Fruit volume and width at harvest can be used to predict shelf life in pepper (*Capsicum chinense* Jacq.)

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Fifteen morphophysiological fresh fruit characteristics were evaluated in purelines derived from 24 accessions of Capsicum chinense (Jacq.) to determine the association of these attributes with shelf life. The accessions were planted under field conditions (Elibox et al., 2015) in a randomized complete block design with three replications; and replicate fruits collected from the fifth harvest were evaluated for color (CIE L*a*b*), weight, length, width, volume, surface area, surface area to volume ratio, epicuticular wax content and thickness of cuticle, exocarp, mesocarp, endocarp, and pericarp. Differences between accessions for all the morphophysiological characteristics were significant at P < 0.001. When the characteristics were subjected to Pearson's correlation with shelf life parameters viz. weight loss, days to 20% pedicel necrosis and days to incipient pericarp necrosis (Elibox et al., 2015), nine (initial color lab space a*, b*, L*; weight, width, volume, surface area, surface area to volume ratio and exocarp thickness) were positively or negatively associated with shelf life. Accessions with wide fruits were generally heavier, had a larger volume, larger surface area, smaller surface area to volume ratio and took longer time to attain 20% pedicel and incipient pericarp necrosis (had longer shelf lives). Accessions with greener, darker fruit colors at physiological maturity had longer shelf lives and those with heavier fruits and possessing thicker exocarps had lower rates of weight (water) loss. Of the characteristics that were associated with shelf life, three, viz. fruit volume, width and surface area to volume ratio were highly autocorrelated and were found to be able to reliably predict shelf life on the day of harvest. As fruit volume and width are easy to measure and showed the strongest correlations with shelf life parameters, they could be used as reliable indicators of shelf life in pepper breeding.

Keywords. Fruit dimensions, pedicel necrosis, pericarp necrosis, postharvest storage, water loss

Pepper (Capsicum chinense Jacq.) is a shortlived, herbaceous perennial (Basu and De 2003) belonging to the plant family, Solanaceae (Bosland et al. 1996; Eshbaugh 1993). The species is indigenous to Central and South America and the Caribbean (Heiser 1976), and possesses considerable variability in fruit characteristics (Bharath et al. 2013). The fruit is botanically classified as an indehiscent berry, borne on a pendulous stalk (pedicel) (Sinha and Petersen 2011). Pepper plants possess many fruits at each node. The fruit has a hollow centre in which the placenta and the seeds are suspended and is enclosed by the pericarp, which is made up of the endocarp, mesocarp, exocarp layers and waxy cuticle that is responsible for the glossiness of the fruit.

In the Caribbean, pepper is grown as an annual crop and is subjected to several harvests during the growing season at the mature green

stage (Elibox et al. 2015; Sinha and Petersen 2011). Fruits that are firm with \leq 20% pedicel necrosis can be sold as fresh vegetables while those without intact pedicels or with pedicel and calyx necrosis not extending into the pericarp can be sold for processing (Elibox et al. 2015; Sinha and Petersen 2011).

Elibox et al. (2015) found a large variation in shelf life among accessions of *C. chinense* (7 to 20 days), with loss of shelf life always initiating as incipient necrosis of the proximal end of the pedicel proceeding along the entire pedicel and calyx and subsequently extending into the pericarp. The study showed that shelf life based on time taken from harvest to (a) 20% pedicel necrosis and (b) incipient pericarp necrosis in pepper fruits were highly correlated with each other, were determined to be the best measures of shelf life, and could be reliably predicted at 8 days after harvest (DAH) based

on proportion of fruits with $\leq 20\%$ pedicel necrosis and at 9 DAH based on proportion of fruits with incipient pericarp necrosis, respectively. Furthermore, the study showed that pepper accessions with larger fruit fresh weights tended to have a longer shelf life than those with lighter fruits. In the closely allied species, chile pepper (Capsicum annuum L.), in addition to fruit fresh weight at harvest, shelf life was found to be positively associated with fruit surface area and surface area to volume ratio (Lownds et al. 1993, 1994); cuticle thickness; but not with pericarp thickness (Lownds et al. 1993). The understanding of morphophysiological characteristics influence shelf life in C. chinense can lead to the development of selection methods in breeding for improved shelf life. Therefore, the objective of this study was to examine morphophysiological characteristics of freshly picked mature fruits of 24 C. chinense accessions and determine their associations with weight (water) loss and shelf life.

Materials and methods

Experimentation. Twenty four purelines derived from landraces of *C. chinense* collected from Trinidad and Tobago were used in the study (Table 1). The nursery practices to generate healthy seedlings and cultural conditions of the field where the accessions were planted were according to Elibox et al. (2015). The purelines were grown in a randomized complete block design with three replications.

Fifteen uniform fruits were selected from each of three replications during the fifth harvest for morphophysiological characterization. Each parameter studied was measured as a mean of three fruits per replicate. Fruit color was determined using the CIE L*a*b* system of Ochoa-Reyes et al. (2013) using a chromometer (CM-200b, Minolta® Company Ltd, Tokyo, Japan), where L* refers to

lightness and a* and b* represent the color opponent dimensions. The equipment was calibrated against a white Minolta® Calibration plate prior to use. Fruit weight (g) was measured gravimetrically using a scale to g accuracy (PGW 753e, Adam Equipment Inc, Connecticut, USA). Fruit length (mm) was evaluated using a meter rule (Helix®, West Midlands, UK). The width of each fruit (mm) was measured half way along the length using a pair of calipers (ENKAY Vernier Caliper 5-inch scale, Lo. 430-C ENKAY, Brooklyn, NY, USA). Fruit volume (cm³) was measured by the water displacement method (Lownds et al., 1993) using a 1000 cm³ measuring cylinder (Kimax®, Kimble and Chase, New Jersey, USA). The surface area per fruit (cm²) was estimated by cutting the mature fruits per accession into flat pieces, then tracing the pieces onto translucent paper and determining the area of the cut-outs using a leaf area meter (ΔT Area Meter MK2 model; Delta-T Devices, England). Fruit surface area to volume ratio was calculated as surface area/ volume. Total fruit epicuticular wax per replicate was determined by dipping the three fruits per replicate in phenol-chloroform (15 s) until all the wax had dissolved, followed by drying of the wax in a rotovac (Haake Buchler Instruments Inc., Saddle Brook, NJ) and weighing the wax using an electronic balance (Acculab; AL-104, Mountville, PA, USA) to 0.0001 g accuracy. The mean wax (g/ mm²) was determined by dividing the mean wax per fruit by the mean surface area per fruit in mm².

To determine fruit wall characteristics, sections of the pericarp were fixed in FAA, prepared for histological examination as outlined by Johansen (1940), embedded in wax sectioned at 20 µm thickness and fixed onto slides based on the methods of Johansen (1940) and O'Brien and McCully (1981). The slides were viewed under x100 magnification using an Olympus CH20 microscope (Olympus Corporation, Tokyo, Japan) and the

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thickness of exocarp, mesocarp, endocarp, cuticle and pericarp were measured using a calibrated eyepiece micrometer.

Shelf life parameter values viz. arcsine percentage weight (water) loss at 4, 6, 8, 10, 12, 14 days after harvest (DAH); days to 20% pedicel necrosis; and days to incipient pericarp necrosis were as reported for the same set of 24 pepper accessions by Elibox et al. (2015).

Data analysis. One-way ANOVA (NCSS 2007) was used to determine whether there were significant differences (P < 0.05) among the morphophysiological characteristics studied. Where a difference was significant, a least significant difference, a within accession coefficient of variation (CV_{within}), a between accession coefficient of variation (standard deviation between the mean of the accessions divided by the general mean) and an index of discrimination (ID = CV between / CV within) were calculated.

Pearson's product moment correlation (NCSS 2007) was used determine to associations among the 15 morphophysiological characteristics as well as between these and shelf life parameters viz. arcsine percentage weight (water) loss at 4, 6, 8, 10, 12, 14 DAH; days to 20% pedicel necrosis; and days to incipient pericarp necrosis reported for the same set of accessions by Elibox et al. (2015). Where a correlation was large, regression analysis was performed to determine the nature of the relationship.

Results

Differences among the pepper accessions for all 15 fresh fruit morphophysiological characteristics were significant at P < 0.001 (Table 1), with considerable variation seen for all the characteristics under study. Fruit fresh weight showed highly significant correlations

(P < 0.01) with fruit volume (r = 0.82), fruit width (r = 0.77), fruit surface area (r = 0.65) and surface area to volume ratio (r = -0.77) (Table 2). Similarly, fruit volume also showed highly significant correlations (P < 0.01) with fruit width (r = 0.90), fruit surface area (r = 0.83), surface area to volume ratio (r = -0.88); and fruit width with fruit surface area (r = 0.70) and surface area to volume ratio (r = -0.83). Fruit length showed a highly significant correlation with fruit surface area (r = 0.66, P < 0.01). Mesocarp thickness showed a perfect correlation with pericarp thickness (r = 1.00, P < 0.01).

When the 15 morphophysiological characteristics were correlated with indicators of shelf life (days to 20% pedicel necrosis; and days to incipient pericarp necrosis) and arcsine percentage weight loss at 4, 6, 8, 10, 12, 14 DAH of Elibox et al. (2015), fruit length, epicuticular wax content and cuticle, endocarp, mesocarp, exocarp, pericarp thickness did not correlate (P > 0.05) significantly with either days to 20% pedicel necrosis or days to incipient pericarp necrosis (data not shown). Color lab space parameter a*, b*, and L* showed moderate correlations with the shelf life parameters (r = -0.42 to -0.52, 0.41 to 0.52; P < 0.05) (Table 3). In general, the darker the green color of the fresh fruit, the lower was the water loss after harvest and the longer was the shelf life. Accessions with heavier fruits (r = -0.61) and with fruits possessing thicker exocarps (r = -0.48) had lower rates of water loss. Fruit volume (r = 0.91, 0.88), fruit width (r = 0.85, 0.84) and fruit surface area to volume ratio (r = -0.82, -0.83) consistently showed the strongest (P < 0.01) correlations with days to 20% pedicel necrosis and incipient pericarp necrosis (Table 3), respectively, with a coefficient of determination larger than 0.65. Consistently, fruit volume was the best predictor of shelf life followed by fruit width.

Fruit volume and width used to predict shelf life in pepper (*Capsicum chinense* Jacq.); *W. Elibox et al* Table 1: Fruit color (CIE L*a*b*), weight (g), length (mm), width (mm), volume (cm³), surface area (cm²), surface area to volume ratio (SA/ V ratio), epicuticular wax content (g/mm²) and cuticle, endocarp, mesocarp, exocarp and pericarp thickness (mm) at physiological maturity for 24 pepper (*Capsicum chinense* Jacq.) accessions

	1	Fruit co	olor		Fruit:						Thickness (mm) of fruit:				
								Surfa							
				*** * * .		*7 *	Widt		G 4 657	Wax					ъ.
Accession	o*	b*		_	Length (mm)	Volume (cm ³)	h (mm)	area	SA/V ratio	(g/ mm ²)	Cuticle	Endo-			
MB14	-14.5	21.9		(g) 11.8			33.5	11.9		0.004		0.02	carp 0.01	-carp	
MB17	-19.2	34.4		13.5		29.6		12.1	0.409	0.004	0.01	0.02			
S20	-19.2	36.3	51.7	14.6	37.1			13.1	0.439	0.013					
F10	-19.1	37.6		9.4	41.7			12.8	0.576	0.003	0.01	0.02			
F11	-15.3	39.4		9.4	_	22.2	36.8 34.5	10.1	0.477	0.004	0.02				0.96
F12	-17.4	27.7		9.9		21.1 23.5		10.1	0.463	0.004	0.01	0.02			
F12	-17.4	34.5	50.1	10.1	37.3			8.9		0.008	0.01	0.02			
F22	-19.4	33.1	48.0	10.1	31.3 39.7	19.2 17.6		9.9		0.021	0.01	0.02 0.02			
F9	-18.6	30.8		7.9				11.2	0.515	0.003	0.01				
L27	-18.0	31.6		12.6	40.5		37.1	9.1	0.404	0.008	0.02	0.02 0.02			
L27 L29	-17.1	32.9		9.2	31.2			10.7	0.509	0.014	0.02				
L30	-17.1	29.3	47.6	13.5		21.1 21.1	33.1 35.7	11.7	0.557	0.027	0.01	0.02			
L30	-17.3	32.8		10.7	41.9		31.1	9.9			0.01	0.01	0.02		
L33	-17.7	33.1	49.8	9.2	40.5			9.7	0.614	0.011	0.01	0.01	0.01		
L53	-14.4	35.8	59.6		42.4 33.5			7.4		0.010	0.01 0.01	0.02			
L52 L53	-15.4	36.9	57.1		33.3 40.9		28.4	9.1	0.690	0.010	0.01	0.02			1.16 1.21
L53	-15.7	24.5	41.5		37.9			9.1	0.543	0.019	0.01	0.02			
L58	-17.1	32.5	49.8		38.4			10.4	0.551	0.008	0.01	0.02			
L59	-16.7	32.3	51.0		35.7			10.1	0.443	0.014	0.01	0.03			
L60	-15.3	40.6	63.8		37.0			7.9	0.664	0.006	0.01	0.02			
L64	-16.3	27.5	45.2		40.8			10.7	0.688	0.011	0.01	0.02			
L66	-16.2	26.6	43.7		40.4			10.4	0.551	0.011	0.01	0.02			
SC5	-14.6	41.7	62.8		43.1	11.4		9.2	0.808	0.009	0.01	0.03			
SP7	-18.7	39.6	50.4	6.1	28.7			7.1	0.660	0.013	0.01	0.03			
Sig. (P <)	0.001	0.001	0.001	0.001	0.001	0.001		0.001	0.001	0.001	0.001	0.001	0.001	0.001	
Mean	-17.1	33.1	50.7	9.5	37.1	18.2		10.2	0.561	0.0107	0.01	0.02	0.01	1.29	1.33
SEM	0.01	0.01	1.03	0.76	1.23	1.29	1.00	0.80	0.062	0.0087	0.001	0.001	0.001	0.042	0.041
LSD	0.01	0.01	2.43	1.80	2.91	3.05	2.35	1.88	0.146	0.021	0.001	0.001	0.001	0.099	0.097
CV within	0.02	0.01	0.04	0.13	0.06		0.05	0.08	0.071	0.0290	0.054	0.088	0.066	0.052	0.049
CV	0.40	0.4-						0.15	0.198		0.00-				
between	0.10	0.15	0.13		0.11	0.30				0.5514	0.235		0.280		
ID	6.64	10.97	3.57	2.02	2.04	2.42	2.30	1.86	2.19	19.01	4.31	3.15	4.26	5.09	5.15

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Table 2: Pearson's product moment correlations between the 15 fresh fruit characteristics at physiological maturity for the 24 pepper (*Capsicum chinense* Jacq.) accessions

Fruit color					Fruit:	Thickness of fruit:								
	b*	L*	Weight	Length	Volume	Width	Surface area	Wax	SA/V ratio	Cuficle	Endo- carp	Exo- carp	Meso- carp	Peri- carp
a*	-0.10	0.31	-0.46	0.16	-0.50	-0.67	-0.39	-0.22	0.50		-0.08	0.01	0.08	0.08
	b*	0.87	-0.38			-0.22	-0.33	-0.17	0.38	0.05	0.19	0.16	-0.03	-0.03
		L*	-0.45	-0.09	-0.42	-0.39	-0.36	-0.35	0.48	-0.09	-0.01	0.19	0.01	0.02
	•		Weight	0.10	0.82	0.77	0.65	0.09	-0.77	0.18	-0.13	0.11	0.09	0.10
		•		Length	0.22	-0.08	0.66	-0.27	0.12	-0.04	-0.20	0.09	-0.15	-0.14
					Volume	0.90	0.83	0.04	-0.88	0.42	0.04	0.01	0.01	0.01
						Width	0.70	0.10	-0.83	0.49	0.15	0.11	-0.02	-0.01
							Surface area	-0.14	-0.52	0.34	-0.04	0.17	-0.12	-0.12
						'		Wax	-0.21	-0.06	0.04	-0.17	0.32	0.32
									SA/V ratio	0.19	0.02	0.18	-0.19	-0.19
								•		Cuticle	0.17	0.26	-0.27	-0.27
											Endo- carp	0.13	-0.27	-0.25
										•		Exo- carp	-0.20	-0.19
			2		1						•		Meso- carp	1.00

SA/V ratio = surface area to volume ratio

Correlation coefficient is significant for 22 d.f. at P < 0.05 and P < 0.01 when r = 0.404 and 0.515, respectively

Table 3: Pearson's product moment correlations between shelf life characteristics (water loss; days to 20% pedicel necrosis, incipient pericarp necrosis) reported by Elibox et al. (2015) with nine fresh fruit characteristics for 24 pepper (*Capsicum chinense* Jacq.) accessions evaluated under controlled storage conditions

		F	ruit colo	or	Fruit:							
Shelf life chara	cteristics	a*	b *	\mathbf{L}^*	Weight	Volume		Surface area		Exocarp thickness		
at	4	0.42	0.28	0.47	-0.53	-0.35	-0.49	-0.24	0.44	0.29		
weight loss day:	6	0.39	0.29	0.48	-0.55	-0.44	-0.53	-0.30	0.52	0.34		
weight day:	8	0.34	0.26	0.41	-0.59	-0.39	-0.52	-0.22	0.50	0.29		
	10	0.35	0.29	0.42	-0.54	-0.42	-0.51	-0.23	0.52	0.46		
Arcsine	12	0.42	0.24	0.37	-0.49	-0.42	-0.49	-0.32	0.45	0.33		
Arc	14	0.52	0.30	0.38	-0.49	-0.69	-0.60	-0.47	0.71	0.52		
Weight loss slope		-0.03	0.27	0.20	-0.61	-0.25	-0.25	-0.19	0.13	-0.48		
Days to 20% pedic	-0.42	-0.50	-0.52	0.64	0.91	0.85	0.71	-0.82	-0.30			
Days to incipient p	-0.29		****	****	0.88			-0.83				

Correlation coefficient is significant for 22 d.f. at P < 0.05 and P < 0.01 when r = 0.404 and 0.515, respectively

The relationship between days to 20% pedicel necrosis and incipient pericarp necrosis with fruit volume and width can be described by the linear equations: days to 20% pedicel necrosis = 0.32* fruit volume + 5.89, $R^2 = 0.83$ (Fig. 1A); days to incipient pericarp necrosis =

0.44* fruit volume + 5.70, $R^2 = 0.78$ (Fig. 2A); days to 20% pedicel necrosis = 0.42* fruit width - 2.11, $R^2 = 0.73$ (Fig. 1B); days to incipient pericarp necrosis = 0.59* fruit width - 5.71, $R^2 = 0.71$ (Fig. 2B), respectively.

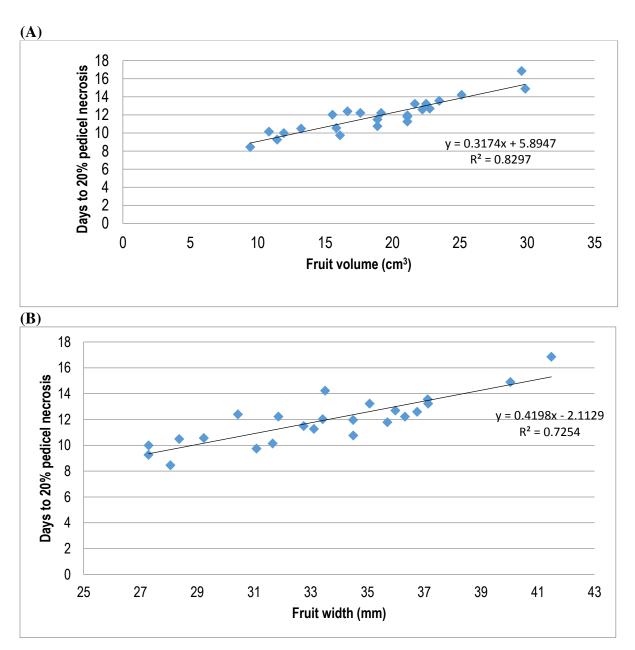


Figure 1: Graph showing the linear regression of days after harvest to 20% pedicel necrosis against (A) fruit volume (cm³) and (B) fruit width (mm) for 24 pepper (*Capsicum chinense* Jacq.) accessions evaluated under controlled storage conditions

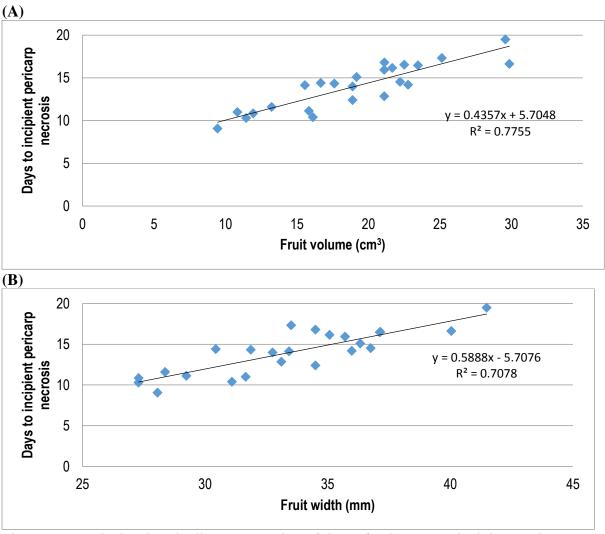


Figure 2: Graph showing the linear regression of days after harvest to incipient pericarp necrosis against (A) fruit volume (cm³) and (B) fruit width (mm) for 24 accessions of pepper (*Capsicum chinense* Jacq.) evaluated under controlled storage conditions

Discussion

There was considerable fruit morphophysiological diversity among the pepper accessions evaluated in this study. This was also reported by Bharath et al. (2013) reflecting the considerable genetic diversity within accessions reported by Moses and Umaharan (2012). In this study, fruit weight was positively associated with fruit width, volume and surface area but was negatively associated with surface area to volume ratio. Hence heavier pepper fruits are wider, have greater surface areas, larger volumes and smaller

surface area to volume ratios. Lownds et al. (1993, 1994) and Maalekuu et al. (2005) found significant associations between fruit weight and pericarp surface area and Díaz-Pérez et al. (2007) also found a strong association between fruit width and weight in *Capsicum annuum*.

This study showed no association for fruit weight, width, volume, surface area and surface area to volume ratio with fruit pericarp, endocarp, mesocarp and exocarp thickness. These results indicate that pepper accessions with heavier, wider fruits do not necessarily have fleshier fruits. In fact, although accession,

L29 possessed the largest pericarp thickness (2.34 mm), it had a relatively low fruit weight (9.2 g) compared to accession L30 which possessed a large fruit weight (13.5 g) but a small pericarp thickness (1.43 mm) (Table 1). The lack of association between fruit weight and pericarp thickness in this study is in variance to the strong association between these two characteristics in C. annuum (Maalekuu et al. 2005). Hence this study suggests that pericarp thickness in C. chinense is genotype specific. In fact, although the pericarp contributes the vast majority of the fruit weight in both C. chinense (González et al. 2004) and C. annuum, the pericarp of C. chinense is very thin (1.33 mm for a mean fresh of 9.5g = 7.1 g/mm, this study) compared to that of C. annuum (4.01 mm for a mean fresh weigh of 106.7g = 26.6 g/mm) (Maalekuu et al. 2005). This shows that C. annuum fruits have almost four times the weight per mm of pericarp compared to fruits of C. chinense.

This study showed that pepper accessions with darker (smaller L* values), greener (greater -a* values) and less yellowish (smaller b* values) fruits were associated with longer shelf life. Pepper fruits are non-climacteric, meaning that they produce very little endogenous ethylene and hence are not capable of continuing their ripening process once detached from the parent plant (Gamage and Rehman 1999; Sinha and Petersen 2011). Hence pepper begins deterioration following harvest due to desiccation, microbial growth, or due to biochemical senescence (Nipersos-Carriedo et al. 1991) and is expressed initially as incipient pedicel necrosis followed by total pedicel/ calyx necrosis and finally as pericarp necrosis (Elibox et al. 2015). That pepper accessions with greener fruit colors at physiological maturity had longer shelf lives may suggest a role for photosynthesis and carbohydrate status in maintaining membrane integrity, thus prolonging shelf life in these accessions. In bell peppers (Capsicum annuum), loss of microsomal membrane integrity has been demonstrated by increased

leakage of electrolytes (Lurie and Ben-Yehoshua 1986) and Maalekuu et al. (2005) found high lipoxygenase activity correlated with cell ion leakage and lower shelf life.

This study and the study of Elibox et al. (2015) showed that C. chinense accessions with heavier fruits had lower water loss after harvest, lower water loss rates and took a longer time to reach 20% pedicel necrosis as well as incipient pericarp necrosis (had longer shelf lives). Based on the research of Maalekuu et al. (2005), water loss and pedicel necrosis are two independent processes and Elibox et al. (2015) showed that they occur simultaneously in C. chinense. In fact, this study showed that only exocarp thickness correlated with weight (water) loss, indicating that accessions with thicker exocarps had lower water loss rates. Maalekuu et al. (2005) found that most water loss in mature bell pepper fruits occur through the pericarp surface with very little or insignificant amounts occurring from the pedicel or calyx. Pericarp thickness in this study was not associated with shelf life.

In this study, accessions with larger fruit width, volume and surface area also had longer shelf lives whereas those with larger surface area to volume ratios had shorter shelf lives. Of the parameters that correlated with shelf life in C. chinense in this study, fruit volume, width and surface area to volume ratio were the best predictors of shelf life as they showed the largest correlation values with days to 20% pedicel necrosis and incipient pericarp necrosis. Of the three parameters, fruit volume and width were highly correlated (r = 0.90, P < 0.01) and can be easily measured, thereby making shelf life determinations quick and easy, requiring very little resources and space. This is beneficial to breeding programmes aimed at developing C. chinense accessions with long shelf lives as well as determining the genetic basis for shelf life.

In conclusion, this study involving 24 pureline accessions of *C. chinense* showed that of the 15 morphophysiological fresh fruit characteristics evaluated, nine (initial color lab

space parameters a*, b*, L*; weight, width, volume, surface area, surface area to volume ratio and exocarp thickness) were positively or negatively associated with water loss and shelf life. Accessions with heavier fruits generally had wider fruits, larger surface area, smaller surface area to volume ratio, greener and darker fruit colors at physiological maturity and longer shelf lives. Of the nine characteristics, three viz. fruit volume, width and surface area to volume ratio were found to be the best predictors of shelf life based on days to 20% pedicel necrosis and incipient pericarp necrosis. Accessions with heavier fruits and possessing thicker exocarps were associated with lower rates of weight (water) loss.

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