.

Research Article

Extending Shelf Life of Tomatoes Using Microbial Antagonists

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Abstract

A study was conducted to determine the effect of citrus peel essential oils on the extending shelf life of f tomatoes. Peel extracts were prepared from sweet orange (Citrus sinensis), lime (Citrus aurantiifolia) and sour orange (Citrus aurantium) peel using a rotary evaporator. Most abundant microbial species were isolated from rotten tomatoes and were tentatively identified as Bacillus spp. and Penicillium spp. The antimicrobial activities of three peel extracts were tested by using agar well diffusion assay. Positive controls for bacteria and fungi were Amoxicillin and Fluconazole respectively, while 50% ethanol was used as negative control. Both concentration and type of extract significantly affected for Minimum Inhibitory Diameter (MID) ($p < 0.05$). Although the highest MID was resulted from positive controls (2.6 ± 0.3 cm, 3.4 ± 0.4 cm for Amoxicillin and Fluconazole respectively), amongst peel extracts, sweet orange peel extracts had shown highest MID of 2.5 ± 0.8 cm and 2.1 ± 0.3 cm (at 2.5 mg/mL) for both bacteria and fungi respectively. A solution of sweet orange peel extracts (2.5 mg/mL) was prepared and sprayed on a batch of ripen tomatoes at same variety and size, while Amoxicillin and Fluconazole solution and distilled water were sprayed on another three batches of tomatoes. The shelf life of sweet orange peel extract solution sprayed batch was exhibited 26 days in average while the shelf life of batches sprayed with Amoxicillin, Fluconazole and water were 19 days, 20 days and 5 days in average respectively. Therefore, sweet orange peel extracts could be used to prepare antimicrobial solutions to extend shelf life of tomatoes.

Keywords: *Tomatoes, Citrus sp. peel extract, Shelf life*

1. Introduction

Tomato (*Lycopersicon esculentum*) is one of the most important supplementary sources of minerals and vitamins in human diet (Bhowmik *et al.*, 2012). Lycopene, found in tomatoes known to act as an antioxidant, neutralizing free radicals that can damage cells in the body (Sies and Stahl, 1998). Since tomato is highly perishable, it

encounters several problems in its transportation, storage and are highly susceptible for microbial damage (Babatola *et al*., 2008).

Bacterial species such as *Bacillus* sp., *Staphylococcus aureus, Staphylococcus epidemidis, Escherichia coli* and fungal species such as *Rhizopus* sp., *Aspergillus* sp., and *penicillium* sp. are significantly known to be responsible for shelf life reduction and quality loss of tomatoes (Gupta *et al*., 2017). Generally fresh tomatoes last for one week and refrigerated one last for 2 weeks Yanuriati, Savage and Rowe, 1999). Currently storage life of tomatoes is improved by cool storage, control atmosphere, modified atmosphere and application of various synthetic fungicides and bactericides (Majidi *et al*., 2014: Palou, 2018 : Ahmed *et al*., 2017). However, these methods are expensive and difficult to handle. Thus inexpensive, simple and nontoxic methods are required to be introduced to improve the shelf life of tomatoes. Citrus peel extracts have been identified as one of the major bio-control method for most of the microorganisms (Dhanavade *et al.*, 2011, Ali *et al.*, 2019). Therefore, in the present study was focused on the use of three citrus peel extracts (Sweet orange : *Citrus sinensise*, Lime :*Citrus aurantiifolia* and sour orange *Citrus aurantium*) for extending shelf life of tomatoes by controlling microbial growth.

2. Materials and Methods

Tomato samples (Padma var.) were collected from the local market at Badulla, Sri Lanka. Collected tomatoes were allowed for rotting and microbial species were isolated, then cultured in Potato Dextrose Agar (PDA) medium and Nutrient agar medium (NA). Cultured plates were incubated at room temperature (25°C). The most common bacterial and fungal species were sub cultured and tentatively identified as *Bacillus* sp. and *Penicillium* sp. respectively.

Sweet orange, lime and sour orange fruits were washed properly with normal water and distilled water as well. Then their peels were collected and their weights were recorded. The peels were soaked in 50% ethanol solution (v/v) and shook for three days as described by Mostafa *et al.,* (2017). The solution was subjected to centrifugation at 3500 rpm for twenty minutes and the supernatant of all three peels soaked ethanol solution was filtered through the filter paper separately and these respective solutions were poured in to flasks and allowed to rotate in rotary evaporator one by one at the rate of 30 rpm and water bath temperature was maintained at 40°C. Finally, extract was obtained and collected in to vials were allowed to dry. After the drying process weight of the extract was measured and concentration series (2.5 mg/mL, 1.25 mg/mL and 0.625 mg/mL) were prepared for all three peel extracts.

The bacterial isolate from overnight cultures was suspended in saline and 50 µL inoculum of bacterium was uniformly spread on separate NA plates (with Fluconazole 8 µg /mL) with the help of glass spreader. Wells (10 mm diameter and about 2 cm a

part) were made in each of these plates using sterile cork borer. after five minutes three wells approximately 6 mm diameter was bored with the help of borer. PDA plates (with Streptomycin 0.03 g/L) of fungi species were prepared using the same methodology.

The wells of both NA and PDA plates were filled with various concentrations of the extracts (2.5 mg/mL, 1.25 mg/mL and 0.625 mg/mL). For other wells in NA plates amoxicillin (2.5 mg/mL) was filled as positive control and 50% ethanol was filled as negative control. For PDA plates fluconazole (2.5 mg/mL) was used as positive control and 50% ethanol was used as negative control. The plates were then incubated at 37ºC for 24 hours. After incubation, Average Mean Inhibitory Diameter (MID) was measured in millimeters. A spray was prepared from the extract with highest MID value with a concentration of 2.5 mg/mL.

MID of triplicates of three peel extracts, microorganisms and concentrations were analyzed using Minitab17 statistical software. Descriptive statistics, and factorial regression methods were used to analyze the data.

Determination of Shelf Life of Tomatoes: Twenty (same fruits in colour, size and ripening age) tomatoes were taken and separated in to four sets. Each set was comprised of five tomatoes. Every set was sprayed with (100 mL) three different solutions as most effective concentration; 2.5 mg/mL, amoxicillin 2.5 mg/mL, fluconazole 2.5 mg/mL and distilled water. Spraying was carried out properly once per day at the same time using a sprayer can. Spraying was carried out until the most effective peel extract solution sprayed tomatoes started rotting. Then the shelf life of tomatoes was determined through visual observation using a colour chart.

3. Results

3.1 Identification of Microorganisms

According to the study on the colony characteristics and microscopic analysis, the most common bacterial and fungal species were tentatively identified as *Bacillus* sp. and *Penicillium* sp. (Fig.1)

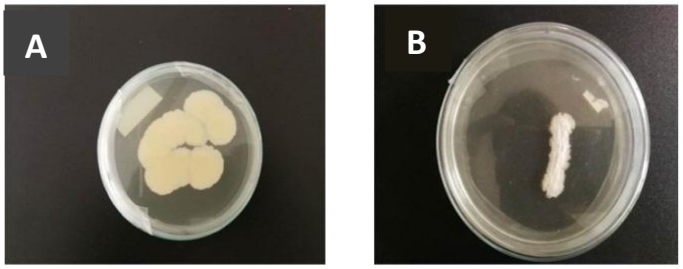

Figure 1: Isolated most common bacterial and fungal species from rotten tomato species. A) Tentatively identified as *Bacillus* sp. B) Tentatively identified as *Penicillium* sp.

3.2 Determination of Most Effective Peel Extract

The minimum inhibitory concentration assay conducted for both bacteria and fungus using solvent extracts are shown in Figure 2, 3 and 4. Both concentration and type of extract significantly affected for MID ($p < 0.05$). Although the highest MID was resulted from positive controls (2.6 ± 0.3 cm, 3.4 ± 0.4 cm for Amoxicillin and Fluconazole respectively), amongst peel extracts, sweet orange peel extracts had shown highest MID of 2.5 ± 0.8 cm and 2.1 ± 0.3 cm (at 2.5 mg/mL) for both bacteria and fungi respectively.

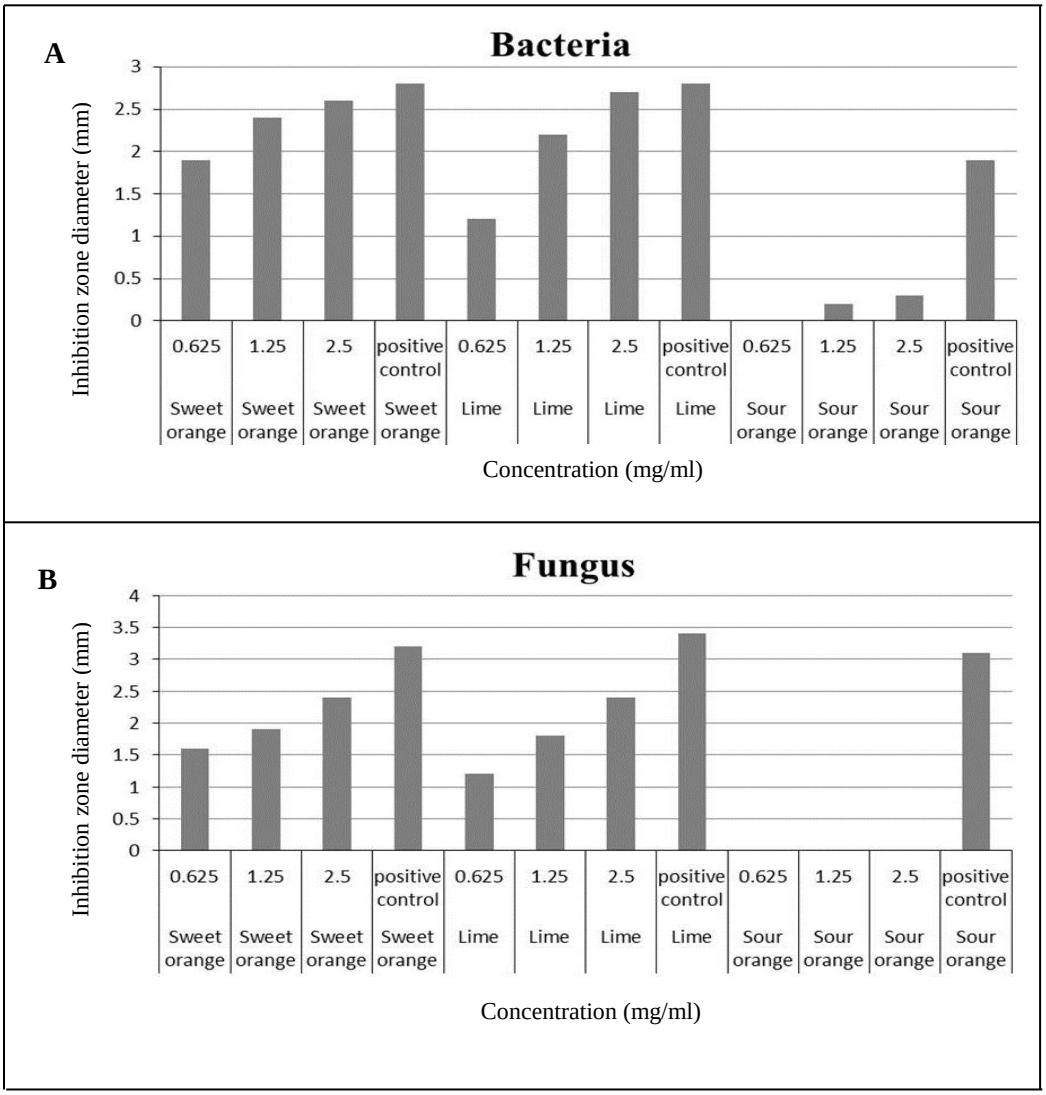

Figure 2: Minimum inhibitory zone diameters for the studied peel extracts A). For bacteria. B). For Fungus

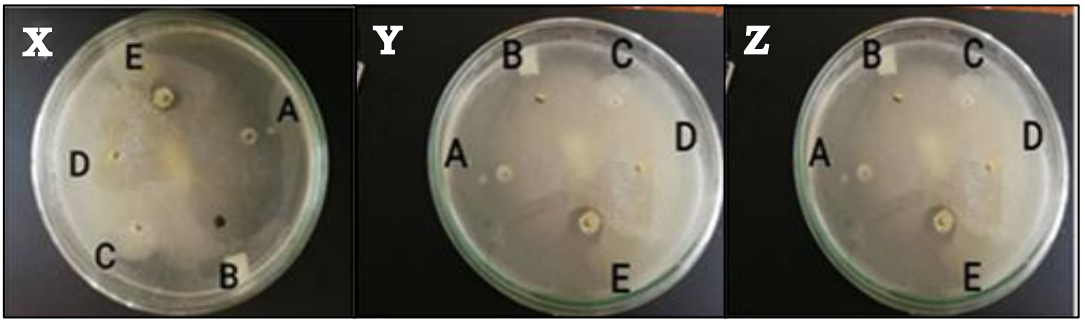

Figure 3: Inhibition zones of three peel extracts against bacterial growth. X: Sweet orange peel extract, Y: Lime peel extract, Z: Sour orange peel extract: (Note : A-2.5 mg/ml, B-1.25 mg/ml, C-0.625 mg/ml, D- Negative control, E- Positive control)

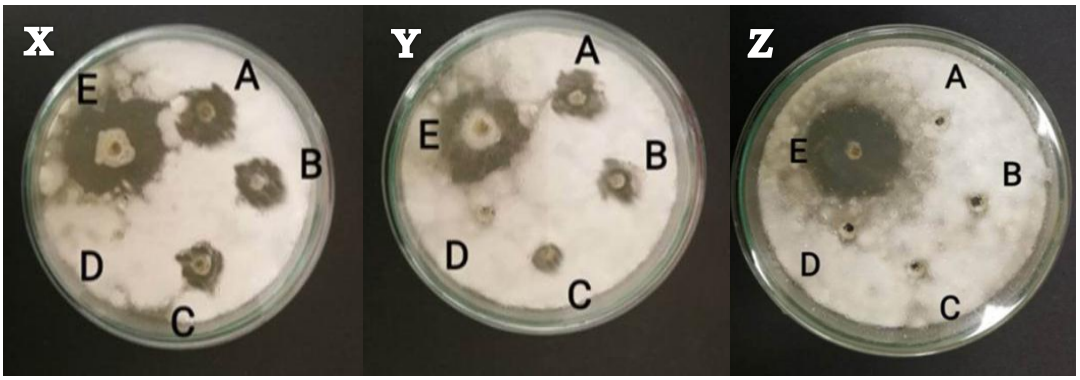

Figure 4: Inhibition zones of three peel extracts against fungal growth. X: Sweet orange peel extract Y: Lime peel extract, Z: Sour orange peel extract (Note: A-2.5 mg/ml, B-1.25 mg/ml, C-0.625 mg/ml, D- Negative control, E- Positive control)

There was no relationship between peel extract and microorganisms, microorganisms and concentration, and peel extract, microorganisms and concentration ($P > 0.05$). However, a significant relationship was observed between peel extract and inhibition diameter ($P= 0.000$).

3.3 Determination of Shelf Life of Tomatoes after Extract Spray Analysis

Water sprayed tomatoes were firstly showed deterioration on 5^{th} day of the spray. Then amoxicillin sprayed tomatoes started to shrink and soften after 13 days from the beginning of spraying. After 19 days they started rotting and became unsuitable for consumption. Moreover, fluconazole sprayed tomatoes started to shrink and soften after 16 days from the beginning of spraying. After 20 days they started rotting and became unsuitable for consumption. Finally, sweet orange peel extract (2.5 mg/mL) sprayed tomatoes started to shrink and soften after 22 days from the

beginning of spraying. After 26 days they started rotting and became unsuitable for consumption (Fig. 5).

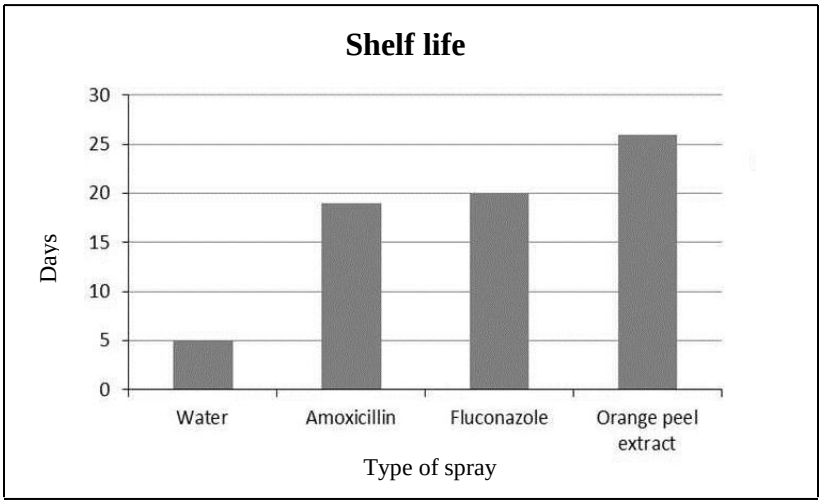

Figure 5: Shelf life of tomatoes with respective sprays

4. Discussion

Tomatoes are highly perishable fruit and give high production during harvesting time which may lead to increase post-harvest losses due to insufficient post-harvest practices (Saeed and Khan, 2010). There were several studies carried out to extend the shelf life of tomatoes by application of irradiated Chitosan (Parvin *et al.,* 2018), changes done in packaging (Geeson *et al.,* 1985) or application of commercially available bactericide and fungicides. Since most of these methods are expensive, toxic and difficult to carry out, in the present study we had focused on biological method to extend the shelf life of tomatoes.

Citrus peel extracts had been studied extremely for its antimicrobial activities. A research (Gupta *et al.,*2017) was carried out to compare and asses the antimicrobial activities of lemon peel extract and lemon oil against the food borne pathogens and to study their potential as food-bio preservatives. The aqueous-ethanolic extract of lemon peel was most effective against *Staphylococcus aureus* followed by *Staphylococcus epidermidis* and *Bacillus subtilis*. Amongst the gram negative bacteria lemon peel extract showed equivalent activity against all the test bacteria *Escherichia coli* and *Enterobacter aerogenes.* Amongst the fungus lemon peel extract was most effective against *Alternaeria* sp and *Rhizopus* sp. However, in the present study Sweet orange peel extract showed highest MID for both studied bacterial and fungal species.

Gram positive bacteria such as *Staphylococcus aureus* and *Bacillus subtilis* contains teichoic acid in the peptidoglycan layer and is therefore inhibited by citrus peel extracts (Matejko and Scaglione , 2000). This could be the reason for inhibition of bacterial species in present study. Further it has been found out that limonene present in Citrus peel extracts prevents the growth of fungal mycelium specially in *Penicillium* sp. (Mahdavi *et al.,* 2011; Matsuoka *et al.,* 1990), which explains the results of the present study on inhibition of fungal growth.

Moreover, the observed antimicrobial activity was probably due to combination of more than one constituent present in the sweet orange peel extract which might have affected bacterial species and fungal species differently. It has been found out that the various components present in extracts may act synergistically (French *et al.,* 1985).

Effectiveness of their antimicrobial activity depends on the constituents present in those peel extracts and also the cell wall structure, resistive mechanisms of the particular microorganisms.

Citrus peel is a valuable source of d-limonene, flavonoids, carotenoids, terpenes, dietary fibers, soluble sugars, cellulose, hemicellulose, pectin, polyphenols, ascorbic acid, methane, and essential oils. d-Limonene is the component present in all three peel extracts. Sweet orange has 83.9% - 95.9% d-Limonene, Lime has 40.4% - 49.4% d-Limonene, Sour orange has 89.7% - 94.7% of d-Limonene (Dosoky and Seltzer, 2018). Further it has been found out that d-Limonene possess antioxidant, anti-inflammatory and anticarcinogenic effects (Bayala *et al.,* 2014; Dosoky and Seltzer, 2018). According to Dosoky and Seltzer (2018) sweet orange peel extract has comparatively higher amount of d-Limonene. This could be the reason for high antimicrobial activity of sweet orange resulted in present study.

In the extract spray analysis also best results were obtained for orange peel extracts. Since Amoxicillin is an antibacterial agent it might have prevented the growth of bacteria in tomatoes batch while fluconazole only controlled fungal growth. Since sweet orange peel extract acted on both species the shelf life might have extended up to 26 days.

5. Conclusion

Shelf life of tomatoes can be extended by using the peel extracts of citrus fruits by controlling the growth of bacteria and fungi. Sweet orange peel extract (2.5 mg/mL) is the most effective extract compared with lime and sour orange peel extracts while extending the shelf life of tomatoes up to 26 days at room temperature.

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