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CONTROL OF PHYSICAL FACTORS FOR ENHANCED POSTHARVEST MANAGEMENT OF GRAPEFRUIT QUALITY

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ABSTRACT

Grapefruit is the fruit of a subtropical citrus tree known for its bitter taste. The variety chiefly grown in Cameroon is yellow skinned and ranges in diameter from 10 –20 cm. They are mainly cultivated in the Southern parts of the country and transported to other regions by trucks and in bags stacked on each other. Due to poor state of farm to market roads, especially in the rainy season, delays in transportation create room for the development of a warm humid environment in the bags. This gives suitable ground for the growth of the green mold, *Penicillium digitatum* (Pers.: Fr.) Sacc., which further causes softening and decay of the fruits.

We studied the effect of temperature and bagging on the growth of *P. digitatum* on grapefruit under different storage conditions for 14 days. Grapefruit was inoculated by *P. digitatum* and water as control. Samples were subsequently packed in paper boxes, bagged and un-bagged before storage at +4ºC and room temperature (RT). Measurements were obtained for weight loss, total soluble solid (TSS), degree of damage and decay development. Results showed that the grapefruits inoculated with *P. digitatum* at room temperature both bagged and un-bagged were completely decayed. However, storage at +4ºC could retard the growth of *P. digitatum* as there was no observable development of decay on the control that showed 40% and 65% decay development when bagged and un-bagged respectively at RT. Storage in bagged conditions reduced weight loss and prevented chilling injury when kept at +4ºC. Results of TSS were not remarkably changed after storage in all treatments while degree of damage was observed to be higher in fruits which were stored at RT in un-bagged boxes. Combination of proper washing,

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bagging in polyethene and cold storage at +4°C inhibit the growth on and spoilage of grapefruits by micro-organisms and will prevent chilling injury.

Keywords: Decay development, P. digitatum, Citrus paradisi, storage

INTRODUCTION

Citrus fruits are considered the most important fruits in the world and grapefruit has a great value in human diet (Ali, 2005). Grapefruit (*Citrus paradisi* Macf.) is the fruit of a subtropical citrus tree known for its bitter taste. The variety chiefly grown in Cameroon is yellow skinned and ranges in diameter from 10 –20 cm. The flesh is segmented and acidic, varying in sweetness and color (white, pink and red) depending on the cultivar and the region where the tree is planted. Grapefruit is a good source of vitamin C, fiber and pectin. The pink and red hues contain the beneficial antioxidant lycopene (Lee, 2000). It is also an excellent source of phyto-chemicals that contribute to a healthy diet (Uckoo *et al.*, 2012).

Fruit quality usually falls gradually after harvesting due to changes in its compositions brought about by mechanical and biological factors (Biolatto *et al.,* 2005). Grapefruit like all citrus fruits is susceptible to chilling injury (CI) during cold storage (Porat *et al.*, 2000). This sensitivity to low temperatures has serious economic implications, since cold storage still provides an important quarantine treatment required to export citrus fruits to many countries (Porat *et al.*, 2000). Several postharvest heat treatments have been reported to induce fruit tolerance to cold temperatures and to reduce the development of CI symptoms during cold storage and cold quarantine treatments (Wang, 1993; Lurie, 1998; Schirra and Ben-Yehoshua, 1999).

In Cameroon, grapefruit can be found on sale in the open, placed on tables or on the ground or in small kiosks almost throughout the year. They are mainly cultivated in the Southern parts of the country and transported to other regions in bags, stacked on each other in trucks. Coupled with delays in transportation due to the poor state of farm to market roads, especially in the rainy season, the bags create an environment with elevated temperatures and moisture. This condition is conducive for growth of micro-organisms which cause softening and decay of the fruits.

During transportation, fruits are bruised easily when packed in bags and stacked on each other without support. Bruising and stacking create wounds, becoming sites for easy penetration of micro-organisms that cause fruit deterioration. *Penicillium digitatum* (green mold) and *P. italicum* (blue mold) are the most important of these micro-organisms in all citrus growing areas of the world (Ballester *et al.*, 2010). However, *P. digitatum* is the most prevalent in locations with arid and subtropical climates (Kanetis and Adaskaveg, 2008). They are ubiquitous and reproduce very rapidly on fruit surfaces hence gain access when the fruits are bruised or wounded. The initial symptom, a softening of the exocarp, is visible within 48 hours. In theory, if fruit injury could be reduced to zero, no disease would be present (Droby *et al*., 2008).

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In fully mature citrus fruits, increased resistance against *P. digitatum* infection can be achieved by application of physical (Arcas *et al*., 2000), chemical (Porat *et al*., 2001, 2002; Venditti *et al*., 2005), or antagonistic microbial (Fajardo *et al*., 1998; Droby *et al*., 2002) treatments. There are several topical and systemic fungicides in the market available for the control of these fungi in storage. However, in Cameroon because the farms and markets are poorly organized and controlled, fungicide use is minimal. The efficacy of these treatments in eliciting induced resistance is variable, and in many instances depends on the maturity of the fruit.

Actual losses due to these diseases are quite variable and depend on the area of production, citrus variety, tree age, weather conditions during the growing and harvest season, the extent of physical injury to the fruit during harvest and subsequent handling, the effectiveness of antifungal treatments, type of packaging used for transportation and storage, postharvest environment and the time it will take from harvest to when it gets to the consumer. Most times, more than 40% of the fruits are lost before they finally get to the consumer and this greatly affects the price per fruit. Between the farmers and the consumers are traders commonly called *buyam-sellams*. These *buyam-sellams* harvest and transport these fruits to retailers who then take the fruits to markets where consumers can buy.

In this study, we evaluate the effect of presence of micro-organisms, packaging conditions and storage temperature on the shelf-life grapefruit.

MATERIALS AND METHODS

Grapefruit for this study were harvested same day from the fruit orchard of the Institute of Agricultural Research for Development (IRAD) Ekona and taken to the JP Johnson Biotechnology Laboratory. A total of 101 fruits were used for this study. Five fruits were used for measurement of total soluble solids (TSS) while the remaining 96 fruits were subjected to bagging, storage at +4^{o}C and room temperature (RT) and inoculation of fruits with *P. digitatum* and water.

Inoculation with water served as control for these treatments. Table 1 below summarizes the different treatments and the quantities used.

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Table 1: Summary of treatments effected

INOCULUM	Bagged fruits		Unbagged fruits	
	Storage at +4ºC	Storage at RT	Storage at +4ºC	Storage at RT
P. digitatum spores	12	12	12	12
Water	12	12	12	12

Five fruits from each treatment were marked and weighed using a balance to assess weight loss.

2.1 Inoculum preparation and fruit Inoculation

Grapefruit was cut and allowed to decay. Decaying portions were inoculated on Potato Dextrose Agar (PDA)containing 1g/L of Cefotaxime to isolate pure cultures of fungus *P. digitatum,*. The plates were incubated at 30ºC for 5 days. Subcultures were made to obtain pure colonies were. On fresh plates, pure cultures were left to overgrow for ten days. The spores were harvested by flooding the pure culture plate with sterile distilled water containing 0.3%Tween 20. The spore suspension was then adjusted to a concentration of 10^6spores/mL using a haemocytometer.

Spots were marked on the equator of each fruit to serve as sites for inoculation. Inoculation was effected by dipping a sterile pin into the inoculum and puncturing the fruits at the marked sites. A total of 48 fruits were inoculated with spores and the balance 48 inoculated with water. Each of these sets was subdivided into 4 groups of 12 fruits each.

2.2 Measurement of weight loss

Five fruits were selected from each treatment and marked. Their masses were measured on the first and the last day of the experiment using a balance and recorded. The difference in weight was recorded as weight loss.

2.3 Decay development

Decay development was carried out by visually inspecting the fruits for fungi growth on the surface, and a further touching for softening. Results were positive when fruits presented softening with or without visible fungal growth. Decay was absent if fruits remained firm with no visual indication of fungal growth. The percentage of fruits decayed was then calculated as follows.

$$\%\ of\ decayed\ fruits = \frac{number\ of\ decayed\ fruits}{12} \times 100$$

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2.4 Measurement of Degree of Damage (DOD)

This measurement was done on 05 fruits from each of the eight treatments using a locally fabricated pressure machine. On its one end weights can be added to exert pressure on the fruit and on the other end where the fruits are placed there is a nipple to read fruit diameter in centimeters. The fruit was placed on the machine, the machine zeroed and the nipple reading recorded. A pressure was induced on the fruit by placing a 02Kg mass on the weight end. After 20 seconds, the nipple reading was recorded. The DOD was calculated as the difference of the initial and final readings.

2.5 Measurement of Total Soluble Solids

Total Soluble Solids (TSS)was measured using a digital refractometer. The machine was zeroed using distilled water. Fruits were cut transversely and the juice was squeezed just to fill the chamber for measurement. The degree Brix was read directly from the instrument and recorded.

3.0 RESULTS

From analysis, the following results were recorded.

3.1 Weight Loss

Greater weight loss was generally incurred for fruits that were inoculated with fungi, un-bagged and stored at RT as opposed to those inoculated with water, bagged and or stored at +4°C respectively. Considerable loss was seen for fruits inoculated with fungi, left un-bagged and stored at RT (5.2g) while the fruits inoculated with water, bagged and stored at +4°C presented least weight loss (0.2g) as shown in figure 1 below.

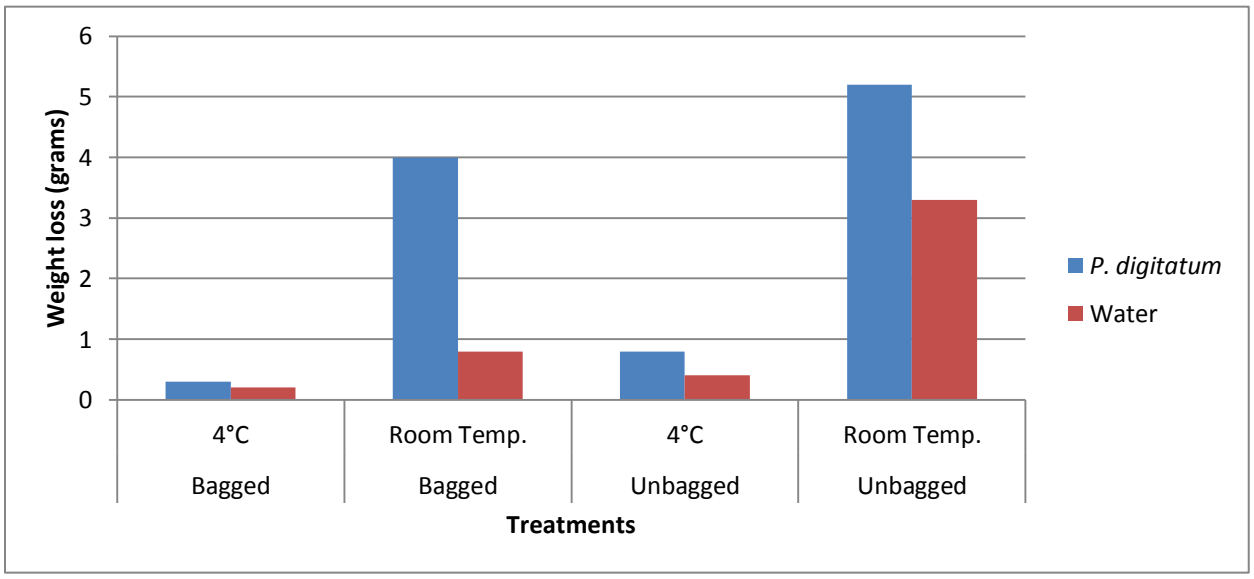

Figure 1. Weight loss (grams) of grapefruit inoculated with *P. digitatum* and water after 14days

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3.2 DECAY DEVELOPMENT

After 14 days of storage, it was observed that all fruits inoculated with *P. digitatum* were soft and decaying. Those stored at RT showed advanced colony development and spore formation seen as a greenish colour whereas those at +4ºC, showed mild mycelia development and no spores formation.

Despite the inoculum used, all fruits stored at RT presented signs of decay, whereas for those stored at +4ºC, only those inoculated with the fungus presented mild mycelia development.

As concerns the effect of bagging, 65% of un-bagged fruits inoculated with water and stored at RT were soft and decaying as opposed to a lesser 40% with bagged fruits.

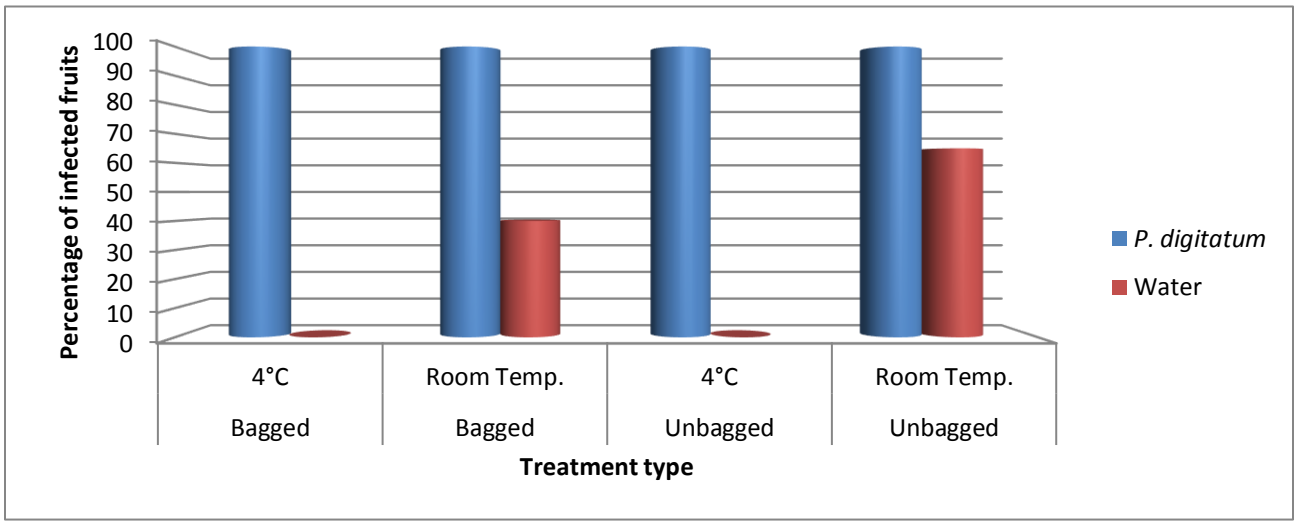

Figure 2 Effect of treatments on decay development.

3.3 Firmness

All fruits inoculated with *P. digitatum* softened and decayed as shown in figure 3 below. Fruits inoculated with water, bagged and storage at +4ºC remained firm even after 14 days.

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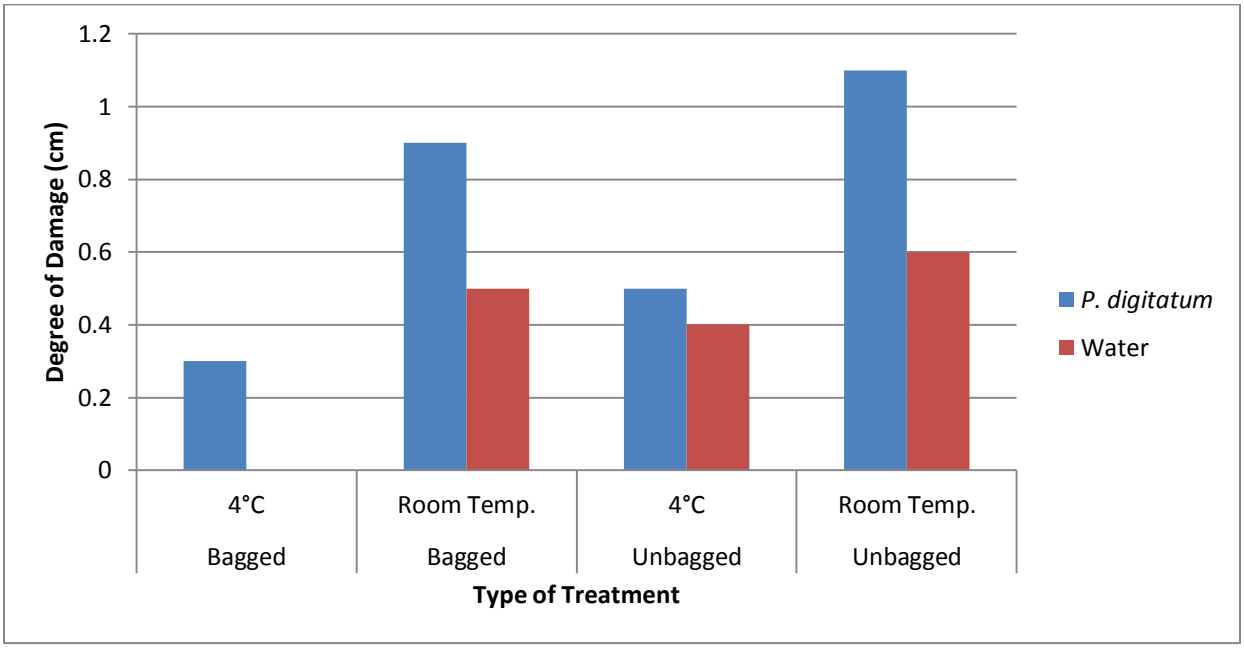

Figure 3 Measurement of DOD (cm) after 14 days storage of fruits.

3.4 Total Soluble Sugars (TSS)

Higher TSS values were observed with fruits that were un-bagged. Fruits stored at RT equally presented higher TSS values as compared to those stored at +4°C.

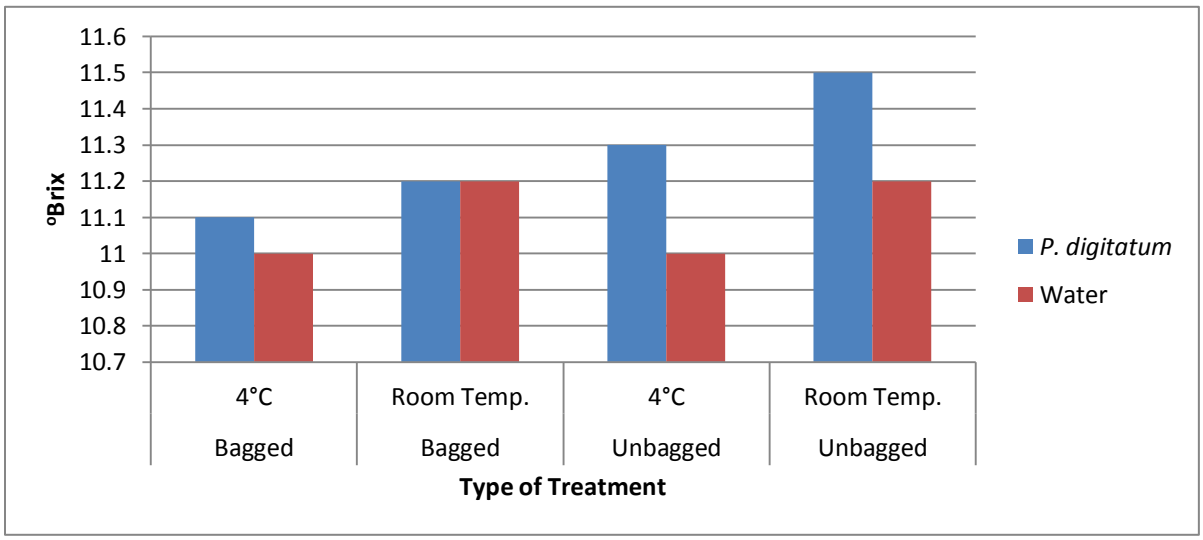

Figure 4 Total Soluble Sugars after incubation

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DISCUSSION

Penicillium digitatum is the major cause of citrus fruit deterioration, and is responsible for about 90% of production losses during post-harvest handling (Macarison *et al*., 2007). In our study, all of the fruits inoculated with the fungus and stored for 14 days were spoilt. Decay development on fruits inoculated with *P. digitatum* occurs in a circular manner beginning with the softening of the fruit as the mycelia spread followed by patch of visible mold growing on fruit surface.

Results we obtained showed that fruits stored at +4ºC presented a lesser average weight loss of 0.45g as opposed to 10.5g when left at RT. None of the fruits inoculated with water and stored at +4ºC showed any symptom of *P. digitatum* infection compared to 40% and 65% of fruits bagged and un-bagged respectively, when stored at RT. The cold storage of grapefruits reduces the rate of spoilage. In their study, Porat *et al.* (2000) termed cold storage as well as cold quarantine as important requirements for the exportation of citrus fruits. To achieve this, small buyam-sellams need to get together as a cooperative. Only when this is done, can they effective build and run appropriate packing houses and get government support for their activity (Levai *et al.*, 2015).

A further confirmation of the destructive effect of leaving fruits un-bagged is seen with the average DOD of 0.65cm observed when the fruits were left un-bagged compared to a lower 0.43cm when bagged during storage. The average weight loss of fruits when bagged is lower (1.45g) than when left un-bagged (2.4g). Bagging therefore limits weight loss, softening and decay of fruits.

Some of the grapefruits which were un-bagged and stored at +4ºC suffered from CI. However, fruits bagged and stored at the same temperatures did not show any sign of CI, suggesting that citrus can be stored at lower temperatures if bagged in the appropriate material. Previous studies have shown that the optimum temperature for storage of grapefruit is between 10 – 15ºC (Shellie, 2002). In this study, we observed that storage at temperatures as low as +4ºC in polythene bags will prevent grapefruit from CI. This temperature also reduces the metabolic rate of *P. digitatum*.

Lower TSS values were generally observed when the fruits were bagged than when left un-bagged, when stored at +4ºC than at RT, and when inoculated with water than fungi. Bagging creates an environment low inO_2 and a probable increase in the amount of CO_2as the fruits start anaerobic respiration. The cold limits all metabolic activities and the absence of additional microbes contribute to a lesser breakdown of the fruit substrates to yield simple sugars. Washing of fruits before bagging will reduce the microbial load on the fruit surface. Hence the absence of fungi, bagging and storage at +4ºC are favourable for grapefruit storage.

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CONCLUSION

Combination of proper washing, bagging in polyethene and cold storage at +4ºC inhibit the growth on and spoilage of grapefruits by micro-organisms. Bagging in plastic wrappings and storage at +4ºC reduces chilling injury on grapefruit.

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