Food Chemistry 112 (2009) 786–794

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Chemometric profiling of pre-climacteric Sri Lankan mango fruit (*Mangifera indica* L.)

T. Thanaraj [a], L.A. Terry [a,*], C. Bessant [b]

[a] Plant Science Laboratory, Cranfield University, Bedfordshire MK43 0AL, UK
[b] Bioinformatics Group, Cranfield University, Bedfordshire MK43 0AL, UK

ARTICLE INFO

Article history:
Received 26 March 2008
Received in revised form 16 April 2008
Accepted 12 June 2008

Keywords:
Principal component analysis
Hierarchical component analysis
Sugars
Starch
Organic acids
Total phenolics

ABSTRACT

There is no published information on the genotypic variation of major biochemical constituents in mango fruit endemic to Sri Lanka. Accordingly, non-structural carbohydrates, non-volatile organic acids and total phenolics were determined from the peel and pulp of pre-climacteric Sri Lankan mango cultivars (*viz.* Willard, Karutha Colomban, Vellai Colomban, Ampalavi, and Malgova) at three different maturity stages. Principal components analysis revealed distinct clustering of samples according to their biochemical profiles of peel and pulp at three maturity stages. Sugar concentrations generally declined with maturity in both peel and pulp except for cv. Willard. Fructose was the predominant sugar in both peel (56.2–106 mg/g dry weight (DW)) and pulp (67.4–141 mg/g DW), followed by glucose and sucrose. Starch concentration increased with maturity and was higher in pulp (26.0–55.0% DW) than peel (18.2–38.9% DW) at full mature stage. Dry matter as a proportion of fresh weight (FW) increased with maturity.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Total mango production in Sri Lanka is around 96,500 tonnes per annum. Mangoes are grown on 26,000 ha of land and derived from three different agro–ecological regions; dry zone, wet zone and intermediate zone. Unique colour, flavour, taste and aroma are distinct properties of mangoes harvested in the dry zone of Sri Lanka, which attain greater consumer demand. However, lower production volumes along with higher pre- and postharvest losses limit processing and export possibilities (FAO, 2005). A seasonal mango harvesting pattern is observed in Sri Lanka as crops bloom from January to March and are harvested in May to July in wet and intermediate zones (Yala season crops). Mangoes from the dry zone bloom between July to September and are harvested November to January (Maha season crops). Therefore, production drops in between the two seasons, resulting in higher prices (Peiris & Senevirathna, 2001).

Mangoes should be harvested at a physiologically mature, hard, green stage for subsequent postharvest ripening. However, harvesting criteria vary according to local consumption patterns and distance to market. Fruit maturity at harvest is judged by the shape of fruits, however, days after flower set (DAFS) is one of the most critical factors affecting subsequent ripening, flavour development and postharvest utilisation (Lizada, 1993). According to Kosiyachinda, Lee, and Poernomo (1984), external properties such as number of days after full bloom (DAFB) or DAFS, protrusion of shoulder, peel colour and a degree of 'bloom' on the fruit surface have also been used as predictors of harvest maturity. However these properties have no direct impact on eating quality.

Mature hard green mangoes attain a superior eating quality when ripe whilst immature ones do not; thus measuring the harvest quality of hard green mango fruit is very important in order to discriminate immature and mature fruits at harvest (Saranwong, Sornsrivichai, & Kawano, 2004). The concentration of accumulated starch and dry matter should be understood to determine harvest quality (Tandon & Kalra, 1983; Ueda, Sasaki, Utsunomiya, Inaba, & Shimabayashi, 2000). Starch is the major carbohydrate present in mature green mangoes and is hydrolysed into sugars during ripening (Lima, Chitarra, & Chitarra, 2001). Only trace amounts of starch and reduced amylase activity can be detected in over-ripe mangoes (Lima et al., 2001).

Much research has been undertaken to quantify non-structural carbohydrates (NSCs), organic acids and total phenolics (TP) in various pre-climacteric mango cultivars (Table 1). However, published information on chemical profiling is very rare for mangoes that are endemic to Sri Lanka. The aim of this study was to improve the understanding of spatial distribution and temporal changes of chemical composition in pre-climacteric Sri Lankan mangoes using chemometric analysis, which may assist in predicting optimum harvest maturity.

* Corresponding author. Tel.: +44 7500 766490.
E-mail address: l.a.terry@cranfield.ac.uk (L.A. Terry).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.06.040

Table 1
Concentration of sugars, starch, organic acids, and total phenolics of different pre-climacteric mango cultivars

Cultivars	Concentrations	References
Sugars		
Tommy Atkins	Reducing sugars: 15 mg/g FW Non reducing sugar: 39 mg/g	Lima et al. (2001)[a]
Mahajanaka	Total sugars (FW): 60.06 mg/g (105 DAFS), 63.64 mg/g (140 DAFS) Fructose: 30.03 mg/g (105 DAFS), 31.09 mg/g (140 DAFS) Glucose: 2.93 mg/g (105 DAFS), 1.63 mg/g (140 DAFS) Sucrose: 27.1 mg/g (105 DAFS), 30.91 mg/g (140 DAFS)	Saranwong et al. (2004)
Delta R2E2	Total sugars (DW): 208.8 mg/g Fructose + glucose: 95.1 mg/g Sucrose: 113.7 mg/g	Lalel, Singh, and Tan (2003)
Alphonso	Total sugars (FW): 10.1 mg/g Fructose: 6.1 mg/g Glucose: 3.8 mg/g Sucrose: 0.2 mg/g	Yashoda et al. (2006)[b]
Starch		
Tommy Atkins	Starch: 11.2% DW	Lima et al. (2001)
Mahajanaka	Starch: 46.31% DW (105 DAFS) and 55.17% (140 DAFS)	Saranwong et al. (2004)
Tommy Atkins	Starch: 29.88% DW	Vergara-Valencia et al. (2007)
Organic acids		
Choke anan	Ascorbic acid (mmol/kg FW): 14 DAFB: 0.28 (peel), 1.05 (pulp) 56 DAFB: 0.58 (peel), 0.69 (pulp) 84 DAFB: 0.79 (peel), 0.72 (pulp)	Kondo et al. (2005)
Raspuri	Ascorbic acid (DW): 0.188 mg/g	Ajila et al. (2007)
Badami	Ascorbic acid (DW): 0.315 mg/g	Ajila et al. (2007)
Alphonso	Citric acid (DW): 24.8 mg/g Malic acid (DW): 0.3 mg/g Succinic acid (DW): 2.9 mg/g	Yashoda et al. (2006)
Total phenolics		
Raspuri	TP (DW): 29.4 mg/g (buffer extract) 73.88 mg/g (alcohol extract) 109.7 mg/g (acetone extract)	Ajila et al. (2007)
Badami	TP (DW): 20.1 mg/g (buffer extract) 37.92 mg/g (alcohol extract) 90.18 mg/g (acetone extract)	Ajila et al. (2007)
Choke anan	14 DAFB: 477 μmol/kg FW (peel), 313 μmol/kg FW (flesh) 56 DAFB: 1263 μmol/kg FW (peel), 855 μmol/kg FW (flesh) 84 DAFB: 1142 μmol/kg FW (peel), 802 μmol/kg FW (flesh)	Kondo et al. (2005)
Tommy Atkins	Total extractable phenolics (DW): 16.14 mg/g.	Vergara-Valencia et al. (2007)

[a,b] These references used 85 and 70% EtOH (v/v) extractions, respectively.

2. Materials and methods

2.1. Plant material

Five prominent mango cultivars, endemic to Sri Lanka (Willard, Karutha Colomban, Vellai Colomban, Ampalavi and Malgova), were randomly selected at three different harvest maturities (*viz.* immature (*I*), half mature (M_H) and fully mature (M_F; 130–140 DAFB)) from the Eastern University Agronomy Farm (Batticaloa, Sri Lanka). Fruit maturity was based on the DAFB, fruit shape and maturity of seed stone during destructive opening. Samples were prepared from fruits immediately after harvest. Each fruit (n = 9 per cultivar) was divided into nine sections. Vertical transects (VT) were made by cutting each fruit longitudinally using a sharp knife adjacent to the seed coat and a 3 cm-wide longitudinal section removed. This section was then equally divided into stem end, middle and distal end pieces. Horizontal transects (HT) were taken from each section of mango as peel (*P*), outer pulp (OF) and inner pulp (IF). The average fresh weight of each peel and pulp sample was in the range of 5–10 g.

Samples were immediately snap-frozen in liquid nitrogen and then stored briefly at −20 °C. Deep-frozen samples were put on dry ice in an insulated expanded polystyrene box before being transported (8 h journey) from Eastern University to Colombo by road. Upon arrival, samples were then put on fresh dry ice before being air freighted from Sri Lanka to London. Upon arrival in the UK, samples (n = 405) were driven by road to Cranfield University, UK and stored briefly at −40 °C. Samples remained fully frozen during the 2 days transit. Frozen samples were subsequently freeze-dried in an Edwards Modulyo (Crawley, UK) freeze drier, milled to a fine powder before being returned to −20 °C until use. Dry matter was calculated as a proportion of fresh weight (FW). All chemicals used were purchased from Sigma unless otherwise stated.

2.2. Extraction and quantification of sugars

Fructose, glucose and sucrose were extracted and quantified from mango peel and pulp tissue according to Terry, Chope, and Giné Bordonaba (2007), with slight modifications. Briefly, lyophilised samples (100 mg) were mixed well with 3 ml of 62.5:37.5 methanol:HPLC grade water (v/v) in 7 ml polystyrene bijou vials (Sterilin, Stone, UK) and then placed in a shaking water bath (HAAKE SWB 20, Germany) for 15 min at 55 °C. Vials were

removed briefly every 5 min and agitated using a vortex mixer (Vortex Genie 2, G-560 E, Scientific Industries, Bohemia, NY) for 20 s to prevent layering. Samples were removed from the water bath and allowed to cool at room temperature for 10 min before subsequently being filtered using 0.2 μm Millex-GV syringe driven filter (Millipore Corp., Billerica, MA). Resulting filtrates were then stored at −40 °C until needed. Extracts were diluted 1:10 with HPLC-grade water immediately before analysis.

Diluted crude mango extracts (20 μl) were injected automatically into a HPLC system, comprising a P580 pump, Gina 50 autosampler (Dionex, Sunnyville, CA, USA) and Rezex RCM monosaccharide Ca^+ size exclusion column, of 300 mm × 7.8 mm diameter and 8 μm particle size (Phenomenex, Torrance, CA; Part No. 00H-0130-K0), fitted with a Carbo–Ca^+ security guard cartridge of 4 mm × 3mm diameter (Phenomenex; Part No. AJ0-4493). The mobile phase was degassed HPLC-grade water at a flow rate of 0.6 ml/min. Column temperature was held at 75 °C using a Dionex STH column thermostat. Eluted sugars from extractions were monitored by an evaporative light scattering detector (ELSD 2420, Waters, Milford, MA), connected to the Dionex system using a UCI-50 universal chromatography interface (Davis, Terry, Chope, & Faul, 2007). The presence and abundance of fructose, glucose and sucrose were automatically calculated by comparison of peak area with peak area of external standards, using Chromeleon version 4.6 software (Dionex).

2.3. Extraction and quantification of starch

Starch concentration in lyophilised samples was measured using a total starch assay kit (Megazyme International Ireland Ltd., Bray, Republic of Ireland) according to the manufacturer's instructions (AOAC method 996.11 (1998); AACC method 76.13, ICC standard method no. 168 (1976); Saranwong et al., 2004). Starch was analysed in only three cultivars (Willard, Karutha Colomban and Ampalavi), as they were identified as having the greatest differences in chemical composition.

2.4. Extraction and quantification of organic acids

Organic acids were extracted and quantified from mango peel and pulp tissue, according to Terry et al. (2007), with slight modifications. Freeze-dried samples (50 mg) were mixed well with 3 ml of HPLC-grade water in vials and the slurry left to stand for 5 min at room temperature. Samples were then agitated for 30 s using a vortex mixer. The slurry was filtered using a 0.2 μm filter into vials and then stored at −40 °C until use within 6 months.

Mixed calibration standards of organic acids, such as ascorbic, citric, malic, oxalic and tartaric acid were prepared at concentrations of 0.05, 0.1, 0.25, 0.5, 1.25, and 2.5 mg/ml. Mango extracts (20 μl) were injected automatically into the HPLC system previously described, with an Alltech Prevail Organic Acid column (250 mm × 4.6 mm diameter, 5 μm particle size; Grace, Deerfield, IL) with an Alltech Prevail Organic Acid guard column of 7.5 mm × 4.6 mm diameter. Analytical grade 0.2% HPO_3 (v/v) was used as the mobile phase at a flow rate of 1 ml/min, and filtered through a filtering mechanism (Charles Austin Pump Ltd, B105 D/E, England) and degassed for 20 min before being used. Column oven temperature was held at 35 °C. Eluted organic acids were detected using a UVD 170 S/340 S (Dionex). Organic acids were automatically calculated by comparison of peak areas with peak area of external standards.

2.5. Extraction and quantification of total phenolics (TP)

Total phenolics were extracted and measured according to the Folin–Ciocalteu Method (FCM) (Singleton & Rossi, 1965) with slight modifications (Terry et al., 2007), based on the reduction of a phosphowolframate–phosphomolybdate complex by phenolics to blue reaction products. Briefly, freeze-dried mango samples (50 mg) were dissolved in 3 ml of aqueous ethanol (80:20, v/v) and held in a water bath for 2 h at 70 °C, mixing every 20 min. The solution obtained was filtered as before and the clear filtrate analysed. Twenty microlitres of filtrate and 3.2 ml of distilled water were mixed with 200 μl of Folin–Ciocalteu's phenol reagent, followed by 600 μl of sodium carbonate (1.9 M). After 2 h incubation at room temperature (20 °C) in the dark, absorbance was measured at 765 nm using a Camspec M501 UV/Vis spectrophotometer (Camspec Ltd., Cambridge, UK). Phenol content was estimated from a standard curve of gallic acid and results expressed as mg gallic acid equivalents (GAE)/g DW.

2.6. Statistical analysis

Data was subjected to analysis of variance using Genstat for Windows Version 10 (VSN International Ltd., Hemel Hempstead, UK). Least significant difference values (LSD; $p = 0.05$) were calculated for mean separation, using critical values of t for two-tailed tests. Variations among principal treatment combinations were plotted in SigmaPlot 9.0 (Systat Software, Inc., London, UK). Tests for correlations between mean values for concentrations were made using Spearman's Rank Correlation. Correlations are presented with the Spearman's Correlation Coefficient (r) and p value based on a two-tailed test. Unless otherwise stated, significant differences were $P < 0.001$. Both principal components analysis (PCA) and hierarchical component analysis (HCA) (using group average linkage) were carried out on the auto-scaled data set of each cultivar separately using MATLAB version 7.3.0.267 (R2006b), in order to understand the chemometric profile of spatial and temporal variation within each cultivar.

3. Results

3.1. Non-structural carbohydrates (NSC)

Fructose, glucose and sucrose varied significantly among cultivars (Table 2). Fructose was the dominant sugar (63.7–130 mg/g DW) in all cultivars and contributed to more than half of total sugar present, followed by glucose (18.6–83.6 mg/g DW) and sucrose (19.8–50.5 mg/g DW). Total sugar was highest in cv. Malgova (260 mg/g DW) followed by cv. Willard (205 mg/g DW) and cv. Ampalavi (190 mg/g DW). There was no significant variation in sugar concentration according to vertical sectioning (stem end, middle and distal end). Sugar concentration was significantly lower in peel (119 mg/g DW) than in pulp (202 mg/g DW). However, there was no significant variation between inner and outer pulp. In general, total sugar concentration declined significantly from immature stage (199 mg/g DW) to fully mature stage (162 mg/g DW) of mango. However, sugar concentration was relatively high in the fully mature stage *versus* the immature stage of mango cvs. Willard and Ampalavi fruit.

Starch concentration was significantly higher in cv. Malgova (35% DW) than cvs. Karutha Colomban (29% DW) and Willard (21% DW) fruit. Even though sugar concentration was relatively low in cv. Karutha Colomban as compared to cv. Willard, starch concentration was high. Starch levels varied significantly between fully mature (38.6% DW) and immature stages (19.7% DW). Mango peel (24.4% DW) had a significantly lower concentration of starch than pulp tissue (30.2% DW); however, there was no noticeable difference in starch concentration between peel and pulp at immature stage. It was also noticed that outer pulp (31.6% DW) contained more starch than inner pulp (28.7% DW) (Table 2). Dry

Table 2
Mean concentration of non-structural carbohydrates of peel and pulp of pre-climacteric Sri Lankan mango cultivars at different maturity stages

Cultivar	Fructose (mg/g) DW (FW)		Glucose (mg/g) DW (FW)		Sucrose (mg/g) DW (FW)		Total sugars (mg/g) DW (FW)		Starch (%) DW (FW)		Proportion dry weight (g/100 g FW)	
	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Malgova												
Immature	113.65 (32.97)	156.60 (31.75)	69.74 (20.14)	123.42 (25.15)	27.59 (8.05)	84.69 (16.94)	210.98 (61.16)	364.71 (73.84)	18.66 (5.30)	20.05 (3.99)	28.61	19.40
Mature Half	113.47 (33.01)	149.61 (34.46)	60.34 (17.75)	103.33 (24.06)	4.22 (0.94)	59.77 (13.85)	178.03 (51.70)	312.71 (72.37)	37.75 (12.38)	44.72 (9.56)	33.47	20.65
Fully Mature	91.15 (34.41)	117.80 (33.23)	43.35 (16.26)	62.60 (17.75)	7.66 (3.02)	44.19 (10.12)	142.15 (53.69)	224.59 (61.00)	37.01 (14.60)	45.47 (10.6)	38.90	22.17
Mean	106.09 (33.46)	141.34 (33.15)	57.81 (18.05)	96.45 (22.32)	13.16 (4.00)	62.88 (55.52)	177.05 (55.52)	300.67 (69.07)	31.14 (10.76)	36.75 (8.04)	32.90	20.39
Willard												
Immature	60.68 (13.68)	85.99 (14.59)	40.78 (9.17)	68.59 (11.64)	26.10 (5.32)	67.56 (11.58)	127.57 (28.17)	222.13 (37.81)	17.36 (4.15)	17.73 (2.82)	23.86	15.27
Mature Half	62.33 (15.67)	90.22 (14.36)	42.76 (10.55)	76.93 (12.22)	22.09 (5.36)	68.34 (10.94)	127.18 (31.58)	235.49 (37.52)	17.46 (4.27)	23.65 (3.88)	24.04	16.31
Fully Mature	80.39 (15.64)	111.74 (22.23)	53.37 (10.25)	94.03 (18.68)	41.12 (8.01)	46.01 (8.92)	174.87 (33.90)	251.79 (49.83)	18.17 (4.02)	25.99 (4.71)	25.67	17.96
Mean	67.80 (15.00)	95.98 (17.06)	45.63 (9.99)	79.85 (14.18)	29.77 (6.23)	60.64 (10.48)	143.21 (31.22)	236.47 (41.72)	17.66 (4.15)	22.46 (3.81)	24.52	16.51
Karutha Colomban												
Immature	55.04 (31.22)	48.14 (16.47)	29.87 (16.91)	23.61 (8.08)	3.82 (2.14)	51.41 (17.32)	88.74 (50.27)	123.16 (41.87)	20.47 (11.62)	22.40 (7.63)	38.21	25.32
Mature Half	64.72 (26.47)	91.09 (23.02)	24.92 (10.19)	41.55 (10.76)	2.16 (0.86)	26.43 (6.29)	91.81 (37.53)	159.07 (40.07)	13.96 (5.98)	16.43 (4.05)	35.33	22.95
Fully Mature	48.94 (18.32)	63.07 (22.56)	17.06 (6.23)	5.52 (2.21)	9.57 (3.51)	33.46 (11.73)	75.57 (28.06)	102.06 (36.49)	38.92 (16.86)	55.00 (15.9)	43.12	27.94
Mean	56.23 (25.34)	67.43 (20.68)	23.95 (11.11)	23.56 (7.02)	5.18 (2.17)	37.10 (11.78)	85.37 (38.62)	128.10 (39.48)	24.45 (11.49)	31.28 (9.19)	38.89	25.40
Ampalavi												
Immature	83.88 (41.0)	148.86 (45.01)	45.11 (22.0)	164.06 (49.41)	0.78 (0.42)	19.08 (5.81)	129.77 (63.42)	332.00 (100.23)	not measured		48.33	29.74
Mature Half	41.17 (14.5)	61.12 (17.12)	23.94 (8.52)	51.51 (13.57)	6.13 (2.81)	16.68 (5.30)	71.24 (25.85)	129.30 (35.97)			47.50	27.87
Fully Mature	105.87 (36.2)	131.54 (38.87)	28.14 (9.93)	23.25 (7.35)	16.45 (5.25)	63.86 (17.26)	150.45 (51.43)	218.65 (63.47)			48.83	29.55
Mean	76.97 (30.6)	113.84 (33.67)	32.40 (13.48)	79.61 (23.44)	7.79 (2.83)	33.21 (9.46)	117.15 (46.9)	226.25 (66.56)			50.66	31.05
Vellai Colomban												
Immature	49.84 (21.0)	71.32 (20.92)	24.59 (10.43)	43.94 (13.15)	11.21 (4.80)	16.11 (4.45)	85.64 (36.19)	131.37 (38.52)	not measured		41.74	27.49
Mature Half	55.67 (24.6)	78.39 (27.09)	14.74 (6.54)	10.29 (3.46)	2.64 (1.19)	15.53 (4.67)	73.05 (32.32)	104.21 (35.21)			44.16	29.38
Fully Mature	39.32 (17.2)	67.19 (25.50)	8.91 (3.98)	5.54 (2.06)	4.31 (1.79)	48.54 (18.46)	52.54 (23.00)	121.26 (46.02)			49.27	29.88
Mean	48.28 (20.9)	72.30 (24.50)	16.08 (6.98)	19.92 (6.22)	6.05 (2.59)	26.73 (9.19)	70.41 (30.50)	118.95 (39.92)			45.06	28.92

Table 3
Mean concentration of organic acids and total phenolics of peel and pulp of pre-climacteric Sri Lankan mango cultivars at different maturity stages

Cultivars	Organic acids, DW (FW)								Total Acids (mg/g) DW(FW)		TP (mg GAE/g) DW (FW)	
	Citric acid (mg/g)		Malic acid (mg/g)		Oxalic acid (mg/g)		Tartaric acid (mg/g)					
	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Malgova												
Immature	18.7 (3.79)	105.2 (14.01)	3.65 (0.74)	7.2 (0.15)	0.41 (0.09)	0.36 (0.03)	3.7 (1.1)	5.1 (1.0)	26.46 (5.72)	117.9 (15.19)	8.80 (1.60)	11.57 (2.15)
Mature Half	11.6 (2.38)	122.7 (16.02)	1.97 (0.43)	4.47 (0.10)	0.6 (0.12)	0.43 (0.06)	2.9 (1.0)	3.9 (0.8)	17.1 (3.93)	131.5 (16.98)	8.25 (1.60)	11.86 (2.25)
Fully Mature	9.8 (2.27)	139.7 (21.49)	2.74 (0.73)	2.19 (0.33)	0.54 (0.12)	0.72 (0.12)	4.1 (1.6)	3.2 (0.2)	17.18 (4.72)	145.81 (22.2)	8.68 (2.13)	9.37 (2.24)
Mean	13.4 (2.81)	122.52 (17.17)	2.79 (0.63)	4.62 (0.19)	0.52 (0.11)	0.5 (0.07)	3.6 (1.2)	4.1 (0.9)	20.25 (4.79)	131.74 (18.1)	8.58 (1.78)	10.93 (2.21)
Willard												
Immature	17.9 (4.29)	139.35 (23.4)	0.23 (0.05)	3.7 (0.53)	2.35 (0.6)	2.62 (0.42)	12.9 (2.9)	13.05 (1.9)	33.38 (7.84)	158.72 (26.2)	14.65 (3.08)	15.75 (3.2)
Mature Half	5.4 (1.33)	81.7 (13.04)	2.04 (0.5)	0.94 (0.14)	1.23 (0.3)	1.27 (0.2)	3.8 (0.9)	4.03 (0.7)	12.5 (3.03)	87.94 (14.1)	14.88 (3.42)	16.91 (3.5)
Fully Mature	12.4 (2.25)	37.8 (6.63)	1.05 (0.23)	1.76 (0.35)	1.19 (0.27)	0.83 (0.15)	6.4 (1.4)	2.2 (0.4)	21.04 (4.15)	42.6 (7.53)	16.43 (3.79)	15.71 (3.1)
Mean	11.9 (2.62)	86.28 (14.34)	1.11 (0.26)	2.13 (0.34)	1.59 (0.39)	1.57 (0.48)	7.7 (1.7)	6.43 (1.0)	23.31 (4.01)	96.42 (15.95)	15.32 (3.43)	16.12 (3.3)
Karutha Colomban												
Immature	29.0 (8.72)	271.3 (46.96)	2.01 (0.61)	3.83 (0.65)	1.5 (0.45)	1.63 (0.28)	3.1 (1.8)	3.7 (1.2)	35.61 (11.58)	280.46 (49.1)	7.93 (2.14)	7.91 (2.1)
Mature Half	10.7 (1.91)	204.0 (15.9)	0.93 (0.17)	1.52 (0.12)	2.4 (0.55)	1.86 (0.23)	4.3 (1.8)	3.5 (0.8)	18.33 (4.43)	210.9 (17.05)	15.68 (2.76)	14.11 (3.1)
Fully Mature	14.3 (3.66)	76.4 (14.38)	0.32 (0.09)	0.28 (0.05)	1.3 (0.37)	1.18 (0.25)	0.7 (0.3)	1.0 (0.3)	16.62 (4.42)	78.9 (14.98)	9.92 (2.80)	7.06 (1.8)
Mean	18.0 (4.76)	183.9 (25.75)	1.09 (0.29)	1.88 (0.27)	1.73 (0.46)	1.56 (0.25)	2.7 (1.3)	2.7 (0.8)	23.52 (6.81)	190.05 (27.04)	11.18 (2.57)	9.69 (2.3)
Vellai Colomban												
Immature	11.8 (2.99)	233.4 (36.12)	0.73 (0.18)	4.4 (0.67)	1.43 (0.35)	1.34 (0.21)	4.2 (1.8)	6.5 (1.9)	18.16 (5.32)	245.7 (38.9)	13.87 (3.64)	14.14 (3.6)
Mature Half	6.2 (1.8)	124.8 (22.7)	2.08 (0.6)	1.8 (0.34)	0.64 (0.19)	1.16 (0.22)	0.8 (0.4)	0.8 (0.2)	9.72 (2.99)	128.6 (23.46)	16.19 (4.84)	15.68 (4.5)
Fully Mature	8.1 (2.54)	122.5 (23.28)	2.16 (0.69)	1.96 (0.38)	1.42 (0.44)	0.99 (0.19)	1.2 (0.6)	0.8 (0.3)	12.88 (4.27)	126.3 (24.15)	7.21 (2.15)	7.82 (2.3)
Mean	8.7 (2.44)	160.2 (27.37)	1.66 (0.49)	2.73 (0.46)	1.16 (0.33)	1.64 (0.21)	2.1 (0.9)	2.7 (0.8)	13.59 (4.19)	166.85 (28.84)	12.42 (3.54)	12.55 (3.4)
Ampalavi												
Immature	1.3 (0.35)	76.8 (12.97)	11.37 (3.41)	3.96 (0.62)	1.47 (0.41)	1.5 (0.26)	2.3 (1.1)	2.8 (0.9)	16.44 (5.27)	85.1 (14.75)	10.28 (2.81)	15.66 (3.7)
Mature Half	16.2 (2.79)	162.2 (12.3)	2.17 (0.44)	2.2 (0.2)	1.86 (0.6)	1.1 (0.2)	6.3 (3.0)	5.9 (1.7)	26.5 (6.31)	171.4 (14.4)	8.17 (2.41)	10.55 (2.9)
Fully Mature	6.8 (1.78)	89.3 (12.58)	2.78 (0.69)	1.54 (0.22)	1.52 (0.42)	1.22 (0.23)	2.5 (1.0)	2.2 (0.6)	13.6 (3.89)	94.26 (13.63)	5.07 (1.31)	8.97 (2.0)
Mean	8.1 (1.64)	109.4 (12.62)	5.44 (1.51)	3.3 (0.35)	1.62 (0.47)	1.27 (0.23)	3.7 (1.7)	3.6 (1.1)	18.88 (5.16)	116.92 (14.26)	7.87 (2.18)	11.73 (2.9)

matter as a proportion of FW increased with maturity and was significantly higher in peel (24.5–50.7 g/100 g FW) than pulp (16.5–31.1 g/100 g FW) tissue. Mango cv. Willard had significantly lower dry matter than that of other cultivars (Table 2).

3.2. Organic acids

Citric acid contributed about 95% of total organic acids present in mango cultivars tested whilst malic, oxalic and tartaric acids were found in relatively lower concentrations. Ascorbic acid was expected to be at a considerable concentration in all cultivars, however it was not properly resolved, since it was unstable and coeluted with dehydroascorbic acid and derivatives thereof. Total acidity was significantly higher in cvs. Karutha Colomban (135 mg/g DW) and Vellai Colomban (116 mg/g DW) than that of other cultivars tested. Peel had lower acidity (13.6–23.5 mg/g DW) than that of pulp (96.4–190 mg/g DW). In general, total acidity reduced with maturity in both peel (25.9–16.2 mg/g DW) and pulp (178–97.5 mg/g DW). However, and in contrast, acidity increased with maturity in cvs. Malgova (118–146 mg/g DW) and Ampalavi (85.1–94.6 mg/g DW). Inner pulp had a significantly higher concentration of acids (169 mg/g DW) than outer pulp (112 mg/g DW) (Table 3).

3.3. Total phenolics

There were no significant differences ($p > 0.05$) in TP among mango cultivars, maturity stages, vertical and horizontal transactions. However, TP was relatively high in cv. Willard (15.9 mg GAE/g DW) as compared to other cultivars (10.2–12.5 mg GAE/g DW). Fully mature mangoes contained slightly lower TP (9.68 mg GAE/g DW) than immature ones (12.4 mg GAE/g DW). In general, TP was weakly correlated ($r^2 = -0.47$) with starch and not correlated with sugars and acids. However, citric acid was negatively correlated with TP in mango peel and pulp (Table 3).

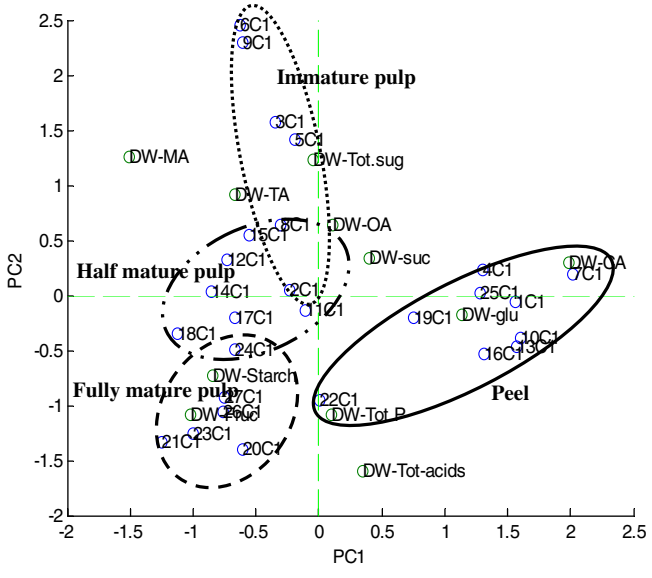

Fig. 1a. PCA bi-plot for PC1 (72.65%) versus PC2 (22.93%) of pre-climacteric mango cv. Willard (C1). Samples (1C–27C; n = 27) from peel and pulp of mango fruits at different maturity stages considered for the analysis. Grouping of samples on the loading and score plot of PCA is based on the similarities in spatial and temporal variation of sugars, acids, TP and starch. The outlines of the clusters have been added manually to aid interpretation.

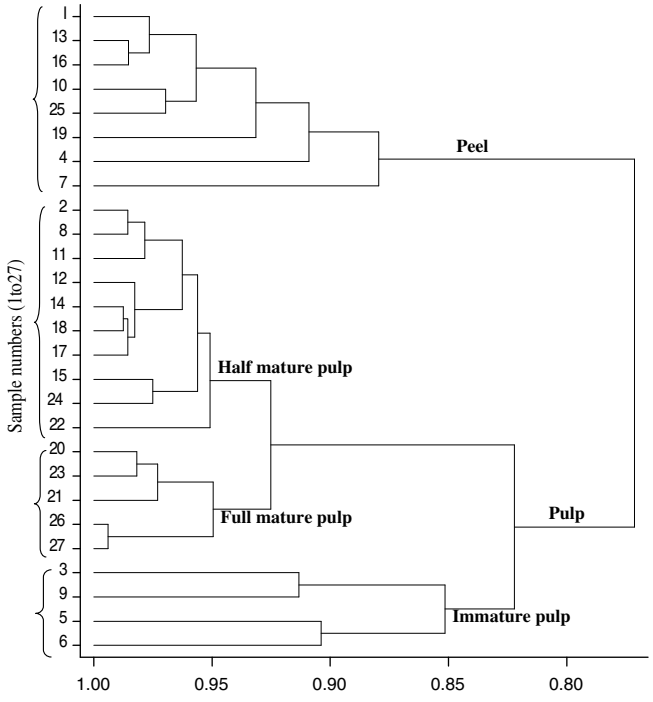

Fig. 1b. HCA dendrogram of pre-climacteric mango cv. Willard. Samples (1–27; n = 27) from peel and pulp of mango fruits at different maturity stages considered for the analysis. Dendrogram exhibits the clustering of samples based on their similarities.

3.4. Chemometric analysis

An investigation of all variables simultaneously was necessary to fully explore the spatial and temporal variation in the chemical composition of each cultivar. Spearman's Rank Correlation analysis typically showed significant positive correlations among variables (sugars and acids). PCA bi-plots and HCA dendrograms revealed clustering of samples according to the spatial distribution and temporal variation of NSC, organic acids and TP.

Principal components analysis of cv. Willard clearly demonstrated the clustering of samples on PC1 (which captured 72.7% of the variance) and PC2 (22.9% of the variance). Peel samples were grouped away from the pulp samples along PC1, indicating that the largest contribution to biochemical variance in this study was the fruit tissue type from which the sample was taken. Although pulp samples could not be discriminated along PC1, the samples were grouped separately into immature, fully mature and half mature pulp samples on PC2. There was no distinguishable variation of chemical profile observed among peel samples to discriminate different maturity stages (Fig. 1a). A HCA dendrogram also demonstrated the same clustering of samples (Fig. 1b), providing further confidence in these findings.

Data from the other tested cultivars were also separated into clusters, however the clustering was not as distinct as for cv. Willard. Generally, peel samples contained significantly lower concentrations of NSC and acids, and hence were clustered separately away from pulp samples. Immature pulp samples containing higher concentration of sugars and acids and were separated away from fully mature pulp samples, which had relatively lower concentrations of sugars and acids (Fig. 2).

4. Discussion

This is the first piece of research reported showing that spatial and temporal variation in chemical composition of pre-climacteric

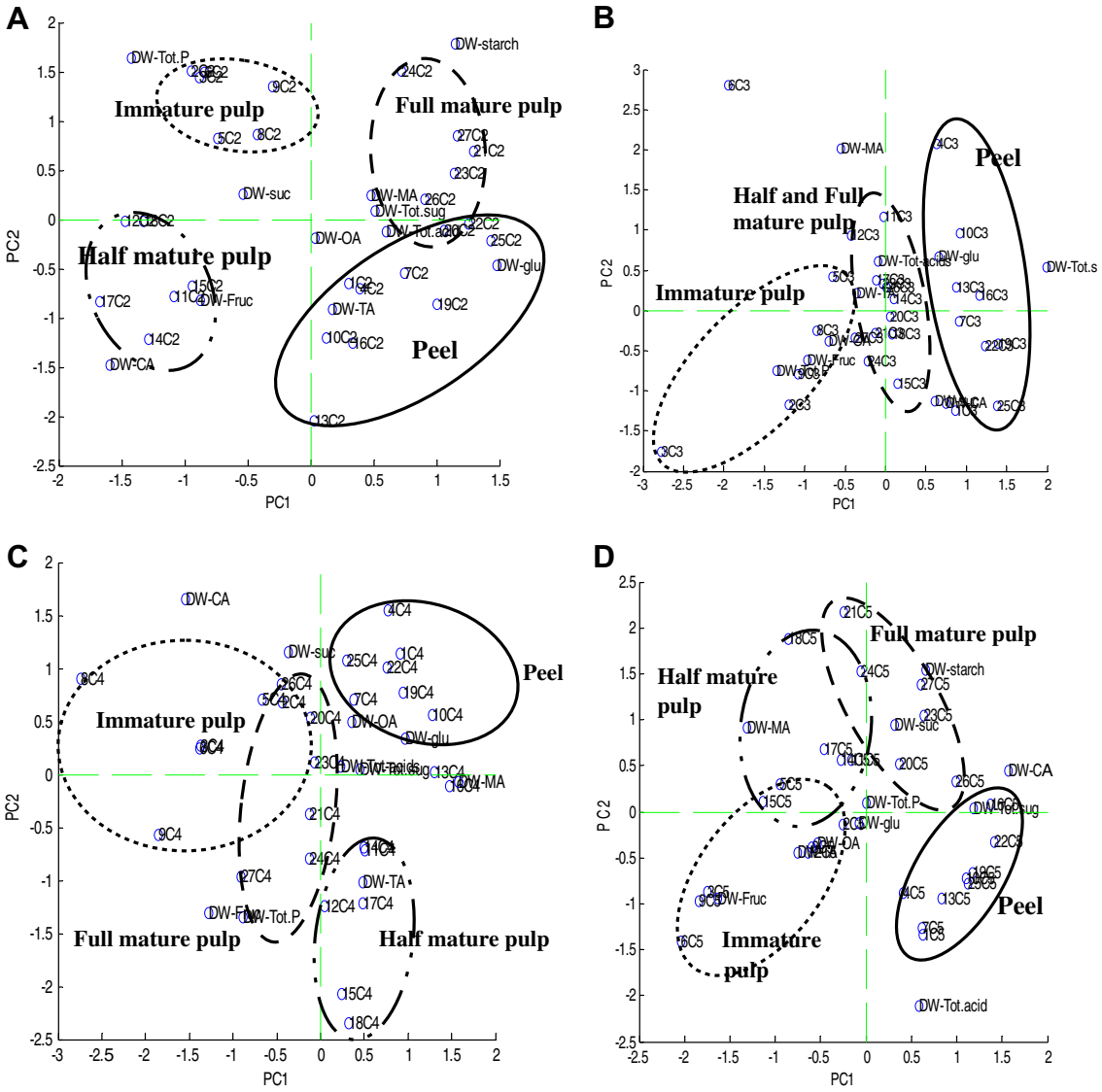

Fig. 2. PCA bi-plots of pre-climacteric mango cvs. Karutha Colomban (A), Vellai Colomban (B), Ampalavi (C), and Malgova (D). Clustering of 27 samples (1C–27C) from peel and pulp of mangoes at different maturity stages demonstrated on the loading and score plot of PCA based on the similarities in spatial and temporal variation of sugars, acids, TP and starch. Starch was not detected in cvs. Vellai Colomban and Ampalavi. The outlines of the clusters have been added manually to aid interpretation.

mangoes can be classified using chemometric analysis. Harvest maturity determines the eating quality of fruits. Concentrations of sugars and acids of ripe fruits are the prime factors which determine taste and consumer demand. Harvest maturity of Sri Lankan mango cultivars has been primarily determined using morphological factors (shape, colour, shoulder protrusion and appearance of the fruit) and DAFB. Since these factors vary with genotype and environment, determining the appropriate harvest maturity becomes a challenging task.

Sugar concentrations generally reduced with maturity while starch concentration increased. In contrast, sugar concentration increased with maturity in both peel and pulp of cv. Willard. Similarly, Saranwong et al. (2004) observed that total sugars increased from 105 to 140 DAFS in the mature green pulp samples of Thailand mango cv. Mahajanaka. Even though past studies have demonstrated that sucrose concentration is 2 to 6-fold higher than that of reducing sugars in the pulp samples of mature green mango cvs. Tommy Atkins, Delta R2E2, Baneshan, Suvarnarekha, Topapuri and Kensington Pride fruit (Hymavathi & Khader, 2005; Lalel, Singh, & Tan, 2005; Lima et al., 2001; Malik & Singh, 2006), reducing sugars contributed to >80% of total sugar concentration in the Sri Lankan cultivars tested herein, with the dominance of fructose most evident. Since the Sri Lankan mango cultivars tested in this study had higher amounts of reducing sugars, it may be expected that they perhaps achieve higher sweetness when ripe. That said, NSCs were extracted and quantified using an aqueous methanol-based method in this study rather than using the more commonly employed ethanol-based extraction. Reported sugar concentrations can vary significantly depending on which extraction method is used (Davis et al., 2007).

Mango pulp samples generally contained higher concentration of NSCs than peel samples. Peel samples of cvs. Karutha Colomban, Vellai Colomban and Ampalavi had relatively high concentrations of glucose at their fully mature stage. Though starch concentration increased with maturity in both peel and pulp, the variation between peel and pulp was relatively low especially at immature stage. This finding is supported by Saranwong et al. (2004) whereby starch concentration (using the same assay) of mango cv. Mahajanaka pulp increased by 8.86% (dry basis) from 105 DAFS to its fully matured stage at 140 DAFS. Starch is the main carbohydrate

accumulated in pre-climacteric mature mango fruit, which subsequently reduces during ripening since it is hydrolysed into sugars; only trace amounts can be detected in over-ripe mangoes with reduced amylase activity (Lima et al., 2001; Mattoo et al., 1975; Selvaraj, Kumar, & Pal, 1989; Subramanyam, Gouri, & Krishna Murthy, 1976). Accumulating sufficient amounts of starch at the mature stage would allow ripe fruit to assimilate a larger amount of sugar during postharvest ripening. Starch concentration of cvs. Willard (23.4%), Malgova (42.7%) and Karutha Colomban (49.6%) at fully mature stage (Table 2) are comparable with cvs. Tommy Atkins (29.8%, Vergara-Valencia et al., 2007) and Mahajanaka (55.17%, Saranwong et al., 2004) (Tables 1 and 2). Mangoes harvested at late maturity stage are expected to have superior eating quality when ripe. It has been found that more than 75% of fruits harvested at 133 and 140 DAFS had excellent eating quality (Saranwong et al., 2004). Dry matter was proportional to the starch concentration and thus maturity. Both starch and dry matter should be at high concentration for determination of harvest quality (Tandon & Kalra, 1983; Ueda et al., 2000).

Generally, organic acid concentration was significantly higher in the pulp of the Sri Lankan mango cultivars tested. Since other acids were in relatively low concentrations in both peel and pulp, citric acid is mainly responsible for the acidic nature of mangoes (Table 3). Citric acid concentration of unripe mango cv. Alphonso pulp (24.8 mg/g FW, Yashoda, Prabha, & Tharanathan, 2006) is in line with the cultivars tested in this study at their full mature stage (Table 1).

There was no significant spatial and temporal variation observed in TP concentration (Table 3). However, half mature mangoes had a slightly higher concentration of TP than mangoes at immature and fully mature stages, which concurs with the findings of Kondo, Kittikorn, and Kanlayanarat (2005). It was expected that TP would have been higher in mango peel than pulp, however the opposite was observed. Combined peel and pulp tissue of hard green pre-climacteric mango cv. Tommy Atkins had a similar concentration of TP (16.1 mg GAE/g, DW) (Vergara-Valencia et al., 2007) with tested cultivars using a similar assay. However, TP was lower in peel and pulp samples of mature mangoes cv. Deshi, Langra, Chausa, Mallika, Deshahari and Amprapali (<3 mg/g) (Singh et al., 2004). TP in the acetone extract of cvs. Raspuri and Badami peel were higher than tested cultivars (Ajila, Bhat, & Prasada Rao, 2007) (Table 1).

The mango fruit analysed in the present study were at pre-climacteric stage, such that sugars and acids levels reduced with maturity, whilst starch concentration increased. Variation in chemical composition between peel and pulp was the major discriminatory factor amongst cultivars. The analysis differentiated pulp according to different maturity stages. PCA and HCA are 'unsupervised' approaches, meaning that no prior knowledge of the sample types is used in the analysis. The benefit of such approaches is that the clustering of samples demonstrates intrinsic variance between the samples without being biased towards desired outcomes. Chemometric analysis of foodstuffs has usually been conducted on volatile fingerprints/profiles and rarely on aqueous compounds. Therefore, increased understanding of these differences and how taste-related compounds relate to one another may assist practitioners in selecting and harvesting pre-climacteric mangoes at the optimum period.

5. Conclusions

Fully mature pre-climacteric mango fruit (130–140 DAFB) had the highest concentration of starch and lowest concentration of sugars and acids. Chemometric analysis clearly revealed distinct differences according to cultivar, tissue type and maturity, based upon non-structural carbohydrates, acids and total phenolics. Since these combined variables and dry matter at harvest are partially responsible for the final quality of ripe fruit, they could be used to select pre-climacteric Sri Lankan mango fruit at the optimum stage for subsequent postharvest ripening. Selecting the appropriate harvest maturity subsequently optimises postharvest quality of ripe fruit.

Acknowledgement

The authors thank the World Bank aided IRQUE project (IDA Credit No. 3781 CE) of Faculty of Agriculture, Eastern University, Sri Lanka and Cranfield University, UK for financial support. Mr. Allen Hilton and Mrs. Balwinder Cook are thanked for their technical assistance.

References

Ajila, C. M., Bhat, S. G., & Prasada Rao, U. J. S. (2007). Valuable components of raw and ripe peels from two Indian mango varieties. *Food Chemistry, 102*, 1006–1011.

AACC. (1976). *Approved methods of the AACC, method 76.11*. American Association of Cereal Chemists.

AOAC. (1998). *AOAC methods*. Washington, DC: Association of Official Analytical Chemists.

Davis, F., Terry, L. A., Chope, G. A., & Faul, C. F. J. (2007). The effect of extraction procedure on measured sugar concentrations in onion (*Allium cepa* L.) bulbs. *Journal of Agricultural and Food Chemistry, 55*, 4299–4306.

FAO, (2005). FAOSTAT database collections, Food and Agriculture Organization (FAO) of the United Nations. <http://www.faostat.fao.org/faostat/collections?subset=agriculture>.

Hymavathi, T. V., & Khader, V. (2005). Carotene, ascorbic acid and sugar content of vacuum dehydrated ripe mango powders stored in flexible packaging material. *Journal of Food Composition and Analysis, 18*, 181–192.

Kondo, S., Kittikorn, M., & Kanlayanarat, S. (2005). Pre-harvest antioxidant activities of tropical fruit and the effect of low temperature storage on antioxidants and jasmonates. *Postharvest Biology and Technology, 36*, 309–318.

Kosiyachinda, S., Lee, S. K., & Poernomo, P. (1984). Maturity indices for harvesting of mango. In B. D. Mendoza, Jr. & R. B. H. Wills (Eds.), *Mango: Fruit development. Postharvest physiology and marketing in ASEAN* (pp. 33–38). Kuala Lumpur: ASEAN Food Handling Bureau.

Lalel, H. J. D., Singh, Z., & Tan, S. C. (2003). Effects of maturity stage at harvest on fruit ripening, quality and biosynthesis of aroma volatile compounds. *Journal of Horticultural Science & Biotechnology, 78*, 225–233.

Lalel, H. J. D., Singh, Z., & Tan, S. C. (2005). Controlled atmosphere storage affects fruit ripening and quality of 'Delta R2E2' mango. *Journal of Horticultural Science and Biotechnology, 80*, 551–556.

Lima, L. C. O., Chitarra, A. B., & Chitarra, M. I. F. (2001). Changes in amylase activity, starch and sugars contents in mango fruit pulp cv. Tommy Atkins with spongy tissue. *Brazilian Archives of Biology and Technology, 44*, 59–62.

Lizada, C. (1993). Mango. In G. B. Seymour, J. E. Taylor, & G. A. Tucker (Eds.), *Biochemistry of fruit ripening* (pp. 255–271). London: Chapman and Hall.

Malik, A. M., & Singh, Z. (2006). Improved fruit retention, yield and fruit quality in mango with exogenous application of polyamines. *Scientia Horticulturae, 110*, 167–174.

Mattoo, A. K., Murata, T., Pantastico, E. B., Chachin, K., Ogata, K., & Phan, C. T. (1975). Chemical changes during ripening and senescence. In Er B. Pantastico (Ed.), *Postharvest physiology, handling and utilization of tropical and subtropical fruits and vegetables* (pp. 103–127). Westport: The AVI Publication.

Peiris, K.H.S., & Senevirathna, S. A., (2001). Flower induction studies for off-season fruit production in mango. Research Report 2000, Field Crop Research and Development Institute (FCRDI), Maha Illuppallama, Sri Lanka.

Saranwong, S., Sornsrivichai, J., & Kawano, S. (2004). Prediction of ripe–stage eating quality of mango fruit from its harvest quality measured non-destructively by near infrared spectroscopy. *Postharvest Biology and Technology, 31*, 137–145.

Selvaraj, Y., Kumar, R., & Pal, D. K. (1989). Changes in sugars, organic acids, amino acids, lipid constituents and aroma characteristics of ripening mango (*Mangifera Indica* L.) fruit. *Journal of Food Science and Technology, 26*, 308–313.

Singh, U. P., Singh, D. P., Singh, M., Maurya, S., Srivastava, J. S., Singh, R. S., et al. (2004). Characterization of phenolic compounds in some Indian mango cultivars. *International Journal of Food Science and Nutrition, 55*, 163–169.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Subramanyam, H., Gouri, S., & Krishna Murthy, S. (1976). Ripening behaviour of mango graded on specific gravity basis. *Journal of Food Science and Technology, 13*, 84–86.

Tandon, D. K., & Kalra, S. K. (1983). Changes in sugars, starch and amylase activity during development of mango cv. Dashehari. *Journal of Horticultural Science, 58*, 449–453.

Terry, L. A., Chope, G. A., & Giné Bordonaba, J. (2007). Effect of water deficit irrigation and inoculation with *Botrytis cinerea* on strawberry (*Fragaria* × *ananassa*) fruit quality. *Journal of Agricultural and Food Chemistry, 55*, 10812–10819.

Ueda, M., Sasaki, K., Utsunomiya, N., Inaba, K., & Shimabayashi, K. (2000). Changes in physical and chemical properties during maturation of fruit (*Mangifera indica* L. 'Irwin') cultured in a plastic greenhouse. *Food Science and Technology Research, 6*, 299–305.

Vergara-Valencia, N., Granados-Perez, E., Agama-Acevedo, E., Tovar, J., Ruales, J., & Bello-Perez, L. A. (2007). Fibre concentrate from mango fruit: Characterization, associated antioxidant capacity and application as a bakery product ingredient. *LWT – Food Science and Technology, 40*, 722–729.

Yashoda, H. M., Prabha, T. N., & Tharanathan, R. N. (2006). Mango ripening: Changes in cell wall constituents in relation to textural softening. *Journal of the Science of Food and Agriculture, 86*, 713–721.