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RESEARCH ARTICLE

EFFECT OF PAPAYA RING SPOT VIRUS INFECTION ON THE FRUIT QUALITY OF A TOLERANT PAPAYA VARIETY, 'RED LADY'

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ABSTRACT

Carica papaya L. 'Red Lady', a *papaya ring spot virus (PRSV)*, tolerant variety was introduced to Sri Lanka in early 2000. However, recently, plants with severe symptoms of *PRSV* were observed in the fields while fruits in local markets were seen with abundant ring-spot symptoms. As the virus is known to have no considerable effect on the yield of tolerant varieties, this investigation was focused on verifying the effect of *PRSV* on the quality of "Red Lady" fruits. Since the initial screening showed that plants with no virus symptoms also carry *PRSV*, they were categorized into mild and severe disease levels based on the results of "DAS-ELISA" test. Severe *PRSV* infections significantly decreased the contents of total soluble solids (TSS), total amino acids, methionine, tryptophan, sodium and flesh brightness. Furthermore, contents of β-carotene, ascorbic acid, crude proteins and certain minerals (K, Mg, Ca, Fe, Zn) were also affected but no marked effect was observed on titratable acidity, pH, flesh firmness and lycopene contents between two disease levels. *PRSV* infection of 'Red Lady' papaya considerably reduces the fruit nutritional quality with respect to its total free amino acids, methionine, tryptophan and Na contents and postharvest parameters such as TSS and flesh brightness.

INTRODUCTION

Papaya (*Carica papaya* L.) is grown to a commercial level in many countries due to its high nutritive and medicinal value and economic potential. In Sri Lanka, it is cultivated in homes as a garden crop as well as in large-scale fields especially in the dry zone. Flesh of well ripen papaya serves as a good source of vitamins, particularly A and C, minerals such as K and Mg, and antioxidants such as flavonoids, carotene, micronutrients and many more (da Silva *et al.*, 2007). The vitamin C content of papaya flesh is higher than 0.50g/kg of fresh weight (FW) (Wall, 2006, Souza *et al.*, 2008, Sancho *et al.*, 2011, Silva-Sena *et al.*, 2014), providing the daily adult Recommended Dietary Allowance (RDA) for L-ascorbic acid of 45 mg day^{-1} (Sahabi *et al.*, 2013). It is one of the main fruits recommended for preventing vitamin A deficiency among preschool children in Sri Lanka (Chandrika *et al.*, 2003). Since papaya consists of highly bio-available β-carotene, β-cryptoxanthin and lycopene than other popular carotenoid sources such as carrot and tomato (Schweiggert *et al.*, 2014), it has good potential in therapeutic aspects.

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However, papaya is affected by a destructive disease caused by *papaya ring spot virus (PRSV)* wherever in the world it is grown (Gonsalves and Ishii, 1980). It causes mosaic and chlorosis of leaves, oily water-soaked streaks in the stem, shoe-string appearance of leaves at severe stages and distinguished ring spots on the skin of the fruits (Bau *et al.*, 2004). The *PRSV* is a potyvirus with a monopartite positive sense ssRNA of approximately 10 Kb. (Yeh *et al.*, 1992). Two *PRSV* strains, *PRSV*-P and *PRSV*-W have been reported so far, of which only *PRSV*–P infects both papaya and cucurbits while *PRSV*–W infects only cucurbits (Bateson *et al.*, 1994). Plants that are susceptible to *PRSV* but produce only mild symptoms and yield a reasonable quantity of fruits are considered tolerant plants (Gonsalves *et al.*, 2010). However, tolerant varieties may carry the virus either without expressing any symptoms (latency) or showing only mild symptoms. Genetically modified varieties with improved resistance, such as 'Sun Up' and 'Rainbow', have been developed (Ferreira *et al.*, 2002) and successfully grown in certain countries. Of the numerous papaya varieties that have been introduced to Sri Lanka, the variety 'Red Lady' governs the cultivations. This is mainly due to its tolerance to *PRSV* and ability to produce good quality fruits without any malformation or reduction in size of the fruit compared to traditional varieties infected by the virus. ‘Red Lady’ is an F_1hybrid suitable for cultivation in the dry zone of Sri Lanka which has unfavorable climatic conditions for the *PRSV* infection. However, in 2011, we noticed that ‘Red Lady’ papaya plants in many plantations in the dry zone expressed severe symptoms of *PRSV*. Moreover, most papaya fruits received by the local markets of Sri Lanka were found to be infected with the *PRSV* as indicated by numerous ring spots on the skin. It is well documented that severe infections of viruses greatly reduce the yields of various crops (Sastry, 2013). However, the information on the nutritional quality of these crops is scarce. Similarly, no published information is available on the postharvest quality or physicochemical properties of *PRSV*-infected papaya fruits. Therefore, the major objective of this study was to investigate the effect of *PRSV* infection on the fruit quality of the tolerant papaya variety, ‘Red Lady’. We report here the effect of the virus on postharvest quality parameters such as total soluble solids (TSS), titratable acidity (TA), pH, flesh firmness, flesh color and nutritional parameters such as total crude proteins, amino acids, ascorbic acid, β-carotene, lycopene and mineral content of ‘Red Lady’ papaya fruits harvested at 10% skin yellow stage.

MATERIALS AND METHODS

Sampling method

Fruit samples were collected from ‘Red Lady’ papaya fields in the Pelwehera CIC farm, Dambulla, Sri Lanka (7°53'44.5"N 80°40'04.2"E). The CIC farm is a fully-fledged, dry zone agricultural farm which consists of more than 25 acres of papaya cultivation with major varieties of ‘Red Lady’, ‘Sinta’ and ‘Sun Rise’. A papaya field solely consisting of ‘Red Lady’ plants with an area of approximately 0.25 acres and 170 plants was selected for this study. Plants were under drip irrigation system and each plant was supplied with 250 g of the fertilizer “CIC-Papaya special” at monthly intervals. Additionally, each plant was supplied with 10 g of both borate and zinc sulphate at intervals of three months. The aphid vectors of *papaya ring spot virus* were controlled by spraying the insecticide ‘CIC dimethoate’ fortnightly. Sampling was done within two fruiting seasons in year 2013 and 2014 (19–05–2013, 23-12-2013, 28-04-2014, 29-09-2014, and 21-11-2014). Fruits were harvested from randomly selected plants of two groups: apparently ‘healthy’ (no virus symptoms) group and ‘diseased’ group bearing severe symptoms of *PRSV*-like infection (severe leaf mosaic and ring spots on fruit skin). Harvesting was done manually at 10% yellow stage. Harvested fruits were wrapped in several layers of packing paper and leaf samples from the same plants were packed in poly bags. These packed samples were labeled with a reference number, placed in cardboard cartons and safely transported to the laboratory. Each plant selected for harvesting was tagged with a code number for future references. Harvested fruits were incubated in room temperature and humidity until they reach 100% yellow (fully ripened) stage.

Enzyme Linked Immuno-sorbent Assay (ELISA) test

Standard double antibody sandwich – enzyme-linked immune-sorbent assay (DAS-ELISA) test using Agdia™ reagent kit was employed to screen papaya plants for *PRSV*. The virus-status of each plant during the time of harvest was examined using leaf samples. Test wells of ELISA plates were coated with capture antibody dissolved in carbonate coating buffer (pH = 9.6, dilution 1:200). Samples were extracted in phosphate buffered saline (PBS at pH = 7.4 containing 2 % PVP, 0.2% egg albumin and 0.05% Tween 20) at 1:10 ratio (w:v). Secondary antibody – enzyme (alkaline phosphatase) conjugate was diluted with enzyme conjugate immunoassay (ECI) buffer (PBST containing 2% PVP and 0.2% bovine serum albumin at 1:200 ratio, pH 7.4), while the substrate PNP (paranitrophenyl phosphate) was used at a concentration of 1mg mL^{-1}. Lyophilized positive and negative controls from Agdia™ accompanied the test. The plates were incubated at room temperature and results were evaluated 2 hours after substrate addition.

A_{test}(absorbance)> 2× $A_{negative\ control}$ was considered as *PRSV* infected samples.

Our preliminary ELISA results showed that apparently “healthy” samples collected from different plantation sites at Dambulla as well as those collected from distant areas of CIC farms also carried the virus. As no “healthy” samples could be obtained, the infected plants were categorized into two distinguished disease levels based on quantitative ELISA test, considering the absorption values at $A_{405\ nm}$ 2 hour incubation period after adding the substrate.

1. Severe infection (SI) ; $A_{405\ nm}$> 1.2
2. Mild infection (MI) ; $A_{405\ nm}$< 1.0

Determination of physicochemical parameters (TSS, TA and pH, flesh firmness and colour)

Twenty grams of the mid 1/3 section of a fully ripened papaya were homogenized with 80 ml of distilled water in an electric blender for 5 minutes. The homogenate was filtered using two layers of muslin cloth and the filtrate was used in determining the TSS, TA and pH of the pulp. Dilutions were used when necessary and the dilution factor was incorporated in calculations. The TSS was directly measured from the homogenate using a hand-held refractometer. Thrice-diluted homogenate was titrated against 0.1 M NaOH with phenolphthalein indicator for determination of TA. The TA was calculated using citric acid factor and expressed as % citric acid (Ranganna, 1997). The pH of the filtrate was measured using a digital pH-meter. Papaya flesh firmness was measured using a fruit firmness tester (model: FT 327 mounted on manual test stand) with a 6 mm probe. Three measurements were obtained through the equator of the fruit and the average was calculated. Each region was peeled using a fruit peeler prior to the penetration. Fruit flesh color was measured using a digital chroma meter (Konica Minolta CR-400, sensing inc., Japan). Three regions of middle axis were measured and readings for the L* a* b* color space were obtained. Hue angle (h*) was calculated using a* and b* attributes of L*a*b* colour space.

Determination of crude protein and amino acid contents

The ripened papaya flesh was diced and dehydrated in a dehydration cabinet. Crude protein content of papaya flesh was determined by the method of micro kjeldahl analysis. Agilent high performance liquid chromatography (HPLC HP1100 system; Agilent Technologies Inc.) equipped with a fluorescence detector was used for free amino acid analysis. Automated, online derivatisation of the amino acids with o-phthalaldehyde (OPA) was carried out with an autosampler (G1313A; Agilent Technologies inc.) prior to injecting the sample into the columns. The mobile phase consisted of 40mmol Na_2HPO_4 (pH=7.8 adjusted with NaOH) (solvent A) and acetonitrile: methanol: water (45:45:10, v/v/v) - (solvent B). Solvent gradient was used for the elution of amino acids. The OPA-derivatized amino acids were monitored at an excitation wavelength of 340 nm and emission wavelength of 450 nm, respectively (Henderson *et al.*, 2000).

Determination of ascorbic acid content

The ascorbic acid content of the fruits was determined according to the titration method recommended in AOAC method 43.064 (A.O.A.C., 1984). Twenty five grams of papaya flesh was homogenized and raised up to 50mL with 3% metaphosphoric acid. Ascorbic acid was titrated with 2, 6-dichlorophenolindophenol.

Determination of β-carotene and lycopene contents

Extraction and determination of carotenoids was carried out according to Rodriguez-Amaya (2001). Five grams of papaya flesh were homogenized with cold acetone using a mortar and pestle, and filtered through a buchner funnel. Grinding and filtration were repeated until the residue was colorless. The filtrate was transferred to petroleum ether with the addition of small amounts of acetone extract to petroleum ether (100mL) in a separatory funnel. The petroleum ether layer was separated and dried using a rotary evaporator. The residue was dissolved in 1mL acetone and used for quantification. Lycopene and β-carotene quantification was carried out using high performance liquid chromatography [HPLC series 1200 (Agilent, Germany)] with diode array detection (DAD) with ChemStation software, degasser G13322A, quaternary gradient pump G1311A, auto-sampler G1329A, column oven G1316A and diode array detector G1315B. Acetonitrile, methanol and ethyl acetate containing 0.05% triethylamine were used as the mobile phase at a flow rate of 0.5 ml/min with a gradient from 95: 05: 00 to 60: 20 : 20 for 20 minutes. A monomeric C_{18} column (Agilent, 3μm, 4.6 × 250 mm^2) was used in DAD. Lycopene and β carotene contents of each sample were measured using external standards (Chandrika *et al.*, 2009, Chandrika *et al.*, 2011).

Determination of mineral content

Mineral contents of *PRSV*-infected papaya fruits were determined according to the AOAC method 3.014 (A.O.A.C., 1984). An aliquot of 400mg of papaya flesh was wet digested with conc. HNO_3 in a microwave digester (Milestone START D Microwave). The filtrate was topped up to 25mL with distilled water. Concentrations of the metals sodium (Na), magnesium (Mg), Iron (Fe), zinc (Zn), potassium (K) and calcium (Ca) were determined using an atomic absorption spectrophotometer (GBC 933AA, Australia).

Statistical analysis

We hypothesized the following:

- The degree of *PRSV* infection does not affect the postharvest parameters such as TSS, TA, pH, fruit firmness and flesh colour of the fruit.
- The degree of *PRSV* infection has no effect on the nutritional values (total proteins, free amino acids, minerals, ascorbic acid, and carotene contents) of fruits

The statistical design used was a completely randomized design with 5 replicates. Results were analyzed by one-way analysis of variance (ANOVA) using MINITAB 15 package. Pair wise comparisons were done with Tukey's multiple comparison test ($P \leq 0.05$).

RESULTS AND DISCUSSION

TSS, TA and pH, flesh firmness and colour

The TSS content of fruits from plants with SI was significantly lower (P= 0.000) than that of plants with MI (Table 1). Many pathogens including the leaf roll virus are reported to decrease TSS values in their hosts (Akbaş *et al.*, 2009). The decrease of TSS in MI fruits from 11.20 to 9.96 °Brix in SI fruits may be due to the excessive use of sugar molecules by the host due to interrupted metabolism by the virus. The TSS value of 'Red Lady' papaya treated with chemicals to delay ripening is approximately 10.8 °Brix (Berry *et al.*, 2004) while its value for 'Red Lady' papaya grown in Florida, USA is in the range of 7-10 °Brix (Nunes *et al.*, 2006). The TSS values for other papaya varieties reported are between 10.0-13.9° Brix (Sarananda *et al.*, 2004, Abu-Goukh *et al.*, 2010, Bron and Jacomino, 2006) while no diseased conditions or symptoms have been reported for those varieties. However, the reported values are in agreement with those found for infected fruits by *PRSV* in the present study. However, according to Kader *et al* cited in Abu-Goukh *et al*. (2010), Hawaiian grade standards require fruits with a minimum of 11.5 °Brix for exporting. Therefore, a lower TSS content due to *PRSV* infections has a negative effect on fruit quality. No observable changes were noted in TA, pH and flesh firmness between the two groups of fruits (Table 1). The TA values for 'Red Lady' papaya treated with chemicals to delay ripening (Berry *et al.*, 2004) and different storage temperature regimes (Nunes *et al.*, 2006) are between 0.14-0.17% and 0.14-0.21% citric acid, respectively. Fairly similar values (~ 0.28%) were obtained for fruits from both levels of infection in the present study. The TA values reported for some other papaya varieties are 0.2-0.3% (Abu-Goukh *et al.*, 2010) and 0.09-0.10% citric acid (Bron and Jacomino, 2006), all representing similar values obtained for TA in 'Red Lady' fruits. These results suggest that the virus interrupted metabolism does not have a marked effect on the amount of acid molecules present in the host cells.

The pH value (5.3) of the fruit flesh of *PRSV*-affected 'Red Lady' papaya is more or less similar to the values reported by Berry *et al*. (2004) and Nunes *et al*. (2006) for the same variety. Furthermore, the results also indicated that the degree of infection had no marked effect on fruit flesh firmness. Nunes *et al.* (2006) reported a decrease in fruit flesh firmness during the storage of papaya under different storage temperature systems after harvesting. However, the use of two different methods in determining flesh firmness (finger pressure ranking method in Nunes *et al.* (2006) and fruit firmness tester used in this study), limits the direct comparisons between findings. Brightness of flesh colour significantly (P = 0.023) increased with disease severity, while redness, yellowness and hue angle of the flesh did not differ as such between the two groups of fruits (Table 1). Hue angle values obtained for *PRSV*-affected 'Red Lady' fruits (~ 62.0) were much lower than those documented previously by Berry *et al.* (2004) (43.3) in 'Red Lady' fruits treated with ripening delayers in USA. Brightness (L*) is independent of hue value and illustrates the shade (lightness or darkness) of the same color. No considerable difference in flesh hue value between the two disease levels suggests that the *PRSV* infection has no marked effect on flesh color, which is primarily obtained through the presence of carotenoids.

Crude protein and free amino acid contents

Crude protein content

The results (Table 2) revealed that there was no significant difference in crude protein contents of fruits between the two disease levels although fruits from SI plants showed ~ 28% higher average protein content. Krishna *et al.* (2008) reported a protein content of 6 g/kg of edible part of *Carica papaya* fruits, but neither the variety nor the maturity stage have been mentioned.The total protein content of climacteric fruits sharply increases during the climacteric peak of respiration while ripening in order to facilitate the metabolic reactions occurring inside the cells (Abu-Goukh *et al.*, 2010). Therefore it is important to know the exact maturity stage used in determination of protein content for direct comparisons. The major protein required in large quantities is the coat protein (CP) as the particles of *PRSV* are long, flexuous rod of ~880 nm. The *PRSV*, being a potyvirus, exhibits an inefficient mechanism of controlling gene expression, as it initially produces a polyprotein, which is then cleaved into 10 functional proteins. As a result, for each CP molecule produced, all other gene products are also generated and this causes large quantities of gene products in non-functional states to accumulate in infected cells (Hull, 2009). Thus, the CP together with accumulated viral protein products may account for the increased total protein contents in infected fruits.

Free amino acid contents

HPLC analysis detected 17 amino acids (Table 2). Phenylalanine, lysine and proline were not detected in fruits from plants of either group.The results revealed a remarkable difference in the total free amino acid content between the two groups, where severe infections resulted in ~ 39% decrease in free amino acid level. Methionine and tryptophan contents were significantly lower (P = 0.003 and P = 0.047, respectively) in fruits obtained from severely infected plants.
The significant decrease in total free amino acid content with disease severity may be mainly due to their consumption for viral CP assembly and other virus-coded protein productions. According to Quemeda *et al*. (1990) about 70% of CP are represented by 10 amino acids, namely arginine, asparagine, glutamic acid, glycine, leucine, serine, threonine, aspartic acid, lysine and valine. Except for aspartic acid and valine, the data obtained in our study also indicated decrease of amino acid mentioned by Quemeda *et al*. (1990) while lysine was not present at detectable levels even in fruits with mild infection. The results therefore, suggest that the decrease of total free amino acid content could be probably due to extensive utilization of some of these amino acids by the virus. However, methionine and tryptophan are not reported as abundant amino acids in coat protein of *PRSV* (Quemeda *et al* 1990). Significant decrease in these two amino acids in infected fruits suggests that they have been extensively used probably to synthesize the CP; the major structural protein of the virus. Therefore, it may be possible that there might be a somewhat different amino acid composition in the CP of some of these Sri Lankan *PRSV* isolates, leading to evolve a different variant. Although the Red Lady is considered as a tolerant variety, the severe *PRSV* symptoms observed in some plants during this study may perhaps due to a virulent variable which is capable of reproducing to a higher extent and causing severe symptoms. However, currently we do not have sufficient evidence to confirm this and unable to explain why some plants in the plantation show severe infection in this tolerant variety.

Ascorbic acid content

In relation to the results there were no significant difference in ascorbic acid contents (Table 3) although 15% increase in ascorbic acid level were observed in fruits collected from severely infected plants. The vitamin C content of fruits from MI and SI plants were measured as 0.58 and 0.67 g/kg of FW respectively. These values are in agreement with the published information on the Vitamin C content of 'Red Lady' papaya stored at different temperatures until ripening, which is reported as 0.55 ± 5 g/kg FW at the fully ripen stage (Nunes *et al.*, 2006). This clearly indicate that *PRSV* has no apparent effect on the ascorbic acid content and allows infected papaya fruits to supply a daily dietary supplement of vitamin C.

β-carotene and lycopene contents

The results indicated that there were no meaningful differences in β-carotene and lycopene contents (Table 3) although 19% decrease in β-carotene level was observed in fruits collected from severely infected plants. There are no published data on the total carotenoid content of 'Red Lady' papaya. Some papaya germplasms in Bangladesh are reported to contain 0.16 g/kg FW total carotenoids (Akhter *et al.*, 2012). Wall (2006) reported 0.80 - 4.10×10^{-2} g β-carotene and 1.350 -3.675×10^{-2} g lycopene/kg FW of the red flesh 'Sunrise' and 'Sun Up' varieties, respectively. Their study further illustrates that different agro-ecological regions have a marked effect on both β-carotene and lycopene contents. Further, Chandrika *et al.* (2009) reported 0.7g β-carotene/kg and 1.15g lycopene/ kg in red flesh papaya grown in Sri Lanka. Considering these as reference values, it is apparent that the mean β-carotene content (0.41×10^{-2}g and 0.33×10^{-2}g/kg FW in MI and SI plants, respectively) are somewhat similar to published data with no significant change in carotenoids between disease levels. Similarly no effect of *PRSV* was observed on the lycopene content of fruits from MI and SI plants (1.150×10^{-2} g and 1.126×10^{-2} g/kg FW, respectively).Our results are in accordance with Pazarlar *et al.* (2013) who reported that TMV infection had no marked effect on the carotenoid content of *Capsicum annuum* varieties. Moreover, a strong positive correlation between colorimetric values and carotenoid content in some pumpkins and squash varieties has been reported (Itle and Kabelka 2009). Current study on papaya too reflect this tendency having no significant differences in both flesh color and carotenoid contents between different disease levels.

Table 1. The TSS, TA, pH, flesh firmness and flesh colour of papaya "Red Lady" fruits affected by *PRSV* (fruits harvested at 10 % maturity). Data values are means ± standard errors (n = 5)

Disease severity	TSS (°Brix)	TA (%citric acid)	pH	Flesh firmness (kg cm^{-2})	Flesh color			
					L*	a*	b*	Hue angle
Mild	11.18±0.20^{a}	0.28±0.02^{a}	5.95±0.03^{a}	2.89±0.22^{a}	50.4±0.66^{b}	20.41±0.48^{a}	34.51±0.59^{a}	60.6±0.87^{a}
Severe	9.96±0.20^{b}	0.28±0.01^{a}	5.91±0.04^{a}	3.04± 0.27^{a}	52.72±0.73^{a}	19.84±0.52^{a}	34.20±0.54^{a}	62.9±0.66^{a}

Means having same letter(s) in each column are not significantly different according to Tukey's multiple comparison test ($P \leq 0.05$).

Table 2. The crude protein content and free amino acids content of papaya 'Red Lady' fruits affected by *PRSV*. Data values are means ± standard errors(n = 5)

	Disease severity		Probability
	Mild	Severe	$P =$
Crude protein (g kg^{-1})	4.3 ± 0.9^{a}	5.5 ± 0.6^{a}	0.308
Amino acid content (g kg^{-1} edible papaya flesh)			
Aspartic acid	0.22 ± 0.03^{a}	0.31 ± 0.08^{a}	0.403
Glutamic acid	0.24 ± 0.01^{a}	0.13 ± 0.05^{a}	0.083
Serine	0.17 ± 0.05^{a}	0.08 ± 0.04^{a}	0.201
Histidine	0.05 ± 0.01^{a}	0.10 ± 0.03^{a}	0.194
Glycine	0.15 ± 0.02^{a}	0.14 ± 0.02^{a}	0.510
Threonine	0.06 ± 0.05^{a}	0.04 ± 0.02^{a}	0.385
Arginine	0.10 ± 0.03^{a}	0.07 ± 0.03^{a}	0.658
Alanine	0.10 ± 0.03^{a}	0.15 ± 0.02^{a}	0.208
Tyrosine	0.12 ± 0.01^{a}	0.07 ± 0.01^{a}	0.053
Cysteine	0.30 ± 0.04^{a}	0.20 ± 0.12^{a}	0.490
Valine	0.02 ± 0.02^{a}	0.07 ± 0.03^{a}	0.225
Methionine	0.05 ± 0.01^{a}	0.01 ± 0.01^{b}	0.003
Isoleucine	0.15 ± 0.01^{a}	0.12 ± 0.01^{a}	0.135
Leucine	0.13 ± 0.02^{a}	0.10 ± 0.02^{a}	0.316
Asparagine	0.70 ± 0.29^{a}	0.13 ± 0.07^{a}	0.100
Glutamine	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}	0.802
Tryptophan	0.58 ± 0.13^{a}	0.18 ± 0.08^{b}	0.047
Total Amino acids	3.18± 0.17^{a}	1.95± 0.32^{b}	0.011

Means having same letter(s) in each column are not significantly different according to Tukey's multiple comparison test ($P \leq 0.05$).

Table 3. Ascorbic acid, β-carotene and lycopene contents of *PRSV* affected papaya "Red Lady" fruits. Data values are means ± standard errors(n = 5)

Disease severity	Content in g kg^{-1} of edible flesh		
	Ascorbic acid	β-carotene	Lycopene
Mild	0.58 ± 0.09^{a}	0.41×10^{-2} ± 0.08×10^{-2a}	1.15×10^{-2} ± 0.26×10^{-2a}
Severe	0.67 ± 0.05^{a}	0.33×10^{-2} ± 0.07×10^{-2a}	1.13 ×10^{-2} ± 0.25×10$^{-2\,a}$

Means having same letter(s) in each column are not significantly different according to Tukey's multiple comparison test ($P \leq 0.05$).

Table 4. Mean contents of minerals in papaya 'Red Lady' fruits affected by *PRSV*. Data values are means ± standard errors(n = 5)

Disease severity	Mineral contents (g kg^{-1} edible flesh)					
	Na	K	Mg	Ca	Fe	Zn
Mild	0.12 ±0.02^{a}	1.87 ± 0.35^{a}	0.13 ± 0.03^{a}	0.11 ± 0.02^{a}	0.007 ± 0.001 ^{a}	0.0023 ± 0.0003^{a}
Severe	0.03 ±0.01^{b}	1.65 ± 0.32^{a}	0.06 ± 0.00^{a}	0.06 ± 0.01^{a}	0.004 ± 0.000 ^{a}	0.0013 ± 0.0002^{a}

Means having same letter(s) in each column are not significantly different according to Tukey's multiple comparison test ($P \leq 0.05$).

Mineral contents

The mean sodium content of the fruits was drastically reduced (68%) by severe infection of *PRSV* ($P = 0.005$). Mean contents of Mg, Ca, Fe, and Zn also exhibited a decreasing tendency in severely infected fruits by 37-50% though the differences were not significant compared to those of fruits from plants with MI. However, the potassium content was found to be least affected by the virus infection (Table 4). The mineral content of same papaya variety could be different according to different growing localities (Wall, 2006). When the infection levels are considered in the present investigation, the mean Na content of fruits from SI plants was 68% lower ($P = 0.005$) than that in fruits with mild infection. Although not significant, the severe infection of *PRSV* decreased the mean contents of Mg,Ca, Zn and Fe by 50%, 39%, 40% and 37%, respectively, compared with those values in fruits with mild infection, while no significant change was observed in the mean content of K between the two groups of fruit. The negative effect of *PRSV* infection on some mineral contents of fruits may be due to the interrupted metabolism of host plant, restricting its absorption of minerals. In general phytopathogenic microorganisms hinder translocation or utilization of nutrients (Spann and Schumann, 2013) but according to Huber cited in Dordas (2009) the exact dynamic reaction between pathogenic microorganisms and mineral nutrients are still in debate.

General Discussion

It is documented that *PRSV* has a marked effect on the quality and quantity of free amino acids and protein contents in the leaves of infected papaya seedlings (Wijendra *et al.*, 1995a, Wijendra *et al.*, 1995b). Other studies have reported the effect of this virus on the nitrogen, protein and carbohydrate contents of the leaves and roots of papaya plants (Mathur and Shukla, 1977). Furthermore, only a few studies have been carried out to examine the physico-chemical changes during growth and development of the papaya fruit variety, 'Red Lady'. The present study brings insight to the knowledge on the quality of papaya fruits affected by the *PRSV* infection. It is evident from the current results that the *PRSV* infection significantly reduces the quality of fruits with respect to certain aspects while some other parameters evaluated were reduced to a smaller extent by the infection, deemed insignificant. The most remarkable finding was the effect on the nutritional quality of the papaya fruit with respect to its contents of total free amino acids, methionine and tryptophan, all of which were substantiallyreduced due to severe infection. Methionine and tryptophan are two of the nine essential amino acids that cannot be synthesized *de novo.* On the other hand,the virus has no effect on the vitamin C content, thus, infected papaya could supply its daily RDA. Lowered TSS contents due to *PRSV* infection could be a disadvantage in exporting fruits from plants of SI. Thus, precautions should be taken to avoid mixing fruits from plants of SI and MI. These results would also be useful to thegeneral public in the selection of fruits, helping consumers to avoid fruits with extensive ringspot symptoms from local markets. Future research with virus-free papaya planting material as controls is essential, which may be possible with imported papaya seedlings under controlled conditions. Additionally, the effect of *PRSV* on the quality of fruits harvested at different ripening stages should be explored to investigate whether the negative effects of the virus infection could be minimized. Further research could also be focused on investigating reasons for the expression of severe *PRSV* symptoms in certain plants of Red Lady papaya.

Conclusion

This investigation reveals that severe *PRSV* infection in papaya significantly affects its physicochemical properties by reducing the TSS content and fruit flesh brightness. The infection also affected the nutritional quality of Red lady fruits by significantly reducing the Na content, total amino acid content and two amino acids, namely methionine and tryptophan. No significant effect of the *PRSV* infection on TA, pH, flesh firmness and hue angle of the flesh colour were observed. However, the mean crude protein and ascorbic acid contents increased with increasing disease severity by 28.0% and 15.5% respectively. Lack of virus-free control material was the major limitation in this investigation. Overall, the *PRSV* infection has negative effects on certain nutritional qualities and postharvest parameters of papaya. Avoiding the selection of Red Lady fruits with severe ringspot symptoms on the skin could help consumers enjoy the maximum benefit of the fruit.

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