CHAPTER SEVEN

SEMI COMMERCIAL EVALUATION OF PLANT EXTRACTS ON QUALITY RETENTION IN *CITRUS SINENSIS*

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Abstract

Six postharvest treatments with extracts of *Acacia seyal* Del. var. Seyal and *Withania somnifera* L. Dunal were tested using artificial wounding or dip applications on citrus (*Citrus sinensis* L.). Quality retention effects of extracts were studied with the application of extracts as either a pre-wax, combined with wax or as a plant extract dip alone. Chlorine washed and commercial chemical treated fruits were included as comparative controls. Fruit were stored for 50 days at 25 ºC and 75% RH or at 7 ºC and 80-95% RH to simulate domestic and export conditions. Fruit quality were assessed for incidence of decay, physico-chemical and sensory parameters. Canonical variate analysis of data indicate that *A. seyal* and *W. somnifera* extracts applied as a pre-wax treatment or combined with wax or using the extract alone resulted in more fruit that retained the colour of the skin, odour/ smell and flavour with overall acceptability when kept at 7 ºC and 80-95% RH for 50 days. Fruits were also assessed for disease development but overall natural infection was too low to see any significant effect. The two plant extracts have potential as a safe, cost-effective alternative for protecting the fruit without affecting the quality during long-term storage.

Key words: Plant extracts; Postharvest treatments; Physico-chemical; Sensory evaluation.

7.1 INTRODUCTION

Citrus (*Citrus sinensis* L.) fresh fruit is one of the major export crops in global trade. Citrus is cultivated in the subtropical and tropical regions of the world in 137 countries and on six continents (Salunkhe and Desai, 1984; Ismail and Zhang, 2004). Annually, more than 104 million tons of citrus fruit are produced of which 15 million tones end up in global trade (FAO, 2004). The storage life of citrus is limited to a maximum of eight weeks at low temperature (Mukhopadhyay, 2004), and inferior quality is often observed on 9-25% of the product at export destinations due to postharvest pathogens (*Penicillium digitatum* Sacc., *Geotrichum candidum* Link and *Colletotrichum gloeosporioides* (Penz.) and physiological disorders (peel pitting and browning) (Klieber *et al*., 2002; Alferez *et al*., 2005). Chemical fungicides used to control postharvest diseases are increasingly being lost to the export sector due to increased requirements of more stringent maximum residue levels and re-registration requirements for pesticides (Plaza *et al*., 2004). Further concern over build up of pathogen resistance and negative impact on environmental health (Brown, 1977; Eckert and Ogawa, 1985; Vero *et al*., 2002) necessitate the search for alternative control options.

Application of plant extracts and essential oils have been extensively evaluated for postharvest application on fruits (Dudareva *et al*., 2004; Tripathi and Dubey, 2004). Saks and Barkai-Golan (1995) reported that application of *Aloe vera* L. Webb and Berth gel on wounded grapefruit reduced green mould decay by 75%, six days after inoculation with *P. digitatum*. The essential oil cumin from *Cuminum cyminum* L. Cumin has also been reported to protect citrus fruits from *P. digitatum* (Yigit *et al*., 2000).

Natural plant extracts can successfully replace synthetic fungicides to control postharvest decay if they are applied during the packhouse operation without additional expenditure on new equipment. *Acacia seyal* Del. var. Seyal and *Withania somnifera* L. Dunal are indigenous plants in Ethiopia used as traditional medicines (Demissew, 1989; Bekele, 1993). As shown in chapter 6, extracts from *A. seyal* and *W. somnifera* showed a broad spectrum *in vitro* antimicrobial activity against food borne and plant pathogens. *In vivo* application of these extracts showed up to 75 % reduction of *P. digitatum* incidence when kept for 21 days under simulated export conditions.

In order to make the application efficient in this study, the plant extract was incorporated in to

the commercial wax formulation or were applied prior to wax application to protect the fruit during storage and transportation. The objective of this study was to evaluate the efficacy of extracts from two indigenous Ethiopian plants *A. seyal* and *W. somnifera* on decay control and quality retention of citrus fruits at long-term cold and room temperature storages.

7.2 MATERIALS and METHODS

7.2.1 Fruit collection

Fifty four boxes of Valencia oranges, each containing 88 fresh fruits were randomly collected from J. M. du Toit citrus packhouse (Tzaneen, Limpopo Province, South Africa). Fruit were transported during the winter at 18 ºC to the Plant Pathology Laboratories, University of Pretoria for immediate treatment.

7.2.2 Plant material extraction

Two plant species *A. seyal* and *W. somnifera* collected from Metahara and Hursso, Ethiopia, were air-dried and undamaged leaf parts of these plants were powdered in a blender (Russell Hobbs) and stored at 18 ºC in amber bottles until further use. One part of the dried plant powder was suspended in 20 parts (w/v) methanol solvent mixture [(methanol/ acetone/ water) (7:7:1)] followed by three successive extractions as described in chapter 5 section 5.2.2. The combined supernatants were concentrated to dryness under vacuum at 25 ºC and equal volume of distilled water as to the original extraction solvent system was added to make the final stock solution. The suspension were then filter sterilised using 0.45µm pore size (Sartorius, Germany) into sterilized Schott bottles and stored at 4 ± 1°C until further use.

7.2.3 Postharvest treatments:

7.2.3.1 Wound treatment

In vivo antifungal activities of *A. seyal* and *W. somnifera* against *P. digitatum* were tested using the method described by Poppe *et al*. (2003), with some modifications. Wound (3 x 3 mm) applications of extracts were applied 12 h prior to the inoculation of the pathogen. The culture of *P. digitatum* collected from the culture collections of Plant Pathology laboratories, University of Pretoria, South Africa were used. In order to avoid a variable inoculum pressure, the pathogen concentration was standardized to 10^5 conidia ml^{-1} using a haemacytometer (Janisiewicz *et al*., 2000) and preserved at 4 ºC in an ice box prior to use. Six treatment combinations indicated in table 7.1 were used.

Table 7.1 Plant extracts treatment combinations for wound application on fruit

Code	Treatment description
1	Fruit wound + *A. seyal* + *P. digitatum*
2	Fruit wound + *W. somnifera* + *P. digitatum*
3	Commercial packing line treatment [(Dipping fruit in chlorine water (Sodium hypochlorite, 250 ppm) for two minutes, spore kill (12% didecyl dimethyl ammonium chloride) (Hygrotech (Pty) Ltd., Johannesburg) (900-1400 ppm) for brief time spray (30 seconds), quattro kill (N, N Didecyl-N, N-dimethyl ammonium chloride) (Hyper Agrochemicals (Pty) Ltd., Johannesburg) (1300ppm) at 45 ºC for five minutes, imazalil (Sanachem, Johannesburg) (1350ppm) for brief time spray (30 seconds), air drying for two minutes and waxing with Citrosol (100 000 ppm) (Brenntag, Germany) for two minutes, drying and packing.
4	Untreated not wounded
5	Wound only
6	Wound + *P. digitatum*

Wound inoculation of the pathogen alone was regarded as a negative control. The application of commercial chemicals was regarded as a positive control. Fruit wounding alone was included to confirm the effect of wound treatments. Ten fruits per treatment and four wounds (3 x 3 mm diameter) per fruit were used. Fruits wounded aseptically with picture hooks (3 x 3 mm) were inoculated with 30 µl of the crude plant extract, air dried for 12 h and inoculated with the same volume of the pathogen, *P. digitatum*. Treated fruits were kept for 21 days in citrus boxes at 8 ºC with a relative humidity of >85% (RH) to simulate export conditions. Evaluation of fruits for disease development was done weekly and percentage disease incidence was computed. The experiment was repeated twice.

7.2.3.2 Fruit dipping

For each treatment, a total of 528 fruits were randomly selected. In each treatment application, fruits were dipped in treatment suspensions for two minutes and air-dried for 10 minutes. Fruits were subjected to either one of the following dip postharvest treatments (Table 7.2).

Table 7.2 Plant extracts treatment combinations for dip application on fruit

Code	Treatment combinations
1	*A. seyal* leaf extract application followed by air drying and waxing with Citrosol
2	*W. somnifera* leaf extract application followed by air drying and waxing with Citrosol
3	Combined treatment of *A. seyal* leaf extract incorporated in the commercial waxing
4	Combined treatment of *W. somnifera* leaf extract incorporated in the commercial waxing (Brenntag)
5	Treatment with *A. seyal* leaf extract alone
6	Treatment with *W. somnifera* leaf extract alone
7	Washing in commercial chlorine alone
8	Commercial packing line treatment as described in the previous experiment, subsection 7.2.3.1 (Table7.1)
9	Untreated control

Fruits were dipped in treatment suspensions for two minutes and air-dried for 10 min. A set of 44 fruits were packed in commercial cardboard boxes (300 x 400mm) and stored at 25 °C and 75% RH and a replicate set were kept at 7 ºC and 80-90% RH for 50 days simulating local and export conditions, respectively. The fruits were then evaluated for overall quality retention and organoleptic parameters.

7.2.4 Fruit quality

Postharvest fruit quality was assessed for incidence of browning on a 1-5 rating hedonic scale, where: 1= very poor, 2= poor, 3= fair with limited acceptability, 4= good, and 5= excellent (Alferez *et al*., 2005). Fruit firmness was measured with a penetrometer (Magness-Taylor penetrometer test) equipped with a six mm diameter plunger capable of penetrating through the peel into the pulp (Abbott, 1999). Ten fruits were taken at random from the different postharvest samples, and firmness was measured on opposite sides of each fruit (Sivakumar *et al*., 2005). Fruit percentage weight loss was calculated out of a hundred by subtracting treated

stored fruit weight from untreated fresh fruit weight measurement before storage. Total Soluble Solids (TSS) was determined trice using fruit juice and a hand-held refractometer (Atago, Japan, Brix 0-30%). Results were expressed as percentages of TSS. Titratable acidity (TA) was also determined by titrating 10 ml of the sample filtrate against 0.1 M NaOH with phenolphthalein as indicator. The turning point was taken as the sudden change of the solution to a slight pink colour, with acidity expressed as percentage citric acid equivalent (Schirra *et al*., 2004).

7.2.5 Sensory evaluation

For sensory evaluation, fruit samples removed from cold storage were kept at room temperature (25 ºC). A set of 10 fruit per treatment was placed on white plates and immediately presented to a taste panel of six panellists familiar with the quality and sensory parameters of citrus fruit. The qualitative analysis based on quality parameters (Table 7.3) was done according to Varela *et al*. (2005).

Table 7.3 Sensory attributes selected for descriptive analysis

Attribute	Associate descriptor
Smell	Total intensity of smell
Freshness	Smell of fresh oranges
Colour	Natural colour of the peel, flavedo and edible portion and presence of browning
Appearance	Condition of a fruit whether it is fresh, shriveled, firm or soft
Flavour	Total intensity of flavour during the first chewing
Sweetness	Taste of the fruit: sweet, bitter or sourness

Quality assessment values were given for each treatment using a hedonic scale structured from 1 to 5 (Srinivasa *et al*., 2004), where 1 meant very poor, 2 meant poor, 3 meant fair with limited acceptability, 4 meant good and 5 meant excellent. Prior to the evaluation procedure, the panel was trained with attribute descriptor by profiling fruit sections to associated parameters. Replicate samples were pooled together according to their storage temperature and fifty fruits per sample were used. Each sample was identified by a random three-digit code. The order of presentation of the samples on the plates was randomised for each panellist. Fruits were displayed in lightened room on big dining table using white plates and

panelists were provided with knife and tissue papers for cutting and cleaning; and glass of water for mouth rinsing between samples. Evaluation of samples from both temperature regimes was done at different times for reliability and validity of results.

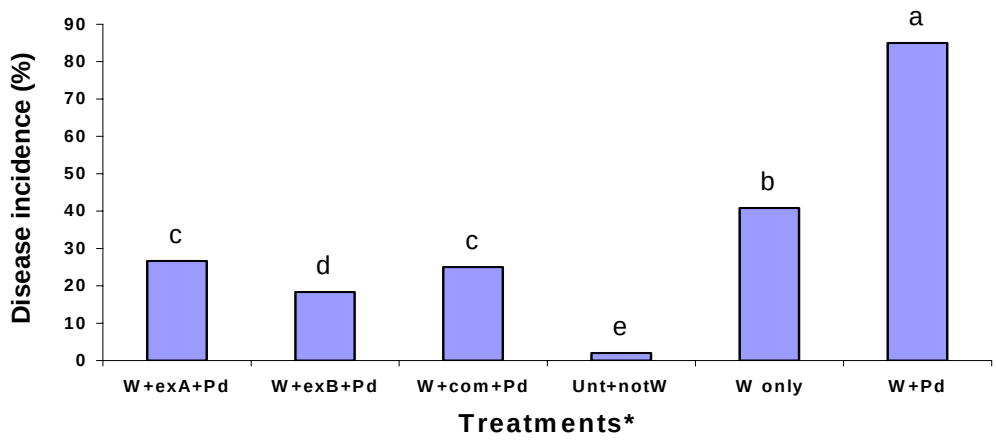
7.2.6 Statistical analysis

The experiments were in a completely randomised design and were carried out twice during the 2004 and 2005 growing seasons. Analysis of variance was used to test for differences between treatments. Treatment means were separated using Fisher's protected t-test least significant difference (LSD) at the 5% level of significance (Snedecor and Cochran, 1980). Data were analysed using the statistical program GenStat for Windows (2004). Multivariate canonical variate analysis (CVA) was used as a useful statistical tool to identify differences between groups of individuals (treatments). It summarises and analysed information contained in the different independent variables, and maximized variation between the groups of individuals while minimising variation within the groups of original variables. Comparisons between the samples and storage conditions and determination of the extent of variation observed in the results were also accounted.

7.3 RESULTS

7.3.1 Postharvest disease incidence and browning evaluation

In vivo wound treated fruits with *A. seyal* and / or *W. somnifera* showed significant (P <0.05) reduction of *P. digitatum* incidence by more than 75% comparable to the effect of commercial chemical treatments when kept for 21 days under simulated export conditions (Fig. 7.1). In fruits subjected to postharvest dip treatments, decay was not observed on fruits held at 7 ºC and 80-90% RH. Incidence of chilling was 6% instead, in untreated fruits stored at this temperature (Table 7.4). Higher incidence of fruit decay (24-36%) was observed on fruits stored at ambient (25 ºC) temperature and 75% RH (Table 7.5). Fruits subjected to a pre-wax application with plant extracts showed relatively higher incidence of browning at 25 ºC (Table 7.5). Significant changes (P <0.05) in firmness and weight loss was observed in extract treated fruits kept at 7 and 25 ºC (Table 7.4 and 7.5). The plant extract *A. seyal* alone or used as a pre-wax application showed significantly (P <0.05) higher retention of fruit firmness at 7 ºC and similar treatments revealed lower firmness at 25 ºC. On the other hand, combined application of *A. seyal* or *W. somnifera* extracts with wax enabled the fruit to retain firmness both at 25 ºC and 7 ºC. Pre-wax application of *A. seyal* extract or *A. seyal* extract alone retained fruit firmness better than the commercially adopted treatment kept at 7 ºC storage.



Legend: Each bar represents treatment means. Means with the same letter are not significantly different by Fisher's protected test at (P <0.05). *Treatment applications are described as follows: W+ exA + Pd = Fruit wound + *A. seyal* extract + *P. digitatum*; W+ exB + Pd = Fruit wound + *W. somnifera* extract + *P. digitatum*; W + com + Pd = Fruit wound + Commercial chemical treatment + *P.digitatum*; Un + not W = Untreated and not wounded fruit; W only = Wounded fruit only; W+Pd = Wounded fruit + *P. digitatum*

Fig. 7.1 *In vivo* wound treatment evaluation of *Acacia seyal* and *Withania somnifera* efficacy against *Penicillium digitatum* on citrus.

A non-significant variation in TSS was observed in fruits subjected to pre-wax application or combined application with plant extracts or plant extracts alone at 25 ºC (Table 7.5). Untreated and chlorine washed fruits showed a significant (P <0.05) increase in TSS unlike other postharvest treatments. Fruits subjected to commercial treatment with waxing showed a significant (P <0.05) decrease in SS and TA levels. Separate application of *A. seyal* or *W. somnifera* extracts resulted in significant (P <0.05) decrease in TA levels in fruits stored at 25 ºC.

7.3.2 Quality assessment and analysis for sensory attributes

Mean separation analyses of sensory parameters showed significant (P <0.05) differences between different types of postharvest treatments with plant extracts alone, or the combination of treatments or with pre-wax treatments and plant extracts (Table 7.6). For further analyses, CVA was carried out to evaluate the differences among the six sensory parameters used and

to show the relative contribution of each variable to the sensory quality on citrus with respect to the different postharvest treatments. The CVA plot axis CA 1 accounts for 61% of the variance and CA 2 for 19% (Fig. 7.2). Together, they account for nearly 80% of the total variance observed. The figure shows six well-separated groups corresponding to samples from different postharvest treatments and storage conditions. The untreated control at 7 ºC is situated to the lower left side of the plot, untreated control and chlorine washed fruit appeared to the lower middle of the plot. The fruit held at 7 ºC and subjected to pre-wax application with of *A. Seyal* or *W. somnifera*, *A. seyal* alone, combined application of wax with *A. seyal* or *W. somnifera* and commercially adopted treatment appeared at the middle left side of the plot. The pre-wax with *W. somnifera*, combined application of wax with *W. somnifera* and the commercially adopted treatment held at 25 ºC were grouped together towards the upper middle part of the plot. The variates responsible for the sensory characters were flavour (r = -0.899), odour (r = -0.789), appearance (r = -0.738), and flavedo colour (r = -0.636). The variate mostly responsible for this was skin colour (r = 0.708). The fruit pre-waxed with *W. somnifera*, combined application with wax and *W. somnifera* and the commercially adopted treatment held at 25 ºC revealed more over matured orangish colour. In this evaluation, pre-wax application of *A. seyal* and *W. somnifera*, combined application of wax with *W. somnifera*, and plant extracts *A. seyal* or *W. somnifera* alone retained the quality of the fruit at ºC.

Table 7.4 Effect of semi-commercial application of plant extracts (*Acacia seyal* Del.var.Seyal and *Withania somnifera* L. Dunal) on postharvest decay control and overall quality retention of citrus fruits during long-term (50 days) cold storage (7 °C)

Postharvest treatments	Penicillium decay incidence (%)	Chilling effect (%)	Weight loss (%)	Firmness (N)	Soluble solids concentration (%)	Titratable acidity (%)
Pre-wax application of A. seyal extract	0.00	0. 00	$0.01^{d} \pm 0.0$	$38.54^{a} \pm 0.4$	$12.66^{c} \pm 0.3$	$1.28^{e} \pm 0.1$
Pre-wax application of *W. somnifera* extract	0.00	0. 00	$0.01^{d} \pm 0.0$	$34.79^{cd} \pm 1.0$	$13.47^{ab} \pm 0.3$	$1.37^{bcd} \pm 0.0$
A. seyal extract + wax mix	0.00	0. 00	$0.00^{d} \pm 0.0$	$36.53^{abc} \pm 0.6$	$13.47^{ab} \pm 0.2$	$1.40^{abc} \pm 0.0$
W. somnifera extract + wax mix	0.00	0. 00	$0.02^{c} \pm 0.0$	$35.80^{bc} \pm 1.4$	$13.21^{abc} \pm 0.3$	$1.35^{cd} \pm 0.0$
A. seyal extract only	0.00	0. 00	$0.00^{d} \pm 0.0$	$38.45^{a} \pm 0.9$	$13.32^{ab} \pm 0.4$	$1.34^{de} \pm 0.1$
W. somnifera extract only	0.00	0. 00	$0.01^{d} \pm 0.0$	$32.69^{e} \pm 1.0$	$13.44^{ab} \pm 0.2$	$1.41^{ab} \pm 0.0$
Control						
Untreated	$10..5^{a} \pm 1..3$	$6.67^{a} \pm 2.08$	$0.06^{a} \pm 0.0$	$33.51^{de} \pm 2.0$	$13.74^{a} \pm 0.2$	$1.35^{d} \pm 0.0$
Chlorine washed only	0.00	0. 00	$0.04^{b} \pm 0.0$	$32.88^{de} \pm 0.6$	$13.51^{ab} \pm 0.6$	$1.47^{a} \pm 0.1$
Commercial	0.00	0. 00	$0.01^{d} \pm 0.0$	$36.99^{ab} \pm 2.4$	$13.09^{bc} \pm 0.3$	$1.40^{bcd} \pm 0.1$

Legend: [x] Means in each column followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected least significant test. Relatively high incidence (10.5%) of fruit decay was observed in untreated fruits. Chilling injury column indicates incidence of chilling injury-affected fruits only in untreated fruits. Abbreviations described as follows: *A. seyal* = *Acacia seyal* Del. var Seyal, *W. somnifera* = *Withania somnifera* L. Dunal.

Table 7.5 Effect of semi-commercial application of plant extracts (*Acacia seyal* Del.var.Seyal and *Withania somnifera* L. Dunal) on postharvest decay control and overall quality retention of citrus fruits at long-term room (25 °C) temperature storage

Postharvest treatments	**Penicillium decay incidence (%)**	**Browning effect (%)**	**Weight loss (%)**	**Firmness (N)**	**Soluble solids concentration (%)**	**Titratable acidity (%)**
Pre-wax application of *A. seyal* extract	0.00	$6.3^{a} \pm 2.3$	$0.11^{b} \pm 0.0$	$29.68^{cde} \pm 0.7$	$13.21^{bc} \pm 0.6$	$1.43^{ab} \pm 0.1$
Pre-wax application of *W. somnifera* extract	0.00	$6^{a} \pm 1.0$	$0.12^{b} \pm 0.0$	$26.57^{ef} \pm 1.1$	$13.41^{bc} \pm 0.3$	$1.41^{abc} \pm 0.1$
A. seyal extract + wax mix	0.00	0. 00	$0.11^{b} \pm 0.0$	$36.07^{a} \pm 1.7$	$13.87^{ab} \pm 0.6$	$1.40^{abc} \pm 0.0$
W. somnifera extract + wax mix	0.00	$2.33^{b} \pm 2.3$	$0.11^{b} \pm 0.0$	$34.98^{ab} \pm 2.5$	$13.40^{bc} \pm 0.1$	$1.30^{cde} \pm 0.1$
A. seyal extract only	0.00	0. 00	$0.12^{b} \pm 0.0$	$27.67^{def} \pm 0.9$	$13.71^{ab} \pm 0.8$	$1.21^{e} \pm 0.1$
W. somnifera extract only	0.00	0. 00	$0.12^{b} \pm 0.1$	$30.77^{cd} \pm 1.6$	$13.13^{bc} \pm 0.3$	$1.16^{e} \pm 0.1$
Control						
Untreated	$36^{a} \pm 3.0$	$0.3^{bc} \pm 0.6$	$0.17^{a} \pm 0.1$	$29.77^{cde} \pm 1.8$	$14.43^{a} \pm 0.4$	$1.52^{a} \pm 0.1$
Chlorine washed only	$9^{b} \pm 2.4$	0. 00	$0.11^{b} \pm 0.0$	$32.24^{bc} \pm 1.9$	$14.34^{a} \pm 0.0$	$1.38^{bcd} \pm 0.1$
Commercial	0.00	$5.33^{a} \pm 0.0$	$0.15^{a} \pm 0.0$	$25.93^{f} \pm 3.0$	$12.93^{c} \pm 0.2$	$1.25^{de} \pm 0.1$

Legend: [x] Means in each column followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected least significant test. High incidence (36%) of fruit decay was observed in untreated fruits. Chilling injury column indicates incidence of chilling injury-affected fruits. For abbreviations, see table 7.4 legend.

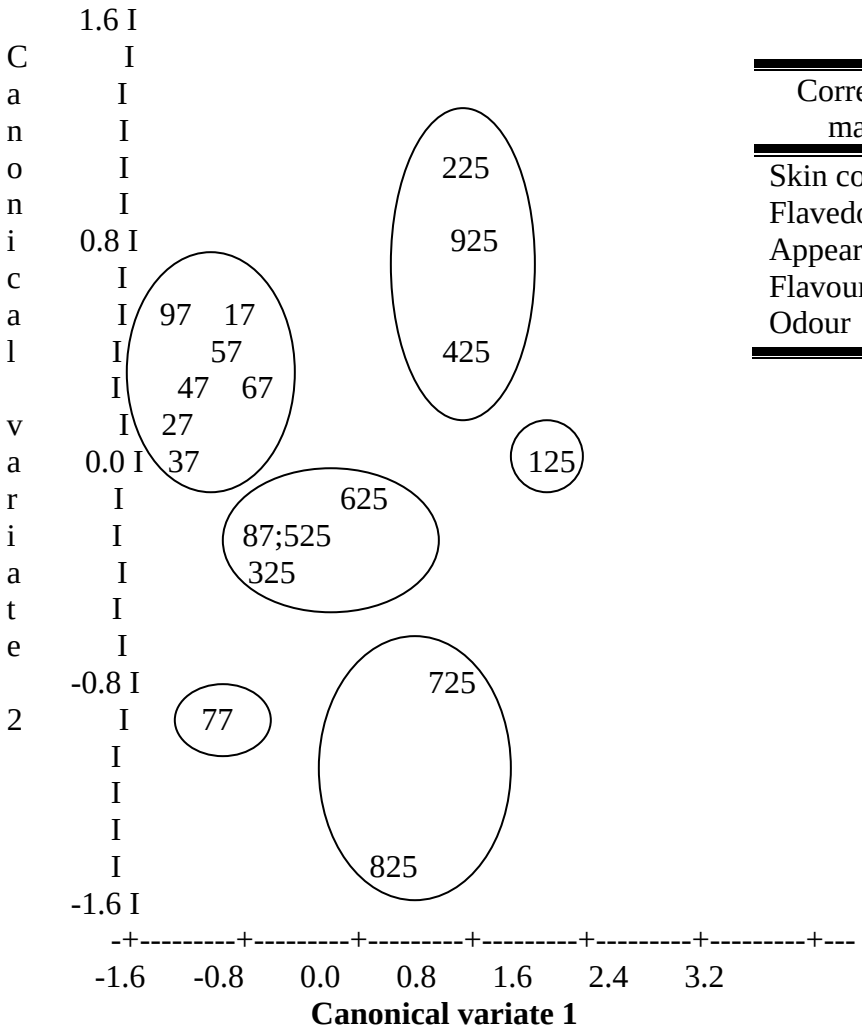
Table 7.6 Sensory evaluation of fruits treated with plant extract treatment combinations and stored at 7 and 25 °C for 50 days

Treatments	Sensory evaluation parameters (7 °C storage temperature)		Sensory evaluation parameters (25 °C storage temperature)			
	Skin colour	Odour/Smell	Skin colour	Appearance	Flavour	Odour/ Smell
Pre-wax application of *A. seyal*	4.3ab	3.8abc	2.7ab	2.5ab	2.2b	2.8ab
Pre-wax application of *W. somnifera*	4.2abc	4.7a	3.8a	3.3ab	2.3b	2.8ab
A. seyal + wax mix	4.0abc	4.3ab	3.5ab	3.5a	4.0a	4.0a
W. somnifera + wax mix	4.2abc	4.0abc	3.5ab	3.0ab	2.7ab	3.0ab
A. seyal extract alone	4.2abc	3.7bc	3.7a	3.5a	4.0	4.0a
W. somnifera extract alone	4.2abc	4.2abc	3.5ab	3.2ab	3.3ab	3.5a
Untreated control	3.3c	4.0abc	2.8ab	2.3ab	3.2ab	3.0ab
Chlorine washed	3.5bc	3.3c	2.3b	2.2b	3.5ab	3.2ab
Commercial line treatment	4.5a	4.3ab	3.3ab	3.3ab	2.2b	2.0b

Legend: Means of the same letter are not significantly different by Fisher's protected test at $P < 0.05$. Sensory parameters, which showed no significant differences, are avoided for simplicity. Postharvest fruit quality was assed using 1 –5 rating hedonic scales, where: 1= very poor, 2 = poor, 3 = fair with limited acceptability, 4 = good, and 5 = excellent. For abbreviations see table 7.4 legend.

W. somnifera and commercially adopted treatment appeared at the middle left side of the plot. The pre-wax with *W. somnifera*, combined application of wax with *W. somnifera* and the commercially adopted treatment held at 25 ºC were grouped together towards the upper middle part of the plot. The variates responsible for the sensory characters were flavour (r = -0.899), odour (r = -0.789), appearance (r = -0.738), and flavedo colour (r = -0.636). The variate mostly responsible for this was skin colour (r = 0.708). The fruit pre-waxed with *W. somnifera*, combined application with wax and *W. somnifera* and the commercially adopted treatment held at 25 ºC revealed more over matured orangish colour. In this evaluation, pre-

wax application of *A. seyal* and *W. somnifera*, combined application of wax with *W. somnifera*, and plant extracts *A. seyal* or *W. somnifera* alone retained the quality of the fruit at 7 ºC.



Correlation matrix	(r) value
Skin colour	0.708
Flavedo colour	-0.636
Appearance	-0.738
Flavour	-0.899
Odour	-0.769

Legend: The first CV (horizontal axis) mainly contrasts with cold (7 ºC) and room (25 ºC) temperatures. The second CV (vertical axis) contrasts mainly to temperature and varies mostly between 2 and 9 or 7 and 8 treatments. Numbers are designated for each treatment in accordance with storage temperature used. Two digits for cold and three digits for room temperature storages are given. The first digit represents a treatment order from (1-9) and the next digit (s), 7 for cold temperature and 25 for room temperature storages, respectively. Cold storage (7 °C) treatments designation represented by the following order as follows: 17- pre-wax application of *A. seyal*; 27 pre-wax application of *W. somnifera*; 37- *A. seyal* + wax mix; 47- *W. somnifera* + wax mix; 57- *A. seyal* extract alone; 67- *W. somnifera* extract alone; 77- Untreated control; 87- Chlorine washed; 97- Commercial line treatment. Room temperature (25 ºC) storage treatments designations represented in the following order: 125- pre-wax application of *A. seyal*; 225 pre-wax application of *W. somnifera*; 325- *A. seyal* + wax mix; 425- *W. somnifera* + wax mix; 525- *A. seyal* extract alone; 625- *W. somnifera* extract alone; 725-Untreated control; 825-Chlorine washed; 925-Commercial line treatment.

Fig. 7.2. Sensory evaluation canonical variate analyses.

7.4 DISCUSSION

It is evident from this study that the two selected plant extracts, *A. seyal* and *W. somnifera* reduce disease incidence and retained the overall quality of citrus when used as a postharvest decay control protective agent during long term cold (7 ºC) and ambient (25 ºC) temperature storages. Pre-wax, wax-mix and/or *A. seyal* and *W. somnifera* extracts alone resulted in significant disease incidence reduction and quality retention of citrus fruits stored under simulated export conditions. These results were comparable and often better than the commercial chemical treatments. This is the first report where these plant extracts were used in citrus postharvest trials and showed potential to retain quality and prevent decay.

Higher incidence of postharvest *Penicillium* decay (36%) was detected in untreated fruits kept at long-term ambient temperature unlike other treatment coatings. The separate application of *A. seyal* and/or *W. somnifera* alone and/or in combination with wax showed significant reduction of *Penicillium* disease incidence, which could involve either the suppression of spore germination and/or the inhibition of mycelial growth. Browning was detected in some treatments such as pre-wax applications of *A. seyal* (6.3%), wax-mix and/or pre-wax application of *W. somnifera* (2.33-6%), untreated fruits (0.3%) and commercial chemical treated fruits (5.33%) stored at room temperature. According to Petracek *et al*. (1998), high temperature storage of waxed fruits stimulates postharvest browning by decreasing peel gas permeability and desiccation.

Realtively higher incidence of *Penicillium* decay (10%) and browning (chilling injury) (6.7%) was detected in untreated orange fruits kept at long-term cold storage. According to Biolatto *et al*. (2005) development of peel pitting on untreated fruits at long-term cold storage are associated with the accumulation of aldehydes and alcohol produced by anaerobic respiration. The chilling effect was not detected in the plant extract or commercial wax treated fruits stored at the same temperature. Postharvest treatments have been known to reduce fruit chilling injury incidences i.e. ethylene degreening prior to cold storage (Grierson, 1974), waxing (Davis and Harding, 1959) and fungicide application (Schiffman-Nadel *et al*., 1972; Petracek *et al*., 1998; Schirra *et al*., 2004). In this study, the postharvest application of plant extracts showed a similar effect in inhibiting pitting and fruit decay, which signifies their commercial value as a postharvest treatment option.

Commercial chemical treated fruits showed a decrease in percentage concentration of SS, TA, fruit firmness and augmenting weight loss on fruits stored at room temperature. According to DeEll *et al*. (2001), firmness depends on cell size, cell wall thickness and strength, turgor pressure and the manner in which cells bind together. In this particular experiment, the low percentage concentration of acidity, SS, firmness and percentage weight loss in commercial chemical treated fruits were associated with waxing (Davis *et al*., 1967; Hagenmaier and Baker, 1993; Hagenmaier, 2002). It has been reported by Ben-Yehoshua *et al*. (1994) that waxing of fruits results in the build up of high carbon dioxide and low oxygen concentrations, which help delay the rate of respiration, senescence and resulting in firm fruits as observed at low storage temperatures. However, an increase in carbon dioxide or ethylene within the wax layer could cause anaerobic stress and result in less firm fruits as studied in apples (Knopacka and Plocharski, 2004) with off flavour fruits like banana (Satyan *et al*., 1992), kiwifruit (Marsh *et al*., 2004) and grape fruits (Biolatto *et al*., 2005; Shi *et al*., 2005) as observed with commercial treatments kept at 25 ºC storage conditions.

Application of *A. seyal* and/ or *W. somnifera* plant extracts with different treatment combinations on citrus fruit showed a significant effect on fruit quality retention as evaluated with flavour, odour, flavedo colour and overall appearance in sensory parameters. These results confirm the data obtained from the physicochemical analysis. It is therefore evident from this study that the application of *A. seyal* and *W. somnifera* extracts would have an effect on the complex biochemical changes associated with ripening but the mechanism of the effect on these changes has not been determined.

This study showed that *A. seyal* and *W. somnifera* can potentially be used as an alternative to synthetic fungicides and waxes to retain fruit quality. Since these plants are used in traditional healing of human aliments, i.e. *W. somnifera* in India (Bhatia *et al*., 1987) and Ethiopia (Demissew, 1989; Bekele, 1993), *A. seyal* in Ethiopia and tropical Africa countries (Duke, 1983; Bekele, 1993), and it could therefore represent a novel postharvest treatment. Further testing of these extracts developed during the current study could be recommended commercially as a safe method for quality retention and postharvest decay control of citrus.

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CHAPTER EIGHT

GENERAL DISCUSSION AND CONCLUSION

In developing countries, where protection and proper handling of fresh fruit are inadequate, losses during transit and storage account for over 50% of the harvested crop (Wisniewski and Wilson, 1992). In this study it was found that actual losses recorded in Ethiopia in citrus storage was 46.7%. This decay was mostly caused by *Penicillium* species, particularly by *Penicillium digitatum* Sacc., the causal agent of citrus green mould. This disease is of economic importance in all citrus producing regions of the world and is mostly related to poor handling and storage practices (Eckert and Eaks, 1989). To prevent or minimise such losses, synthetic chemicals are applied either pre- or postharvestly. However, the application of these chemicals may result in chemical residues on food that affect human health (Roistacher *et al*., 1960; Matsumura, 1972; Houck, 1977; Koeman, 1978; Norman, 1988) and can lead to build up of pathogen resistance or environmental pollution (Janisiewicz, 1987; Wilson and Wisniewski, 1989). The use of biocontrol agents to manage postharvest decay of fruit has been explored as an alternative to synthetic fungicides (Wilson and Wisniewski, 1989; Benbow and Sugar, 1999) and several commercial products are now available (Bull *et al*., 1997; Droby *et al*., 1998; Janisiewicz and Korsten, 2002). The choices of using natural plant products and/or the development of natural microbial antagonists thus could minimise environmental risks.

Results obtained in the present study showed that the selected plant extracts and yeast antagonists have desirable characteristics for postharvest applications to control *P. digitatum* on citrus. From a total of 23 plant species and 242 potential microbial isolates of three citrus growing regions in Ethiopia, screening for their antimicrobial activity yielded two superior plant species [*Acacia seyal* Del. var. Seyal and *Withania somnifera* L. Dunal] and three yeast antagonists [MeJtw 10-2 (*Cryptococcus laurentii* (Kufferath) Skinner, TiL4-2 (*Candida sake*) and TiL4-3 (*C. laurentii*)].

Application of *A. seyal* and/or *W. somnifera* plant extracts with different treatment combinations on citrus fruit showed a significant effect on fruit quality retention as evaluated with flavour, odour, flavedo colour and overall appearance in sensory parameters. These results confirm the data obtained from the physicochemical analysis and show the potential effect of these plant extracts involving complex biochemical changes associated with ripening

and fruit quality. This is the first report where plant extracts from *A. seyal* and *W. somnifera* are described to be used as an alternative to synthetic fungicides and waxes to retain fruit quality. The commercial use of these plant extracts can result in a safe method to protect the citrus from postharvest decay and could represent a novel postharvest treatment. These products are used in traditional healing of human ailments [*W. somnifera* in India (Bhatia *et al*., 1987) and Ethiopia (Demissew, 1989; Bekele, 1993), *A. seyal* in Ethiopia and other tropical African countries (Duke, 1983; Bekele, 1993)]. *In vivo* tests with some selected plant extracts showed remarkable control of fruit decay due to *P. digitatum* in South Africa, which may indicate the promising potential for postharvest disease control, especially for the citrus industry. In addition, the plant extracts provided a shiny gloss to the fruit surface and prevented desiccation, suggesting a potential replacement for wax. Future research advances on this aspect would contribute to determining the active chemical compounds of these plant extracts for commercial use as postharvest applications. In order to test the potential application of these extracts against other pathogens, several fungal and bacterial spp. were inhibited by the extracts. The effective control on important food borne pathogens such as *Staphylococcus*, *Salmonella* and *Shigella* spp., previously associated with citrus and other fruits and vegetables, could also make the commercial product more acceptable for other disease control strategies.

In this study, three potential yeast antagonists [two strains of *C. laurentii* (MeJtw10-2 and TiL4-2) and one of *C. sake* (TiL4-3)], exhibiting the best inhibition of *P. digitatum* and broad-spectrum activity against *Geotrichum candidum* (Link ex Pers) and *Colletotrichum gloeosporioides* Penz., were identified. The potential use and application of yeast strains without antibiosis activity have been demonstrated by many workers to control postharvest decay of fruits and vegetables (Wilson and Wisniewski, 1989, Wisniewski and Wilson, 1992, Janisiewicz and Bors, 1995). It is evident from the *in vitro* study of this experiment that the selected potential antagonists did not show any antibiosis or volatile production against any of the pathogens tested. The isolates also showed a significant rate of disease incidence reduction (70-100%) on fruits incubated at 7 ºC and 25 ºC for >30 days. The application of antagonist TiL4-2 (*C. sake*) suppressed *P. digitatum* growth at a minimum concentration (10^5 spores ml^{-1}) of both antagonist and pathogen, which is a more effective control than previous reports made by Droby *et al*. (1989). The rapid growth of the yeast antagonists without any additive at the wound site indicates their ability and considerable potential use as a biocontrol agent (Vero *et al*., 2002). This would require further commercial testing upon product formulation and registration according to Act 47, 2000 of the Republic of South Africa.

Excluding antibiosis as potential mode of action at the initial screening stages is important when selecting a natural antagonist for postharvest disease control. Although the mechanisms by which yeast biocontrol agents provide decay control are not fully understood, the mode of action of several yeast antagonists was shown in this study not to involve antibiosis. The mechanism involved was found to be competition for nutrients (Benbow and Sugar, 1999; Janisiewicz *et al*., 2000) and space (Janisiewicz *et al*., 2000) at the wound site. In this study, the fast colonisation effect of yeast antagonists by producing extracellular matrix that sticks to the pathogens was evident. This was confirmed by the *in vitro* dual culture experiments supported by electron microscope results. The fast recovery and compatibility of yeast antagonists integrated with plant extracts *in vitro* and *in vivo* treatments showed potential for industrial application to substitute chemical pesticides.

The search for potential antagonists from specific geographic areas based on their distinct mode of actions other than antibiosis against the range of pathogens is crucial for selection of and development of antagonists for postharvest application. The future search and development of biopesticides therefore can be upheld with this strategy to control pre- and postharvest diseases of citrus in particular and other crops in general.

Suggestions for future studies:

The out comes of this study can provide an effective alternation for pesticides. In order to develop these products the following needs to be done:

1. Commercial evaluations of various treatment formulations of *A. seyal* and *W. somnifera* extracts and its assessment under export conditions and overseas.
2. Semi-commercial and commercial evaluations of various yeast antagonist treatment formulations under simulated and export conditions.
3. Evaluate product consistency by repeating semi commercial and commercial trials.
4. Evaluate the efficacy of both plant extracts and yeast antagonists on other crops.
5. Product registration and commercialisation.

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