.2. Incidence and Severity of Crown Rot

Modified atmosphere packaging through its active form of vacuum packaging significantly controlled the incidence of crown rot during the 25-day holding under MAP and during ripening in air at 23 °C (Figure 2A). The supplementary treatment of 1% sodium bicarbonate applied in the form of BS did not contribute to the control of crown rot as suggested by the earlier studies of Alvindia [7]. Vacuum packaging in combination with 1% BS resulted in 5.6% incidence of crown rot in contrast to 2.8% in vacuum packaging alone, although differences between treatments were not significant. Crown rot was observed only on the 3rd day of ripening at 23 °C (post-storage) when bananas would already have been sold to consumers in Japan. Severity of crown rot was likewise very low at 0.2 (Figure 2B), and was observed only as surface molds on the crown which can be readily removed by slight trimming of the crown prior to retail packaging. The earlier proposal of Ke and Kader [9] that MAP can replace the use of postharvest chemicals like fungicides and insecticides for disease and insect control, respectively, was apparent in this study. Kader [10] further recommended that MAP can be a component of integrated pest management in fresh produce since low O₂ and high CO₂ might have fungistatic effects.

On the other hand, treatment with 1% BS alone (non-vacuum packaged) did not control crown rot during storage at 13.0–13.5 °C or during ripening in air at 23 °C. Upon removal from cold storage, incidence of crown rot was already high at 16.7% and progressively increased during ripening, reaching 88.9% on the 5th day (Figure 2A). In the control (non-vacuum packed, no BS), crown rot was absent upon removal from storage at 13 °C and became evident only on the 3rd day of ripening at 23 °C. On the 5th day, incidence of crown rot reached 50% and was lower than bananas subjected to 1% BS alone. The increase in the severity of crown rot during ripening of the control and 1% BS-treated fruits closely followed the increase in incidence (Figure 2B). On the 3rd and 5th day of ripening at 23 °C, the severity rating of crown rot in 1% BS alone reached 3.8 (41%–70% discoloration and mycelial growth on crown area), and decay had spread in the pedicel of some fingers in a cluster. The control fruit had a lower crown rot severity rating than the 1% BS-treated fruit, although mycelial growth on the crown was already prominent.

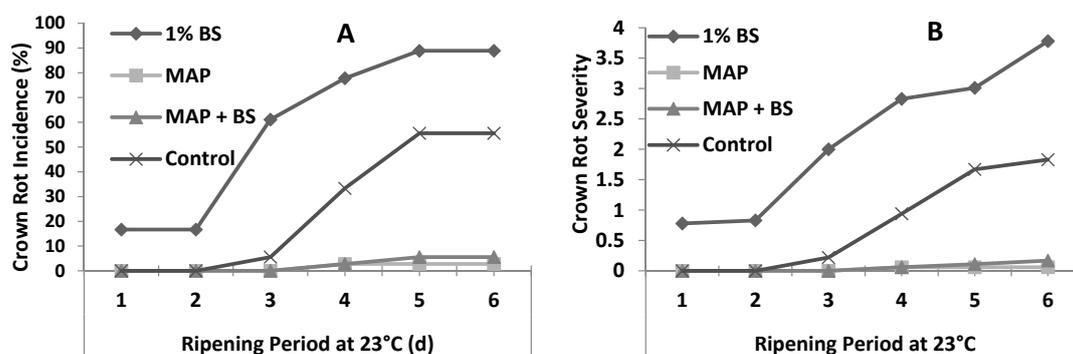


Figure 2. Incidence (A) and severity rating (B) of crown rot of Balangon bananas upon removal from MAP (day 1) and during ripening in air at 23 °C. Bananas were previously kept under MAP for 25 days at 13.0–13.5 °C simulating domestic interisland and export sea shipment to Japan. Each point represents the mean of three replicates per treatment with each replicate consisting of 18 clusters of bananas.

3.3. Rate of Ripening

Upon removal from MAP at 13.0–13.5 °C storage, bananas were still green with one to two fingers in some clusters exhibiting traces of yellow. Generally, however, ripening of Balangon bananas were retarded during the 25-day storage at 13.0–13.5 °C regardless of whether they were held under MAP or not. The prolonged green life of bananas is important in terms of export shipment to Japan where only green or unripe bananas are allowed entry. When bananas were induced to ripen with ethephon and

held in air at 23 °C, the fruit ripened normally attaining the table ripe stage on the 5th day. There were no significant differences in the rate of ripening among treatments indicating that bananas held under MAP were exposed only to mild O₂ stress such that upon return to air, normal metabolism ensued. Mild O₂ stress does not result in injury and can increase longevity and maintain product quality [8,11].

3.4. Visual Quality Rating (VQR)

Due to the low incidence and severity of crown rot of vacuum-packaged Balangon bananas, the decrease in the visual quality or external appearance of the fruit was low during ripening at 23 °C (Figure 3). On the 6th day when bananas were at full yellow peel color, VQR of vacuum-packed banana was 6.2 where fruit were still highly marketable. On the other hand, bananas treated with 1% BS alone and not subjected to vacuum packing exhibited the fastest decline in VQR attributed mainly to the occurrence of moderate to severe crown rot with the pedicel of some fingers in a cluster exhibiting decay. The significantly low VQR of the control fruit was also attributed to crown rot.

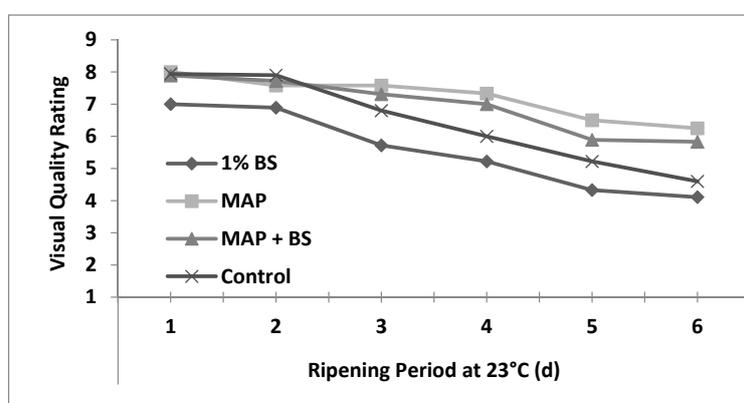


Figure 3. Change in the visual quality rating of Balangon bananas during ripening in air at 23 °C. Bananas were previously kept under MAP for 25 days at 13.0–13.5 °C simulating domestic inter-island and export sea shipment to Japan. Each point represents the mean of three replicates per treatment with each replicate consisting of 18 clusters of bananas.

3.5. Physico-Chemical Changes at The Ripe Stage

Significant difference in firmness at the ripe stage were obtained only between the control and the previously MAP-stored bananas where the control fruits had the higher firmness value (Table 2). The TSS content did not vary significantly among treatments, ranging from 21.2 to 23.5 Brix. Similarly, TA did not vary significantly among treatments. The lack of significant differences in TSS and TA values among treatments indicated that the mild O₂ stress under MAP did not affect the internal quality attributes of the fruit as has been reported by [8].

Table 2. Physico-chemical characteristics of Balangon bananas at the ripe stage¹.

Treatment	Firmness (kg-Force)	TSS (Brix)	TA (%)
1% Baking soda (BS)	0.71 ab	23.4 NS	0.24 NS
MAP	0.66 b	23.5	0.23
MAP + BS	0.71 ab	21.9	0.24
Control (No VP)	0.76 a	21.2	0.25

¹ Balangon bananas were previously stored under MAP for 25 days at 13.0–13.5 °C then withdrawn from MAP and induced to ripen with 2500 µL/L ethephon and ripened at 23 °C. Mean separation within columns for each characteristic at 5% LSD. NS indicates no significant difference among means. Each data represents the mean obtained from 3 replicates per treatment consisting of 3 fruits per replicate.

3.6. Sensory Evaluation at the Ripe Stage

The low O₂ and relatively high CO₂ with MAP storage at 13.0–13.5 °C did not result in anaerobiosis that would lead to development of off-odor and off-flavor at the ripe stage. Bananas when subjected to sensory evaluation were acceptable to the panelists whether fruits were subjected to MAP or not (Table 3). The creamy-white pulp color and aroma did not vary significantly among treatments. Significant differences were obtained in the following sensory attributes: sweetness, balance of sweetness and sourness, flavor, smoothness, and overall acceptability. In all these attributes, MAP-stored and bananas subjected to 1% BS alone had higher scores than the control.

Table 3. Sensory scores of Balangon bananas at the ripe stage ¹.

Sensory Attribute	Sensory Score/Treatment			
	1% BS	MAP	MAP + BS	Control
Pulp color	7.4 NS	7.4	7.5	7.2
Aroma	7.3 NS	7.5	7.5	7.1
Sweetness	7.5 a	6.3 ab	7.1 a	6.0 b
Sourness	6.7 NS	6.8	7.2	6.5
Balance of sweetness & sourness	7.5 a	6.5 ab	6.8 a	5.9 b
Flavor	7.8 a	6.7 ab	7.0 ab	6.3 b
Smoothness	8.0 a	7.3 a	7.6 a	6.3 b
Firmness	7.5 NS	7.3	7.5	6.8
Overall acceptability	7.9 a	7.1 a	7.2 a	6.5 b

¹ Balangon bananas were previously stored for 25 days simulating refrigerated (13.0–13.3 °C) sea shipment from the packinghouse in Bacolod City to the port of Japan then ripened in air at 23 °C. Mean separation within rows for each sensory parameter at 5% LSD. NS indicates no significant difference among means. Each data represents the mean score of 10 sensory panelists. Sensory scores followed: 9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely.

4. Conclusions and Recommendation

Modified atmosphere packaging in its active form as vacuum packaging dramatically reduced the incidence of crown rot of organically-grown Balangon bananas by 94%–97% when used alone, and by 89%–94% when a supplementary treatment with 1% BS was used, indicating that BS can be eliminated. Treatment with 1% BS alone did not control crown rot. Ripening was retarded during the 25-day simulated domestic inter-island and export sea shipment to Japan at 13.0–13.5 °C. The ripening behavior of bananas proceeded normally upon removal from MAP and subsequent exposure to air at 23 °C. The physico-chemical changes in fruit firmness, TSS and TA were not affected, nor were the desirable sensory attributes of bananas at the ripe stage indicating that the mild O₂ and CO₂ stress during storage under MAP did not affect the quality of fruits. These results showed the potential for MAP to reduce losses in Balangon bananas (estimated at 22%) due to delays in shipment to Japan.

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Conflicts of Interest: The authors declare no conflict of interest.

References

1. Valmayor, V.V.; Espino, R.R.C.; Pascua, O.C. *The Wild and Cultivated Bananas of the Philippines*; Philippine Agriculture and Resources Research Foundation, Inc.: Los Baños, Philippines, 2002; p. 242.

2. Alvindia, D.G.; Kobayashi, T.; Yaguchi, Y.; Natsuaki, K.T. Pathogenicity of fungi isolated from non-chemical bananas. *Jpn. J. Trop. Agric.* **2002**, *44*, 215–223.
3. Thompson, A.K. *Controlled Atmosphere Storage of Fruits and Vegetables*; CAB International: New York, NY, USA, 1998.
4. Pesis, E.; Ben Arie, R.; Feygenberg, O.; Villamizar, F. Ripening of ethylene-pretreated bananas is retarded using modified atmosphere and vacuum packaging. *HortScience* **2005**, *40*, 726–731.
5. Kudachikar, V.B.; Kulkani, S.G.; Keshava Prakash, M.N. Effect of modified atmosphere packaging on quality and shelf life of “Robusta” banana stored at low temperature. *J. Food Sci. Technol.* **2011**, *3*, 319–324. [[CrossRef](#)] [[PubMed](#)]
6. Alvindia, D.G. Revisiting hot water treatments in controlling crown rot of banana *cv.* Buñgulan. *Crop Prot.* **2012**, *33*, 59–64. [[CrossRef](#)]
7. Alvindia, D.G. Sodium bicarbonate enhances the efficacy of *Trichoderma harzianum* DGA01 in controlling crown rot of banana. *J. Gen. Plant Pathol.* **2004**, *79*, 136–144. [[CrossRef](#)]
8. Kader, A.A. Regulation of fruit physiology by controlled/modified atmospheres. *Acta Hort.* **1995**, 59–61. [[CrossRef](#)]
9. Ke, D.; Kader, A.A. Potential of controlled atmosphere for insect disinfestation of fruits and vegetables. *Postharvest News Inf.* **1992**, *3*, 31–37.
10. Kader, A.A. The future of modified atmosphere research. *Acta Hort.* **2010**, *857*, 213–217. [[CrossRef](#)]
11. Abdul-Rahaman, A.; Bishop, C. Evaluating the effects of biodegradable and conventional modified atmosphere packaging on the shelf life of organic Cavendish bananas. *J. Postharvest Technol.* **2013**, *1*, 29–35.



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