

**Postharvest quality and sensitivity to anthracnose disease
of *Carica papaya* L. 'Red Lady' affected by
Papaya ring spot virus**



by

Peella Kankanamage Chamika Buddhinie

**Thesis submitted to the University of Sri Jayewardenepura
for the Degree of Master of Philosophy in Botany**

I / we certify that by the candidate has incorporated all corrections, additions and amendments recommended by the examiners.



Prof (Mrs). N. Salim
(Principal Supervisor)

Date: 14th March 2016

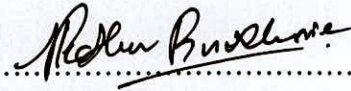


Dr. K. H. Sarananda
(External Supervisor)

Date: 18/3/2016

DECLARATION

The work described in this thesis was carried out by me under the supervision of Prof (Mrs). N. Salim and Dr. K.H. Sarananda and a report on this has not been submitted in whole or in part to any University or any other Degree/Diploma.


.....


P. K. C. Buddhinie

Date: *14th March 2016*
.....

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.



.....
Prof (Mrs) N. Salim
(Principal Supervisor)



.....
Dr. K. H. Sarananda
(External Supervisor)

Date: 14th March 2016

Date: 18/3/2016

Table of Contents

	Page number
Table of Contents	I
List of Tables	VII
List of Figures	IX
List of Plates	XIII
Acknowledgement	XV
ABSTRACT	XVIII
1. INTRODUCTION	1
1.1 Papaya (<i>Carica papaya</i> L.)	1
1.2 Papaya Ringspot virus disease	3
1.3 Postharvest diseases	3
1.4 Scope of the study	5
1.5 Specific objectives	6
2. LITERATURE REVIEW	7

2.1	<i>Carica papaya</i> L.	7
2.2	Papaya Varieties	8
2.2.1	Papaya varieties found in Sri Lanka	10
2.3	Harvesting and postharvest handling of papaya	11
2.4	Pests and diseases of papaya	12
2.4.1	Fungal, Bacterial and Phytoplasma diseases	13
2.4.2	Viral diseases	14
2.4.2.1	Papaya ringspot virus disease	14
2.4.2.2	Biology and epidemiology of PRSV	15
2.4.2.3	Management of PRSV disease	19
2.4.3	Postharvest diseases of papaya	22
2.4.3.1	Anthracnose and Chocolate spot	22
2.4.3.2	Control of postharvest diseases	26
2.5	Nutritional value of Papaya	28
2.5.1	Carbohydrates	28
2.5.2	Protein and lipid	28
2.5.3	Minerals	29
2.5.4	Carotenoids	30
2.5.5	Ascorbic acid	31
2.6	Physico-chemical parameters of papaya	32
2.6.1	Titrateable acidity	32
2.6.2	Fruit firmness	32

2.6.3	Flesh colour	33
2.7	Organoleptic properties of papaya	35
3.	MATERIALS AND METHODS	36
3.1	Sample Collection	36
3.1.1	Field samples	36
3.1.2	Market samples	36
3.2	PRSV disease detection	38
3.2.1	PRSV detection in field samples	38
3.2.2	PRSV detection in market samples	39
3.3	Categorization of plants according to ELISA values	40
3.4	Effect of harvesting stage and degree of PRSV disease on post harvest quality of Red Lady papaya	42
3.4.1	Harvesting stage	42
3.4.2	Total soluble solids (TSS)	43
3.4.3	pH	43
3.4.4	Titrateable acidity (TA)	43
3.4.5	Fruit flesh firmness	44
3.4.6	Fruit flesh colour	44
3.5	Determination of nutritional value of PRSV affected Red Lady papaya of different disease levels	45
3.5.1	Moisture content	45

3.5.2	Dehydration of papaya samples	46
3.5.3	Total crude fat content	46
3.5.4	Ash content	47
3.5.5	Total crude protein content	47
3.5.6	Free amino acid contents	48
3.5.7	Minerals	49
3.5.8	Vitamin C content	50
3.5.9	Lycopene and β carotene content	51
3.6	Sensory evaluation	52
3.7	Sensitivity of PRSV affected Red Lady papaya to anthracnose disease	54
3.7.1	Isolation of <i>Colletotrichum</i> sp. from anthracnose lesions on Red Lady papaya	54
3.7.2	Natural development of anthracnose disease on Red Lady papaya fruits infected with PRSV	56
3.7.3	Anthracnose disease development of artificially inoculated fruits	57
3.8	Statistical analysis	58
4.	RESULTS	59
4.1	Categorization of diseased plants according to ELISA values	59
4.2	Effect of stage of maturity at harvest and degree of disease on post harvest quality of PRSV affected Red Lady papaya	60
4.2.1	Interaction between stage of maturity at harvest and disease severity on post harvest quality parameters	60

4.2.2	Effect of PRSV disease severity on post harvest parameters of Red Lady papaya fruits	64
4.2.2.1	Effect of PRSV disease severity on TSS, TA, pH and fruit firmness of Red Lady papaya fruits	64
4.2.2.2	Effect of disease severity on flesh colour parameters of PRSV affected Red Lady papaya fruits	66
4.2.3	Effect of stages of maturity at harvest on post harvest parameters of PRSV infected Red Lady papaya fruits	68
4.2.3.1	Effect of stages of maturity at harvest on TSS, TA, pH and fruit firmness of field samples of PRSV infected Red Lady papaya fruits	68
4.2.3.2	Effect of stage of maturity at harvest on flesh colour parameters of PRSV infected Red Lady papaya field samples	74
4.3	Effect of PRSV infection level on nutritional parameters of Red Lady papaya	80
4.3.1	Effect of PRSV infection on percentage moisture content of Red Lady papaya	80
4.3.2	Effect of PRSV infection on percentage ash content	82
4.3.3	Effect of PRSV infection on percentage crude fat content	84
4.3.4	Effect of PRSV infection on percentage crude protein content	86
4.3.5	Effect of PRSV infection on free amino acid content	88
4.3.6	Effect of PRSV infection on ascorbic acid content	95
4.3.7	Effect of PRSV infection on carotenoid content	97
4.3.8	Effect of PRSV infection on mineral contents of Red Lady papaya	102
4.4	Effect of PRSV infection on organoleptic properties of Red Lady papaya	107

4.4.1	Effect of PRSV infection on odour, flavour and overall acceptability	107
4.5	Effect of PRSV infection on postharvest infection by <i>Colletotrichum</i> sp. in Red Lady papaya	112
4.5.1	Effect of PRSV infection on development of anthracnose lesion	112
4.5.2	Effect of PRSV on anthracnose lesion development in fruits artificially inoculated with <i>Colletotrichum</i> sp.	116
5.	DISCUSSION	118
6.	CONCLUSIONS	134
	List of References	137
	Appendices	154

List of tables

	Page number
Table 1: Interaction between different stages of maturity at harvest and PRSV disease severity for different post harvest parameters	61
Table 2: Postharvest parameters; TSS, TA, pH, fruit firmness of fruits collected from plants with mild and severe infections of PRSV	64
Table 3: Flesh colour parameters (L*, a*, b* and hue) of fruits from mild and severe infections of PRSV	66
Table 4: TSS, TA, pH and fruit firmness of Red Lady papaya fruits harvested at different stages of maturities	69
Table 5: Flesh colour parameters (L*, a*, b* and hue) of PRSV infected Red Lady papaya fruits harvested at different stages of maturities irrespective of the disease severity	75
Table 6: Percentage moisture and ash contents of Red Lady papaya field samples	81
Table 7: Percentage moisture and ash contents of Red Lady papaya market samples	81
Table 8: Crude fat and protein contents of Red Lady papaya field samples	84
Table 9: Crude fat and protein contents of Red Lady papaya market samples	85
Table 10: Free amino acid contents in fruits of Red Lady papaya field samples	89
Table 11: Free amino acid contents in fruits of Red Lady papaya market samples	90
Table 12: Ascorbic acid content of Red Lady papaya field samples	95
Table 13: Ascorbic acid content of Red Lady papaya market samples	96
Table 14: Lycopene and β -carotene contents of Red Lady papaya field samples	98

Table 15: Lycopene and β -carotene contents of Red Lady papaya market samples	99
Table 16: Mineral contents of Red Lady papaya field samples	102
Table 17: Mineral contents of Red Lady papaya market samples	103
Table 18: Median values of organoleptic properties of Red Lady papaya field samples	107
Table 19: Median values of organoleptic properties of Red Lady papaya market samples	108
Table 20: Anthracnose disease development on PRSV infected Red Lady papaya fruits collected from field with different infection levels	113
Table 21: Anthracnose disease development on Red Lady papaya market samples with and without PRSV symptoms	114

List of figures

	Page number
Figure 1: The interaction plot between different stages of maturity at harvest and degree of disease for the L* parameter of PRSV affected Red Lady fruit flesh colour	62
Figure 2: The interaction plot between different stages of maturity at harvest and degree of disease for the b* parameter of PRSV affected Red Lady fruit flesh colour	63
Figure 3: The boxplot of TSS values against PRSV infection levels of Red Lady papaya fruits	65
Figure 4: The boxplot of colour component L* against PRSV infection levels of Red Lady papaya fruit flesh colour	67
Figure 5: The boxplot of TSS in fruits harvested at different stages of maturity of PRSV infected Red Lady papaya fruits irrespective of the disease severity	70
Figure 6: The boxplot of fruit firmness against different stages of maturity at harvest of PRSV infected Red Lady papaya fruits irrespective of the disease severity	71
Figure 7: The boxplot of TA against different stages of maturity at harvest of PRSV infected Red Lady papaya fruits irrespective of the disease severity	72
Figure 8: The boxplot of pH against different stages of maturity at harvest of PRSV infected Red Lady papaya fruits irrespective of the disease severity	73

Figure 9: The boxplot of L* against different stages of maturity at harvest of PRSV infected Red Lady papaya fruit flesh colour irrespective of the disease severity	76
Figure 10: The boxplot of a* against different stages of maturity at harvest of PRSV infected Red Lady papaya fruit flesh colour irrespective of the disease severity	77
Figure 11: The boxplot of b* against different stages of maturity at harvest of PRSV infected Red Lady papaya fruit flesh colour irrespective of the disease severity	78
Figure 12: The boxplot of hue value against different stages of maturity at harvest of PRSV infected Red Lady papaya fruit flesh colour irrespective of the disease severity	79
Figure 13: The boxplot of moisture content of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	82
Figure 14: The boxplot of ash content of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	83
Figure 15: The boxplot of crude fat content of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	86
Figure 16: The boxplot of protein content of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	87

Figure 17: The boxplot of total amino acid content of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	91
Figure 18: The boxplot of amino acid contents of Red Lady papaya fruits against different PRSV infection levels of field samples	93
Figure 19: The boxplot of amino acid contents of Red Lady papaya fruits against market samples with and without PRSV disease symptoms	94
Figure 20: The boxplot of ascorbic acid content of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	97
Figure 21: The boxplot of Lycopene content of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	100
Figure 22: The boxplot of β -carotene contents of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	101
Figure 23: The boxplot of Na content of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	105
Figure 24: The boxplot of Ca content of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	106

Figure 25: The boxplot of organoleptic property - flavour of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	109
Figure 26: The boxplot of organoleptic property - odour of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	110
Figure 27: The boxplot of organoleptic property – overall acceptability of Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	111
Figure 28: The boxplot of percentage anthracnose lesion development of uninoculated Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	115
Figure 29: The boxplot of percentage anthracnose lesion development of artificially <i>Colletotrichum</i> sp. inoculated Red Lady papaya fruits against different PRSV infection levels of field samples (MI and SI) and market samples with and without PRSV symptoms	117

List of plates

	Page number
Plate 1: Electron micrograph of papaya ringspot virus particles	17
Plate 2: Leaf mosaic and shoestring appearance of PRSV infected papaya leaves	18
Plate 3: Characteristic ringspot symptoms of PRSV infected papaya fruits	18
Plate 4: Typical anthracnose lesion on papaya fruit skin	25
Plate 5: Chocolate spot symptoms on papaya fruit skin	25
Plate 6: Attributes of L*, a*, b* colour space	34
Plate 7: Hue value scale for L*a*b* colour space	34
Plate 8: Red Lady market samples without PRSV disease symptoms	37
Plate 9: Red Lady market samples with PRSV symptoms	37
Plate 10: Micro titre ELISA plate after adding PNP substrate	40
Plate 11: Red Lady papaya fruits harvested at different stages of maturity; 1. Colour break stage, 2. 10% yellow, 3. 20% yellow, 4. 30% yellow, 5. 40% yellow	41
Plate 12: Papaya fruit cubes prepared for sensory evaluation	53
Plate 13: <i>Colletotrichum</i> sp. grown on PDA plate	55
Plate 14: <i>Colletotrichum</i> sp. spores under high power ($\times 40$) resolution of light microscope	55
Plate 15: Red Lady papaya fruits incubated in glass chambers to develop natural anthracnose lesions	56
Plate 16: Anthracnose lesion development on uninoculated Red Lady papaya fruit collected from mildly PRSV infected plant	112

Plate 17: Anthracnose lesion development on a Red Lady papaya fruit collected from mildly infected plant inoculated with spores of *Colletotrichum* sp at 2×10^5 spores/ml concentration.

116

Acknowledgement

I am deeply indebted to my principal supervisor, Prof (Mrs) N. Salim of Department of Botany, University of Sri Jayewardenepura whose stimulating motivation, enormous support and invaluable ideas helped me in completing this research. It is a great honor to work under her supervision and without her guidance and persistent support this thesis would not have been possible. I would like to express my sincere gratitude and heartfelt appreciation to my external supervisor Dr. K. H. Sarananda, Head, Food Research Unit, Gannoruwa for guiding me and providing invaluable advices throughout the research. I am greatly indebted to him for his patients and immense support rendered.

My heartfelt gratitude goes to Mr. Waruna Madawanaarachchi, CEO of CIC Agribusiness (Pvt) Ltd, General Manager Mr. Priyanga Dematawa, Assistant Manager Mr. Sumith Attanayake, Mr. Hemantha, and Mr. Waruna of CIC farm, Pelwehera for allowing me to collect samples from their papaya fields without any hesitation.

I am grateful to Dr. N. Punyasiri, Nature's Secrets (Pvt) Ltd, Dr. U.G. Chandrika, Department of Biochemistry, University of Sri Jayewardenepura, Dr. V. Basnayake of Plant Virus indexing center, Homagama, Dr. M. Iqbal and Dr. M. S. Vithanage of Institute of Fundamental Studies, Kandy, Mr. D.P.C. Swarnasiri, and Miss Suneka of Institute of Postharvest Technology, Anuradhapura for their invaluable cooperation provided to me to fulfill my research objectives.

I sincerely acknowledge the support given by the academic and non academic staff of Department of Botany, University of Sri Jayewardenepura throughout the study.

Generosity of the academic and supporting staff of the Department of Food Science and Technology and Department of Biochemistry is highly appreciated. Without their help, some of the experiments in the study could not have been completed.

Special thank goes to late Mr. G.R.N. Kandambi of Department of Botany, USJP for his enormous support and valuable advices given.

The financial assistant provided by the University Grant, USJP (ASP/PR/06/2010/13) is highly acknowledged.

I am eternally grateful to my dear friend Mrs. Wajira Nandanee of Department of Botany, USJP for her immense support, encouraging words and true affection which was helped me a lot in my difficult times.

I express my sincere thanks to my parents and childhood friends Nuwani, Thanuja, Asanga and Padmanath who has gave me confidence and motivated me in so many ways. Their love and dedication bestowed to me throughout my life is priceless.

I would like to express my deepest gratitude to my loving husband, Najith Wijerathna for his invaluable support and encouragement. Through his love, patience, enthusiasm and unwavering belief in me, I have been able to complete my research work.

Finally my biggest thank goes to my little daughter, Tarinya Monadee, who has patiently endured many long hours alone while I worked on my research. Her unconditional love always inspires me to meet success in my life.

This thesis is dedicated to my lovely, little daughter;

Tarinya Monadee Wijerathna

Postharvest quality and sensitivity to anthracnose disease of *Carica papaya* L. 'Red Lady' affected by Papaya ring spot virus

P. K. C. Buddhinie

ABSTRACT

Papaya (*Carica papaya* L.) Red Lady variety has been identified as a *papaya ring spot virus* (PRSV) - tolerant variety. However, Red Lady papaya fruits with abundant ring spot symptoms on skins are commonly seen in local market since last few years.

Therefore, an investigation was carried out to determine any effects of this virus on post harvest and nutritional parameters of papaya Red Lady fruits. A sensory evaluation also was carried out to determine the effect of PRSV on organoleptic properties of fruits. The investigation was further extended to find out the effect of PRSV on anthracnose disease development of papaya fruits.

Since the initial screening carried out at commercial 'Red Lady' plantations showed plants with no external virus symptoms also carry PRSV, they were categorized into severe and mild disease levels based on the results of double antibody sandwich enzyme linked immunosorbant assay (DAS-ELISA) test. Market samples of this variety were selected depending on the abundance of visual PRSV symptoms on fruit skin.

Of all the post harvest parameters considered, severe infections significantly decreased the contents of Total soluble solids (TSS) and brightness of the flesh colour (L^* of $L^*a^*b^*$ colour space) while other parameters such as titratable acidity (TA), pH, flesh firmness, and a^* , b^* and hue attributes of flesh colour had negligible effect. However, harvesting at different maturity stages had significant effect on TA, pH and all the flesh colour attributes

(L*, a* and hue) except b*. Significant interaction between disease severity and harvesting maturity was resulted only for L* and b* attributes of flesh colour.

Severe infections in field samples had significantly decreased nutritional parameters such as total amino acids, methionine and tryptophan amino acids and sodium (Na) content. Although percentage ash content, β carotene, minerals (K, Mg, Ca, Fe and Zn), ascorbic acid, crude protein contents, were affected by the infection, the effects were not significant. No significant change in quantities was observed on moisture percentage, crude fat, and the lycopene content between the two disease levels. Market samples with PRSV symptoms recorded significant reduction in lycopene, β carotene and Ca content and a significant increase in amino acid tryptophan with compared to those of fruits with no symptoms. Other nutritional parameters assessed did not exhibit any considerable changes in quantity between the two set of market samples.

Sensory evaluation carried out to determine the effect of PRSV on organoleptic properties such as odour, flavour and overall acceptability revealed that there was no significant effect of the virus infection on organoleptic properties considered. Similar to the field samples, there was no significant effect of virus on the organoleptic properties of market samples due to the virus symptoms.

Non inoculated and inoculated anthracnose lesion development also revealed there was no marked effect on disease development between two disease severity levels as well as between market fruits with and without PRSV symptoms.

In general, PRSV infections negatively affected some of the post harvest parameters and nutritional considerations, while it neither had considerable effect on consumer preference and nor an impact on anthracnose disease development in fruits.