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Research Application Summary

Efficacy of sodium bicarbonate and chlorine based sanitizers on postharvest microbial load and quality attributes of packaged tomato fruits

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Abstract

The high potential of tomato production is not realized in Kenya due to various factors in the value chain such as high postharvest losses estimated to be 40-50%. These losses are as a result of many factors including poor postharvest handling, lack of storage facilities and deteriorative agents such as postharvest diseases. Microbial decay alone accounts for 15% of the postharvest losses. Some of the methods and treatments used to manage microbial decay are costly and unhealthy. In this study, sodium bicarbonate (baking soda) which is a low cost sanitizer was evaluated for its effectiveness to manage fungal rots in comparison with the commonly used chlorine based sanitizers. The fungus was isolated on potato dextrose agar media (PDA). The mature green tomato fruits were wounded (4 wounds, 3mm deep near stem end) and artificially inoculated with a spore suspension of 106 conidia/ml of Alternaria alternata. Different sanitization treatments were then applied to control the fungal rots. The treatments included 30,000 ppm sodium bicarbonate solution; 150 ppm chlorine solution; 200 ppm NaOCl solution; water and untreated samples which served as a negative control. The fruits were then air dried and either packaged into special modified atmosphere packaging (MAP) bags or left unpackaged. The treated fruits were then stored under ambient room conditions (Temp 25 ± 10 °C and RH 65± 5%). Physical parameters (color, percentage weight loss, firmness, wilting index), biochemical parameters (total soluble solids and total titratable acidity) and microbial analysis (spore count and disease severity index) were determined. The results showed that sodium bicarbonate whether packaged or unpackaged significantly ($P<0.05$) reduced deterioration compared to chlorine based sanitizers and control as evidenced by the number of live spores. Although deterioration of wounded fruits was more rapid compared to the unwounded ones, application of Na_2CO_3 sanitizers slowed down deterioration. Therefore sodium bicarbonate can be recommended as a low cost and healthier sanitization option for tomatoes.

Key words: Food loss, postharvest handling, rotting, sanitization, tomato

Résumé

Le potentiel élevé de la production de tomates n’est pas réalisé au Kenya en raison de divers facteurs dans la chaîne de valeur tels que les pertes post-récolte élevées estimées à 40-50%.

Ces pertes sont le résultat de nombreux facteurs, notamment une mauvaise manipulation après récolte, le manque d'équipements de stockage et des agents de détérioration tels que les maladies post-récolte. La désintégration microbienne représente à elle seule 15% des pertes post-récolte. Certaines des méthodes et traitements utilisés pour gérer la dégradation microbienne sont coûteux et malsains. Dans cette étude, le bicarbonate de sodium (bicarbonate de soude), qui est un désinfectant à faible coût, a été évalué pour son efficacité à gérer les pourritures fongiques par rapport aux désinfectants à base de chlore couramment utilisés. Le champignon a été isolé sur un milieu de gélose dextrose de pomme de terre (PDA). Les fruits de tomates vertes mûres ont été blessés (4 blessures, 3 mm de profondeur près de l'extrémité de la tige) et inoculés artificiellement avec une suspension de spores de 106 conidies / ml d'*Alternaria alternata*. Différents traitements de désinfection ont ensuite été appliqués pour contrôler les pourritures fongiques. Les traitements comprenaient une solution de bicarbonate de sodium à 30 000 ppm; Solution de chlore à 150 ppm; Solution de NaOCl 200ppm; eau et échantillons non traités qui ont servi de contrôle négatif. Les fruits ont ensuite été séchés à l'air et emballés dans des sacs d'emballage spéciaux sous atmosphère modifiée (MAP) ou laissés non emballés. Les fruits traités ont ensuite été stockés dans des conditions ambiantes (Temp 25 ± 10 °C et HR 65 ± 5%). Les paramètres physiques (couleur, pourcentage de perte de poids, fermeté, indice de flétrissement), les paramètres biochimiques (solides solubles totaux et acidité totale titrable) et l'analyse microbienne (numération des spores et indice de gravité de la maladie) ont été déterminés. Les résultats ont montré que le bicarbonate de sodium, qu'il soit emballé ou non emballé de manière significative (P <0,05), réduisait la détérioration par rapport aux désinfectants à base de chlore et au contrôle, comme en témoigne le nombre de spores vivantes. Bien que la détérioration des fruits blessés ait été plus rapide que celle des fruits non blessés, l'application de désinfectants au Na_2CO_3 a ralenti la détérioration. Par conséquent, le bicarbonate de sodium peut être recommandé comme une option de désinfection peu coûteuse et plus saine pour les tomates.

Mots clés: Perte de nourriture, manipulation après récolte, pourriture, désinfection, tomate

Background

Tomato in Kenya is grown in open field system (95%) or under protected environment (5%) because of its wide adaptability and versatility (Sigei *et al.,* 2014). An annual production of about 494,000 metric tons of tomatoes valued at Kshs.114.bIllion was achieved in the year 2013 in Kenya (HCDA, 2013). Tomato production plays a critical role in poverty alleviation. Despite its key role in economy, it still faces lots of problems. The prospective to manage the ways of enhancing its competitiveness in the value chain is still locked and unexploited owing to a mass of restraining factors along its value chain, which must be dealt with. The constraints in value chain start at farm level with prevalence of pest and diseases and physiological disorders (blossom-end rot, sunbscald, catfacing, boat fruit); poor post-harvest technologies and practices at the farm and market level; price fluctuation; cartels and poor infrastructure. The constraints at every level of the value chain influences the production and marketing of quality tomatoes and so should be properly controlled through periodic inspection and enhancement of every level in order to build up a fruitful, sustainable and vigorous tomato value chain. Recent scientific research on tomatoes has focused on production mainly, and this has resulted in improved and high yielding varieties while neglecting post harvest hence the reason why most good harvests do not translate to profits as most tomatoes are lost after harvest (Arah *et al.,* 2016). Postharvest loss which occurs between harvesting and consumption contributes to 50% of all tomato loss in tropical countries (Kader, 2005; Pila *et al.,* 2014). Postharvest losses can be due to poor sanitation, lack of storage or improper packaging. Poor sanitation leads to deterioration by various post-harvest pathogens that contribute 15% of the postharvest losses in tomatoes, the most common and obvious cause of deterioration is fungal activity (Kader, 1992). Jones *et al.* (1993) noted that in developing countries, commercialization of fruit is limited by rotting which is caused by *Alternaria alternata*.

Alternaria alternata can be controlled using sanitizers at various stages of the value chain. Chlorine, a common sanitizer has a distinctive, powerful and unpleasant odor. Even at low concentrations, chlorinated water may produce undesirable off-flavors in foods (Hassenberg *et al.,* 2008). It has also been established that there are chances that chlorine can react with organic matter in water to produce undesirable byproducts such as chloroform, (CHCl3) or other trihalomethanes that have known or suspected carcinogenic potential at high doses (Kader and Rolle, 2004). The inadequacies of chlorine based sanitizers has stimulated interest in environmentally friendly and effective alternative sanitizers (Sapers, 2003) such as sodium bicarbonate.

Sodium bicarbonate also known as baking soda which is an alkaline salt that is eco-friendly and efficacious in produce wash can be used as an alternative to chlorine based sanitizers. Sodium bicarbonate is recognized as safe (GRAS) according to classification done by the United Food and Drug Administration. United States Environmental Protection Agency has found that sodium bicarbonate leaves no residues on commodities (Palou *et al.,* 2001) hence considered as a natural sanitizer. Palou *et al.* (2001) documented that sodium bicarbonate is inexpensive, readily available and can be used with little risk of fruit injury. Research has shown that sodium bicarbonate is effective in controlling brown rot and green moulds on lemons (Smilanick *et al.,* 1999) and blue mould on oranges (Palou *et al.,* 2001).

The objective of this study waas to determine the effectiveness of sodium bicarbonate in comparison to chlorine-based sanitizers to prevent postharvest rotting and preserving quality of tomatoes under modified atmosphere packaging. We tested the hypothesis that Sodium bicarbonate and chlorine based sanitizers cause similar effects in controlling postharvest rotting and quality preservation of tomatoes.

Study description

The experiment was carried out during the months of July 2017 and December 2017. Tomato fruits, variety Milele were harvested at physiological maturity (mature green) from a commercial farm in Murang'a county which is located at 00 43' 0S, 370 8'60E and 1255M. The harvested fruits were packed in plastic crates and immediately transported within 2 hours to the pathology lab at Upper Kabete University of Nairobi Kenya. The fruits were selected for uniformity in size, color, and freedom from diseases, pests, injuries and blemishes. They were then divided into two batches which were either mechanically injured or left uninjured.

Isolation of *Alternaria alternata* and inoculation. An isolate of *Alternaria alternata* sp. *lycopersici*. was used for this study. The isolate was cultured from an infected ripe tomato. Two to three millimeter sections of infected tomato skin were plated on PDA media and the purified cultures formed stock culture. The isolate from stock culture was subjected to pathogenicity test. Microscopic examination to identify the pathogen was done as described by Misaghi *et al.* (1978). The cultures were left to grow for three weeks so as to get spores that were used for inoculation.

The pure cultures on the petri-dishes were flooded using sterile distilled water, the conidia were loosened up by scrapping using sterile microscopic slide and the suspension filtered through double layers of cheesecloth so as to remove adhering fungal mycelia. The concentration of the spores were counted and adjusted to 1.0 106 spores per ml using a haemocytometer. Subsquently, 1 mm wide and 3 mm deep wounds were made on one batch of the tomatoes near the stem end using sterile dissecting needle. Inoculation was done on both batches (wounded and unwounded) by dipping the fruits in the fungal suspension and later placing them on trays covered with moistened serviettes and left for 48 hours. The fruits were then divided into random batches and subjected to the various treatments using different sanitizers.

Sanitization procedure. Sanitizing was done by mixing each sanitizer treatment in water to obtain desired concentration. The sanitizer treatments were: 30,000ppm Sodium bicarbonate; Chlorine at 150ppm and sodium hypochlorite 100ppm. These concentrations were based on preliminary experiments conducted to determine the most effective concentration of each of the sanitizers. The fruits were submerged into the specific different sanitizers' solutions for two minutes. Plain water was used as the control treatment while one batch of tomatoes was untreated. After the treatment, the tomatoes were air dried and then packaged into special modified atmosphere packaging (MAP) called Xtend® film which were sourced from Amiran Kenya Ltd. A second batch of unpackaged were placed in open trays with stem end facing up and laid out on the bench in laboratory for evaluation of fruit quality and disease development. The experiment was a CRD (Completely Randomized Design) laid down as a factorial with two factors (Wounding and packaging) with two levels each. There were three replicates per treatment.

Parameters measured. The deterioration and overall quality of tomato fruits was evaluated based on color, firmness, wilting index, disease severity, total spore count, total soluble solids, total titratable acidity, and physiological weight loss. Standard procedures were used to measure the parameters.

Data analysis. The data collected were analyzed using Genstat 15th Edition statistical program. Analysis of Variance (ANOVA) was used to test for significant differences among sanitizer treatments and packaging for each parameter and means separated using Fischer's Protected least significant difference at P=0.05.

Results

Disease severity index (DSI) and spore count varied significantly ($P \leq 0.05$) between the treatments. Overall, sodium bicarbonate was the best anti-fungal treatment in both packaged and unpackaged tomatoes. Sodium hypochlorite and chlorine ranked second and third, respectively. At the end of storage (Day 30), DSI and spore count were least in sodium bicarbonate on the tomatoes that were not wounded and packaged in MAP (Table 1). On the other hand, firmness, TSS, color, TTA and weight loss were not significantly ($P \leq 0.05$) affected by the sanitizer, but by MAP packaging. Overall, MAP slowed down the ripening process ($P \leq 0.05$) in terms of color change, firmness, TSS, TTA and ensuring reduced weight loss. At the end of the storage (day 30) not wounded and not packaged fruits had lost 8.1%, wounded and not packaged 9.7%, wounded and packaged 5.9% and not wounded and packaged 5.2% percentage of the initial weight.

Table 1. Spore count and disease severity index (DSI) of different sanitizers at the end of storage period when not wounded and packaged

Sanitizers	spore count	DSI
Sodium bicarbonate	916667^{c}	1.3^{h}
Chlorine	1750000^{ab}	2.3^{gh}
Sodium hypochlorite	1083333^{ab}	3.3^{adefg}
Unwashed	2146667^{a}	2.3gh
Washed	1833333^{ab}	$3.7^{abcdefg}$
LSD (0.05)	1275651	1.413
CV%	43.8	23.2

Key: Values followed by the same letter (s) within a column are not significantly different (Fischer's protected least significant difference test at p 0.05); Mean ± standard error values per treatment analysed

Table 2. Spore count and disease severity index (DSI) of different sanitizers at the end of storage period when wounded and packaged

Sanitizers	spore count	DSI
Sodium bicarbonate	1250000^{c}	4.7abcde
Chlorine	2333333^{abc}	5.0^{a}
Sodium hypochlorite	1583333^{bc}	5.0^{a}
Unwashed	3333333^{a}	5.0^{ab}
Washed	2916667^{ab}	5.0^{abc}
LSD (0.05)	1369495	1.413
CV%	33	23.2

Key: Values followed by the same letter (s) within a column are not significantly different (Fischer's protected least significant difference test at p 0.05); Mean ± standard error values per treatment analysed

Discussion

Sodium bicarbonate was a more effective sanitizer compared to chlorine based formulations possibly due to its alkalinity and ions (Na+ and HCO_{3-}) that form when in aqueous solution. Enfors and Molin (1978a) also reported that sodium bicarbonate works by retorting with carboxyl group of some acidic amino-acid residues of protein in the spore resulting to a less oriented formation. Our results are similar to findings of Cheung *et al.* (1997) for *Bacillus stearothermophilus*, also green and blue molds on citrus research work reported by Palou *et al.* (2001).

Chlorine did not exhibit consistent performance in terms of spore count reduction. This may have been associated with chlorine losing its effectiveness quickly in the presence of dirt and organic material. Chlorine usually combines with inorganic compounds such as sodium or calcium to produce hypochlorites, which are effective disinfectants. Similar findings were obtained by Chen *et al.* (2004) who found that a 5-minute wash in a solution of NaOCl (provides at 200 ppm CI) delayed but did not prevent the growth of *P. expansum* on apples. Likewise, research done by Bartz (1988) showed that water chlorination did not prevent decay in tomato fruit contaminated with *Alternaria alternata* and then washed with chlorinated water at 26 C and pH 6.8 to 9.6. Washing of tomatoes with tap water was not significantly different from keeping the fruits unwashed. Tap water helps reduce dirt load on the fruits but does not kill microorganisms, instead microorganisms can spread during washing causing more damage. Similar findings were recorded when decay in chestnut became prevalent during postharvest storage after washing with tap water (Lee *et al.,* 2016).

The wounded tomato fruits used in the study resembles a fresh harvested tomato that becomes inoculated due to poor postharvest handling. Wounded fruit is susceptible to pathogens beside showing scars that reduce its market value. In the inoculation period, *Alternaria alternata* will have time to germinate and penetrate into the fruit epidermis. For effective control, fruits should be sanitized before the pathogens become embedded on them (Al-Haq *et al.,* 2001).

Disease severity index and spore count was influenced by both packaging and sanitizer treatment and the effect was significant. Lesion diameter and daily growth rate were also higher in wounded fruits compared with not wounded fruit. During the observation period, the sanitizers (especially sodium bicarbonate) reduced the number of spores of *Alternaria* alternate to a safe level. The inhibitory effect of sodium bicarbonate on spore germination reported here is consistent with earlier observations by Enfors and Molin (1978a) who noted that high concentrations (0 42% w/v) of sodium bicarbonate inhibited germination of some Bacillus spores.

The synergetic effect of the sanitizers and packaging in preventing postharvest rotting and preserving quality of tomatoes was also noted. Packaging with Xtendbag ® slows down respiratory activity and ethylene production by creating modified gas atmosphere where CO_2 percentage is elevated and O_2 is reduced (Mekonnen, 2017). Packaging delays senescence by decreasing respiration rate, metabolic activity and microbial growth (Villalobos *et al.,* 2017). Tomatoes stored using MAP had a low reduction in weight loss brought about by the saturated environment inside the bags due to water vapor pressure. This in turn caused high humidity leading to lower transpiration. Packaged fruits had a significant delay in color change from green to red, and this could be attributed to high CO_2 conditions that may have resulted into reduced ethylene synthesis in the MAPs. Ethylene which is a necessary evil in climateric fruits is known to activate enzyme actions involved in color changes during ripening.

Extremely high firmness values signify unripe situations, whereas a sharp decline in the firmness values signify the non-effective storage conditions in sustaining a solid maturity level of tomatoes. Softening of fruits is as a result of partial disassembly of the cell wall as they ripen. The tomatoes not wounded and packaged remained firmer compared to in any other treatment and this could have been as a result of reduced O_2 concentration and elevated CO_2 concentration that obstructs the enzyme activity thereby helping to retain firmness during storage in MAP. Slow reduction in firmness can also be linked to fruit cells remaining more turgid due to the lower rate of moisture loss in packed fruits owing to the reduced transpiration rate.

Soluble solids increase during ripening because of polysaccharide degradation into simple sugars. The tomatoes packaged in MAP had a slow increase in ripening compared to the not packaged and this could be attributed to slow synthesis of the metabolites and their use due to reduced respiration rates caused by low O_2 and high CO_2 concentration. In unpackaged fruits, the high rate of TSS increase is linked to high respiration rate hence the need for respiratory substrates to provide energy for metabolic activities. Increase in TSS correlated positively with weight loss in this study.

Total titratable acidity (TTA) decreased gradually which was an indicator of ripening irrespective of packaging. However, fruits that were packed in MAP had delayed rate of reduction of TTA. Decrease in acidity is attributed to high respiration rate in produce that uses organic acids (malic acid and citric acid) as primary respiration substrates. Reduced respiration rate in MAP can delay the organic acid utilization due to decreased metabolism activities. Citric acid (main acid in tomatoes) percentage increased throughout the storage period. MAP also reduced contamination of the fruits hence reducing pathogen growth on sanitized fruits, however, on infected fruits MAP facilitated rotting by providing high humidity that enabled growth of *Alternaria alternata*. The results obtained showed that the coupled effect of sanitization and packaging was effective.

Conclusion

The use of this affordable sanitizer approach to postharvest disease control offer a promising, safe and effective alternative to chlorine based sanitizers in treatment of postharvest fungal diseases of tomato fruits. Use of sodium bicarbonate does not require expensive resources that the small holder farmer would not afford and would provide a farmer with a longer storage life for tomatoes. Combining sanitization and packaging reduced microbiological contamination and deterioration of physical quality of tomatoes. However, additional studies needs to be done to optimize storage conditions.

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