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Control of Postharvest Crown Rot Disease in Cavendish Banana with Aluminium Sulfate and Vacuum Packaging

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ABSTRACT

Effect of alum in combination with vacuum packaging was investigated in controlling crown rot disease of Cavendish banana (Musa acuminata, AAA, Grande Naine cultivar) at 12-14 ºC. Twelve week mature Cavendish banana fruits were treated with 1% (w/v) alum (Potassium aluminium sulphate), 0.5 g/L carbendazim (Positive control) or distilled water (Negative control). Treated banana samples were packed in Low Density Polyethylene bags(150 gauge) and stored at 12-14 ºC. In-package gases were analysed every seven days up to 28 days of storage.Physicochemical properties (pH, firmness, TSS, TA), sensory properties (peel colour, flesh colour, aroma, flavour, taste, overall acceptability) and crown rot disease severity were determined in ripening induced fruits after each storage period. Data obtained for physicochemical properties and in-package gases were subjected to ANOVA whereas pathological and sensory properties were analysed using Kruskal-Wallis non-parametric statistical test using Minitab. At the end of 28 days of storage, O_2 in all packages remained between 5.6-5.8% while CO_2 remained between 5.0 to 5.1%. Further, treatment of 1% alum alone controlled crown rot disease completely up to 14 days. Most of physicochemical and sensory properties of treated banana were not adversely affected by the treatment.

Keywords: *Crown rot disease, in-package gases, physicochemical, sensory*

INTRODUCTION

Cavendish, a worldwide cultivated commercial subgroup of banana, grown especially for export market, is a pure triploid of *Musa acuminata* (AAA) and its cultivars include, Lacatan, Poyo, Williams, Grand Naine, and Petite Naine (Aurore *et al.,*2009).

Crown rot disease of banana is a serious postharvest disease reducing the storage life which greatly influences export of banana. This disease is caused by a range of different fungi that occur on banana crop debris, including *Colletotrichum musae*, *Lasiodiplodia theobromae* as well as *Fusarium spp*., *Verticillium spp.* and *Cephalosporium spp.* (Abd-Alla *et al.,* 2014).These fungi penetrate directly into the tissues through wounds occurring in the 'dehanding' process and through contaminated water used for washing bananas, which may serve as main source of fungal spores (Hailu *et al.,* 2013).

Chemical control using fungicides such as carbendazim, methyl thiophanate, Imazalil and bitertanol is still the most common practice in controlling crown rot disease of banana**.** An abundance of fungicides are used in banana industry in major Cavendish banana exporting countries in Latin America, Caribbean, Far East and Africa irrespective of its hazardous effects on humans and environment (Lassois and Bellaire, 2014).

However, inorganically grown bananas, wound healing is the key point in preventing crown rot, because fungicide dips are not allowed. Latex

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exudates from the wounds form dark stains, which results in an unattractive appearance of fruits. To prevent this, 1-2% of alum is added to 'delatexing' tanks. Adding of alum helps to remove the latex, controls pathogens in the wash water and promotes the proper healing of the wound at crown (Anyasi *et al.,* 2013).

Alum (Potassium Aluminum Sulfate), double sulfate with the formula of $KAl(SO_4)_2.12H_2O$, is generally an odourless, colourless crystalline solid that turn white in air, which is used as an astringent and antiseptic in various food preparation processes such as pickling and fermentation and as a flocculent for water purification (Clark, 1970). Alum is added in the form of $KAl(SO_4)_2.12H_2O$,which constitutes of tri-valent aluminium (Al^{3+}) in the treatment solution.Tri-valent aluminium is known to coagulate colloidal organic impurities in drinking water, either by forming soluble complexes at pH less than 4.5 or by the adsorption on aluminium hydroxide crystals formed at pH of 5 to 7. In treatment of low turbidity drinking water, alum is used at concentrations of up to 2 ppm Al^{3+}. A solution of 12 ppm Al^{3+}, as used in Philippines in de-handing and flotation tanks, would be expected to assist in the coagulation of banana sap (Speiser and Berge, 2014). Food and Drug Administration (FDA) has recommended alum as category I active ingredient in mouthwashes (Olmez *et al.,* 1998). Potassium Aluminum Sulfate solution has also been used to prolong shelf-life of tomatoes (Cemanes and Gabornes, 2013). Petrovsky and Aguilar (2004) reported on the use of alum as an adjuvant in the production of numerous vaccines including diphtheria-tetanus-pertussis, human papillomavirus and hepatitis vaccines.

However, to the best of our knowledge, there are no reports on the effect of alum as a sole source in controlling crown rot disease of Cavendish banana. Therefore, this research was conducted to identify the efficacy of alum in controlling crown rot disease and extending shelf life of Cavendish banana which were subjected to vacuum packaging and stored at 12-14 ºC up to one month. We aimed to examine headspace respiratory gas composition and crown rot disease severity and to evaluate physicochemical and sensory properties of alum treated Cavendish banana, which were subjected to packaging and storage as above.

MATERIALS AND METHODS

Fruit harvesting, packaging and storage

Twelve week mature Cavendish banana (Grande Naine cultivar) bunches were harvested from CIC banana plantation in Pelwehera, Dambulla, Sri Lanka. Banana bunches were transported to the CIC banana pack house, in CIC Agri Business Centre, Dambulla, Sri Lanka. Bunches were 'dehanded'and approximately 1 kg hands were selected as experimental units. All hands were washed in water to remove dirt and then washed with potassium aluminium sulphate (alum) (1% w/v) except control. A fungicide treatment of carbendazim (0.5 g L^{-1}) and distilled water control were also included as positive and negative controls respectively. Banana hands were allowed to drip dry and placed separately in low density polyethylene (LDPE) bags (0.076 mm thickness) of 31.5 × 32 cm surface area and polyethylene foam liners were placed on top of banana to provide protection to fruits. Air inside the bags was removed using a vacuum cleaner and mouths of bags were tied tightly with rubber bands and packed in (40×29×19 cm^3) ventilated 3-ply fiberboard cartons. Each treatment comprised of four replicate boxes, each with five hands (weighing 5.0-5.5 kg). All treatments were stored at 12-14 ºC in a cold room at 85-90% relative humidity at CIC Agri Business Centre, Dambulla, Sri Lanka (Abeywickrama *et al.,* 2009). Observations were made after 7, 14, 21 and 28 days of storage. The experimental arrangement was a completely randomized design (CRD). This experiment was repeated once under identical conditions.

In-package gas analysis

In-package respiratory gas (O_2 and CO_2) variations within bags were measured on7th, 14th, 21st and 28th days during cold storage using a Digital Oxygen and Carbon Dioxide Head Space Gas analyzer (Model 902 D, Quantek Instruments, Grafton, MA). A needle was inserted into each bag and a small sample of package headspace gas was pumped into the gas analyzer and recorded the oxygen and carbon dioxide measurements (Kudachikar *et al*., 2011). Five replicate measurements were taken per treatment.

Ripening of banana

After each storage period, banana hands were subjected to induced ripening by exposure to ethylene (thrill – 480 g/L ethephon, 1 mL/L of water) for 24-48 h at ambient temperature of 26 ± 2 ºC (Siriwardana *et al.,* 2016).

Pathological properties

Crown rot in each hand was recorded using a standard index developed at the Department of Botany, University of Kelaniya (Crown Rot Severity (CRS) 0=No rot, 1=25% Crown rot, 2=50% Crown rot, 3=75% Crown rot, 4=100% Crown rot) (Abeywickrama *et al.,* 2009; Siriwardana *et al.,* 2016).

Physicochemical properties

Ten randomly selected fingers from each treatment were analyzed for physicochemical properties.

Total Soluble Solids (TSS): A 10 g sample of pulp from the middle of the fingers of banana was homogenized with 40 mL of distilled water in a blender (Black & Decker, BX 250, Hunt Valley, USA) for 2 min. The homogenate was filtered and the filtrate was taken to measure the TSS using a hand-held Refractometer (ATC, ATAGO, Japan, Brix; 0-32%). The actual TSS content was calculated by multiplying each reading with the dilution factor (Abeywickrama *et al.,* 2009; Siriwardana *et al.,* 2016).Ten replicate samples were used per treatment.

pH: pH of the filtrates was measured using a digital pH meter (PC 510, EUTECH Instruments, Singapore). Ten replicate samples were used per treatment (Abeywickrama *et al.,* 2009; Siriwardana *et al.,* 2016).

*Titratable Acidity (TA) (% acid):*Ten (10) mL samples from filtrates prepared for the TSS test were diluted with 20 mL distilled water and titrated against 0.1 M NaOH with phenolphthalein as the pH indicator. The end point was taken as the sudden appearance of slight pink colour in the solution. TA was calculated by multiplying the NaOH volume with the dilution factor and the malic acid factor (malic acid factor=0.0067 g).TA was expressed as % malic acid (Abeywickrama *et al.,* 2009; Siriwardana *et al.,* 2016). Ten replicate samples were used per treatment.

Firmness: Fruit firmness of the pulp was measured using a Fruit Firmness Tester (FT 011, QA Supplies, Italy). The probe was gently pressed against a cross cut section (1 cm thickness) of a finger until it indicated a constant value (Abeywickrama *et al.,*2009; Siriwardana *et al.,* 2016). Ten replicate samples were used per treatment.

Sensory properties

Peel colour, flesh colour, flavour, taste, aroma, texture and overall acceptability of fruits were assessed by a trained ten member sensory panel. Each quality parameter was scored as follows: Excellent=9-10, Good=6-8, Fair=4-5, Poor=1-3 (Abeywickrama *et al.,* 2009; Siriwardana *et al.,* 2016).

Statistical analysis

Data obtained for in-package gases and physicochemical properties were subjected to ANOVA and mean separation was done using Tukey's Multiple Comparison test using Minitab. Data obtained for pathological and

sensory properties were analysed using Kruskal-Wallis non-parametric statistical test.

RESULTS AND DISCUSSION

In-package gas analysis

Oxygen level in all treated and control samples slightly decreased through 28 day storage period and final O_2 levels were between 5.6-5.8% (Fig. 01.). Carbon dioxide level of alum treated banana increased slightly through the test period. However, in fungicide treated and control samples, gas levels increased slightly up to day 21 and thereafter remained more or less constant and final CO_2 levels were within the range of 5.0-5.1% (Figure 01). There was no significant difference of gas levels between treatments and the control.

The net extension in shelf life can be attributed to the overall low O_2 retention that prevailed through the storage of vacuum packed samples. This modification in the gas composition by vacuum packaging reduces the fruit respiratory intensity and hampers endogenous ethylene production, which can considerably increase the length of the preclimacteric phase. Low O_2 may also inhibit the metabolism of some pathogenic agents that can survive on the crown of banana.

In vacuum packaged banana (cv. Pachbale) stored at 13±1ºC for 21 days, O_2 concentrations were between 2-4% while CO_2 ranged between 4-6% (Chauhan *et al.,* 2006). Abdulla *et al*., (1993) reported that banana cv. Berangan packed in polyethylene bags with or without ethylene absorbent maintained the levels of CO_2 and O_2 around 3.4%-6.7% and 1.6%-6.1%, respectively from first to fourth week of storage at 14 ºC.These findings are in accordance with the current research data.

Pathological properties

Alum treatment showed no crown rot disease up to 14 days, but, showed mild crown rot disease (CRS = 0.3; 7.5% crown rot) after 21 days (Figure 02). Fungicide treatment also showed similar crown rot disease severity on day 21. Control banana showed higher crown rot disease severity in all analysis days compared to other treatments, with the highest mean crown rot disease severity of 1.7 (42.5% crown rot) on day 21.Crown rot disease severity of alum and fungicide treated banana was significantly low compared to the control ($P< 0.05$).

In agreement with the present results, Abeywickrama *et al.,* (2009) reported that, 1% alum washed and vacuum packed *Embul* banana showed lower crown rot disease compared to control samples in cold storage at 13-15 ºC. Alum at 1% (w/v) prevented mycelial growth of crown rot pathogen *L. theobromae* and papaya stem end rot pathogen *Phomopsis caricae-papayae* during *in vitro* assay (Abeywickrama *et al.,* 2012).

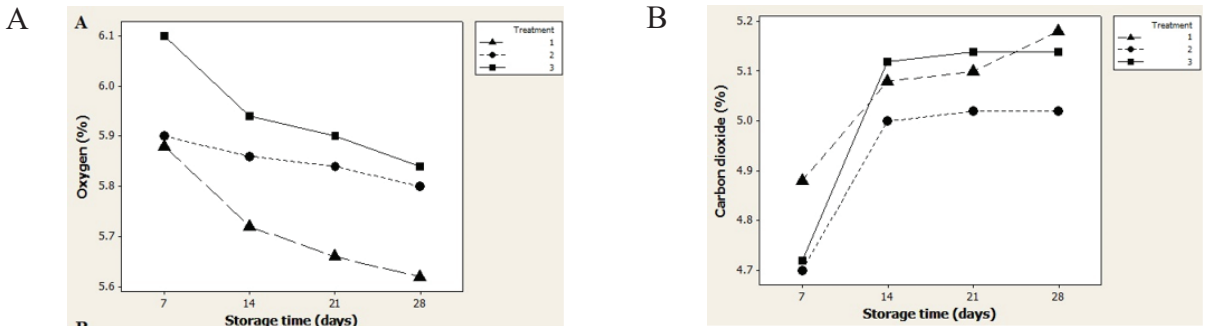

Figure 01: Oxygen (A) and Carbon dioxide (B) gas concentrations of vacuum packaged Cavendish banana(Treatment: 1 – 1% alum, 2- 0.5 g L^{-1}carbendazim 3- control)

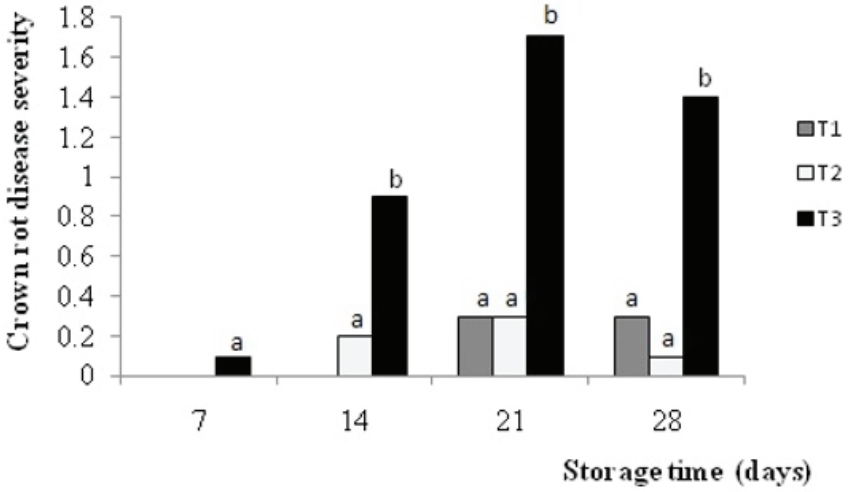

Figure 02: Crown rot disease severity of vacuum packaged Cavendish banana treated with 1% alum (T1), 0.5 g L^{-1}carbendazim (T2) and distilled water (T3) after each storage period at 12-14 ^{0}C and subjected to induced ripening.

Each data point represents the mean of ten replicates.

Bestoon (2012) reported on the antibacterial action of alum solution (1 mg /mL, at pH 3.6) against bacterial isolates found in infected root canals, including facultative anaerobic microorganisms (*Escherichia coli, Staphylococcus aureus* and *Klebsiella* sp.), and aerobic species (*Pseudomonus aeruginosa*), using agar well diffusion test. Alum solution was able to demonstrate antibacterial activity against all the bacteria tested, and produced inhibition zones of 27, 25, 24 and 22 mm against *S. aureus, P. aerogenosa, E.coli* and *Klebsiella* sp. respectively.

Bnyan (2014) tested different concentrations of alum (10, 20, 30, 40 and 50) w/v % against four bacterial isolates (S. *aureus*, *S. epidermidis*, *E. coli*, *Klebsiella pneumonia*) and the results showed that the 20% of alum was found to be Minimum Inhibitory. Also, from the results, it was observed that the bacterial growth inhibition was increased when the alum concentration increased. Exact action of alum on crown rot disease control is not known. However, mechanism of action of alum against microorganisms would be due to the competition for nutrients and sites in the wound so the pathogen is unable to grow.

Physicochemical properties

TSS: During the current research, TSS decreased up to 14th day and then increased in all treatments and in the control**.** Higher TSS values were reported for control samples over alum and fungicide treated samples except on day 7**.** Total Soluble Solid values ranged from 15.50 to 20.80 (^{0}Brix) during the 28-day storage period (Table 01.). Further, TSS values of alum treated banana were not statistically significant, compared to the control except on day 7.

During banana ripening, sugars increase as starch is converted to soluble solids, of which sucrose comprised more than 70% of the total sugars in fully ripe banana, followed by glucose and fructose (Marriott *et al.,* 2006). Total Soluble Solids (TSS) is an important measure related to consumer taste preference and fruits above 12% Brix are considered more acceptable to consumers (Mcglone and Kawano, 1998). According to Opara *et al.,* (2013) TSS of ripe Dwarf Cavendish banana were within 19.6 -

21.2 ^{0}Brix while 14.00 ^{0}Brix was reported for TSS of Grande Naine banana (Dadzie, 1998). Results obtained during current research are compatible with previously published literature.

pH: pH values of all treated and control samples dropped over time except in alum treated samples on day 28 and the values ranged from 4.65-4.93 (Table 01). pH values of alum treated Cavendish banana were not significantly different compared to the control in each analysis day, except on day 21.

pH is high in mature green banana and gradually decreased with ripening. pH of the pulp of the green banana fruit ranged between 5 - 5.8 while pH of the pulp of ripened banana fruit ranged between 4.2 - 4.8 (Hailu *et al.,* 2013). Marin *et al.,*(1996) reported, pH of Grande Naine (AAA) banana after ripening were in the range of 4.94-4.95 while, Opara *et al.,* (2013) reported pH of Dwarf Cavendish banana were to be in the range of 4.98-5.43. According to Dadzie (1998), pH of Grande Naine banana after ripening attained a value of 4.93.Therefore, current results are in agreement with the previous literature.

TA: There were slight variations of titratable acidity over time and values ranged from 0.65 to 0.77 (% Malic acid) (Table 01.).TA values of alum and fungicide treated banana were not significantly different, compared to the control. With ripening of banana, TA increases causing a drop in the pH. According to Dadzie (1998), TA of Grande Naine banana was about 0.30% Malic acid after ripening while Opara *et al*., (2013) reported TA values in Dwarf Cavendish banana to be in the range of 0.34-0.41% Malic acid. Current results are higher than previous literature reports indicating higher acidity of samples.

Firmness: Firmness values of samples ranged from 0.39 to 0.47 (kg cm^{-2}) in all treatments and in the control (Table 01.). Although there were slight variations of firmness over time, by day 28 firmness values decreased. Lowest firmness was seen in the control sample while alum treated banana showed highest firmness on day 28 (Table 01.). Firmness values in all treated and in the control banana were not significantly different.

During ripening of banana, firmness decreased to a relatively narrow optimal eating range of 0.7-0.4 kg cm^{-2} beyond which the fruit becomes senescent. This decrease in firmness is due to solubilization of peptic substances in the cell wall and middle lamella (Dadzie, 1998). In accordance with the current results, Opara *et al.,* (2013) reported that fruit firmness of Dwarf Cavendish banana gradually decreased over 21-day storage period at 11-12 ºC.

Sensory properties

Sensory panelists preferred the alum treated banana over the fungicide treated and the control banana after 28 days of storage which were subjected to induce ripening. Score values obtained for alum treated Cavendish banana were 6 or above indicating samples were of 'good' quality (Table 02.).There was no significant difference of sensory properties of alum treated banana, except for flavour and taste compared to the control. Similarly, Abeywickrama *et al*., (2009) reported that sensory properties of 1% alum washed and vacuum packed Embul banana were slightly affected compared to untreated fruits.

Table 01: Physicochemical properties of vacuum packaged Cavendish banana stored at 12-14 °C after induced ripening.

Treatment	Storage time			
	day 7	day 14	day 21	day 28
	pH			
T1	4.93 [a] ± 0.04	4.85 [a] ± 0.04	4.65 [a] ± 0.03	4.78 [a] ± 0.05
T2	4.91 [a] ± 0.06	4.88 [a] ± 0.00	4.78 [b] ± 0.02	4.74 [a] ± 0.01
T3	4.86 [a] ± 0.02	4.88 [a] ± 0.03	4.74 [b] ± 0.03	4.73 [a] ± 0.04
	TSS (^{0}Brix)			
T1	20.80 [a] ± 0.48	15.80 [a] ± 0.30	17.30 [a] ± 0.58	17.00 [a] ± 0.28
T2	17.80 [b] ± 0.20	15.50 [a] ± 0.43	17.40 [a] ± 0.40	17.30 [a] ± 0.57
T3	18.50 [b] ± 0.48	16.30 [a] ± 0.30	17.90 [a] ± 0.34	17.70 [a] ± 0.54
	Firmness (kg cm^{-2})			
T1	0.41 [a] ± 0.01	0.44 [a] ± 0.00	0.47 [a] ± 0.02	0.43 [a] ± 0.01
T2	0.43 [a] ± 0.02	0.43 [a] ± 0.02	0.47 [a] ± 0.02	0.41 [a] ± 0.00
T3	0.39 [a] ± 0.01	0.43 [a] ± 0.01	0.44 [a] ± 0.02	0.40 [a] ± 0.00
	TA (% Malic acid)			
T1	0.72 [a] ± 0.03	0.72 [a] ± 0.04	0.77 [a] ± 0.04	0.70 [a] ± 0.04
T2	0.73 [a] ± 0.03	0.65 [a] ± 0.04	0.75 [a] ± 0.04	0.68 [a] ± 0.03
T3	0.75 [a] ± 0.03	0.67 [a] ± 0.01	0.75 [a] ± 0.03	0.73 [a] ± 0.03

T1 – 1% alum, T2- 0.5 g L^{-1} carbendazim, T3- control.

*Each data point represents the mean of ten replicates ± standard error.

*Means sharing a common letter (s) in each column are not significantly different by Tukey's multiple comparison test.

Table 02: Sensory scores obtained for treatments stored for 14 days at 12-14 ^{0}C after induced ripening from sensory panel at University of Kelaniya.

Treatment	Sensory property						
	Peel colour	Flesh colour	Flavour	Aroma	Taste	Texture	Overall acceptability
1% alum	6.7 [a]	7.0 [a]	7.3 [a]	6.9 [a]	7.6 [a]	7.1 [a]	7.1 [a]
1% alum	6.6 [a]	6.8 [a]	6.3 [b]	6.7 [a]	6.2 [b]	6.5 [a]	6.4 [a]
control	6.7 [a]	6.9 [a]	6.1 [b]	5.9 [a]	5.8 [b]	6.1 [a]	6.2 [a]

*Each data point represents the mean of twenty replicates.

(Excellent 9-10, Good 6-8, Fair 4-5, Poor 1-3).

*Means sharing a common letter (s) in each sensory property are not significantly different by Kruskal Wallies non parametric statistical test.

CONCLUSIONS

Alum treatment combined with vacuum packaging controlled crown rot disease of Cavendish banana completely up to two weeks in cold storage. Most of the physicochemical and sensory properties of treated samples were not significantly different compared to the control samples, although with slight variations. Alum treated samples were preferred by sensory panelists than the other treatments. Vacuum packaging of banana does not involve application of any potentially toxic chemical but only involves washing with alum (potassium

aluminum sulfate) solution.This treatment of alum combined with vacuum packaging is a relatively cost effective technology, which could be used in organic banana industry where fungicides are not allowed in sea shipments or air freight where two weeks of transit time is required.

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