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Calcium chloride and wax influences the post harvest behaviour of custard apple fruits

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Abstract
Custard apple is one of the most popular tropical underutilized fruit crops of the world appreciated for delicate flavour. The main problem of postharvest losses associated with custard apples is rapid loss of firmness. To combat the problem, the fruits were exposed to pre-storage treatments involving $CaCl_2$ (4%) and wax (1:10) and combination of two for 5 minutes. The fruits were stored in cold storage (10±1ºC) for 12 days. The results indicated, the minimum physiological loss in weight (16.57%), respiration rate (24.24 ml CO_2/kg/h), TSS (22.25°B), titratable acidity (0.27%) and minimum change in instrumental color values *viz.*, peel *L** (52.51), *a** (-2.02), *b** (30.12), *C** (29.24) and h^o angle (93.93) as well as pulp *L** (35.77), *a** (-0.95), *b** (10.68), *C** (9.84) and h^o angle (92.41) values and maximum firmness (2.49N) were noticed in combination treatment as compared to control fruits. The maximum scores (out of 5) for organoleptic qualities with respect to appearance (4.50), texture (4.69) and taste and flavor (4.69) was observed in the fruits treated with a combination of calcium chloride (4%) and wax (1:10) for 5 minutes at the end of 12 days of cold storage at 10±1ºC. In conclusion, combined pre-storage treatment of $CaCl_2$ (4%) and wax (1:10) for 5 minutes effectively delayed the ripening changes in sugar apple.

Keywords: *Annona squamosa*, respiration rate, firmness, organoleptic qualities, storage

1. Introduction
The custard apple (*Annona squamosa* L.), is one of the important tropical fruit crop belonging to the family Anacardiaceae. The fruits are seasonal and possess very short shelf life at ambient storage hence, pre-storage treatments coupled with cold storage is highly beneficial to improve their storability. The pulp has pleasant flavor, excellent taste and texture with some medicinal values. The fruits of the custard apple are very delicate and highly perishable in nature. Being climacteric in nature, ripening of the mature fruits after harvest occur at a faster rate followed by senescence process. At the room temperature, the post harvest lifespan of this fruit is only 3-4 days which is limited by physiological deterioration caused by fruit over ripening, loss of pulp firmness and darkening of the skin caused by the development of pathogens which alter the taste, aroma, flesh consistency and skin colour. Therefore, it is necessary to develop a technology which enables to extend the custard apple post harvest life making to reach the consumers with the minimally altered nutritional and sensory qualities.
Post-harvest applications with calcium chloride have been used to delay aging or ripening, consequently reducing post-harvest decay and controlling many diseases in fruits and vegetables (El-Gamal *et al*., 2007) [1]. Wax coatings have been shown to extend postharvest quality of many fruit and vegetable crops by limiting gas exchange and reducing discoloration, water loss and skin susceptibility to abrasion. Therefore the present study was undertaken to investigate the possibilities of enhancing the shelf life of the custard apple fruits by use of calcium chloride, wax and combination of both to check the efficacy and make the fruits available to the consumers with superior quality.

2. Materials and Methods
Fruits of uniform size, shape and maturity were harvested in the evening and brought to the laboratory of the Department of Post Harvest Technology in plastic crates. The damaged, bruised, punctured and infected fruits were discarded manually. Then, the healthy fruits were pre-cooled in cold storage at 10±1°C for twelve hours to remove field heat. After removal from the cold room, fruits were thoroughly washed in 0.2 per cent sodium hypochlorite solution for five minutes to remove the surface microbial load and dirt adhered to the fruit surface. Immediately, fruits were air dried under fan to remove the surface moisture. These fruits were then used for further experimentation to impose different postharvest treatments to study their influence on behaviour and shelf life during storage.
The fruits were into four lots of 80 fruits each, the first being control, the second lot was

treated with wax (1:10), the third lot was treated with calcium chloride (4%) and the fourth with the combination of both wax (1:10) and calcium chloride (4%) for 5 minutes with 5 replications. After subjecting to dipping for specific duration in the respective treatment, custard apple fruits were surface dried under electric fan. Then the fruits were packed in ventilated corrugated fibre board boxes. Paper lining was provided between the two layers of the fruits and paper shreds were used to provide cushioning and avoid fruits directly coming in contact with each other.

2.1 Physical parameters

2.1.1 Physiological loss in weight (PLW %)

In each replication, 4 fruits were ear marked to record the PLW. The marked fruits in each replication of the respective treatment were weighed individually at the beginning of storage to record the initial weight. On subsequent days of observation, the fruits were weighed again. The cumulative losses in weight of fruits were calculated and expressed as per cent physiological loss in weight.

2.1.2 Firmness (N)

Firmness of custard apple flesh was measured on both sides of the fruit at regular intervals taking a fruit from each replication by using force guage (Make: Lutron FG-5000A). It was recorded in Newton.

2.1.3 Respiration rate (ml CO_2/kg/h)

Respiration rate was measured with a CO_2 gas analyzer (Make: PBI Dansensor, Check Mate - II) in static method. The fruit was weighed and placed in a hermetically sealed container of 1250 ml capacity for 60 minutes. At the end of incubation period, gas sample was drawn from the container head space using gas tight syringe and injected into the CO_2 analyzer. The change in CO_2 gel concentration in the head space and time was read in the instrument was recorded. The respiration rate of the fruit was expressed as ml CO_2/kg/h.

2.1.4 Colour (L* a* b*, C* and h^o)

The colour of the samples was measured using a Lovibond colour meter (Lovibond RT300, Portable spectrophotometer, The Tintometer Limited, Salisbury, UK) fitted with 8 mm diameter aperture and the instrument was adjusted at 10° observer and D65 primary illuminant. The instrument was calibrated using the black and white tiles provided. Colour was expressed in Lovibond units *L** (Lightness/darkness), *a** (redness/greenness), *b** (yellowness/blueness), *C** (chroma) and h^o angle (hue). Custard apple fruits were directly placed under the aperture of the colour meter.

2.2 Biochemical parameters

2.2.1 TSS (^{o}B)

The juice extracted by squeezing the homogenized fruit pulp through muslin cloth was used to measure the TSS. It was determined by using ERMA hand refractometer, replicated three times and the mean was expressed in ^{o}B.

2.2.2 Titratable acidity (%)

A known volume of juice sample (10 ml) was taken and titrated against standard NaOH using phenolphthalein indicator. The appearance of light pink colour was marked as the end point. The value was expressed in terms of citric acid as per cent titratable acidity of juice (AOAC, 1984) [2].

2.3 Organoleptic evaluation of fruits

Organoleptic evaluation of fruits was carried out by a panel of 6 semi-trained judges at weekly intervals from third week. The sensory characters like skin colour as well as appearance, texture and taste and flavour were evaluated on a 5 point Hedonic scale.

Statistical analysis

Statistical analysis was performed using Web Agri Stat Package (WASP) Version 2.0 (Jangam and Thali, 2010) [3]. All data the collected were analysed by one-way analysis of variance (Anova). Significant differences among means at $P \leq 0.05$ were determined by post hoc tests using Duncan's multiple range test.

3. Results and Discussion

The surface treatments significantly affected the post harvest life of custard apple fruits. The physiological loss in weight of the custard apple fruits increased with the increase in storage period (Table 1). The significantly minimum weight loss was observed in the combination treatment of calcium chloride and wax treated fruits throughout the storage period of twelve days whereas maximum was observed in untreated fruits. Higher weight loss of untreated fruits may be due to increased storage breakdown associated with higher respiratory rate as compared to treated fruits. Retardation in transpirational losses on treatment of permeable edible coating has been reported in custard apple (Patel *et al*., 2011 and Masalkar and Garande, 2005) [4, 5]. As calcium is known to increase fruit cell wall turgidity, it is also supposed to reduce water diffusion over the fruit cuticle to reduce the differences in osmotic potential that are the driving force for water diffusion, and to strengthen the walls of epidermal cells that might had resulted in improved resistance to the fruit cell degradation, when the cells meet free flow of water (Sekse, 1998) [6]. This could be one of the reasons for reduction in PLW with storage time in 4% calcium chloride treated custard apple fruit up to 12 days.

Fruit firmness is the most important parameter in custard apple fruits during storage and marketing, because flesh softening is associated with senescence and increased susceptibility of fruits to injury during handling. There was decrease in the fruit firmness along the storage period (Table 1). Softening of fruits is associated with dissolution of the middle lamallae with turnover in the composition, structure and linkages between polysaccharides (Vicente *et al*., 2007) [7]. However, maintenance of firmness is also a result of direct interaction of calcium with the cell walls, where it binds to pectins and forms bridges between pectic acids. Calcium also stabilizes pectin–protein complexes in the middle lamallae thereby acting as an intermolecular binding factor (Dey and Brinson, 1984) [8], and may thicken the middle lammelae due to increased calcium pectate deposition (Gupta *et al*., 1984) [9]. The coating of fruits with wax resulted in higher fruit firmness, during storage, which might be due to reduction in moisture loss and respiratory activity and thus maintained the turgidity of the cells. Application of wax coatings have been reported to maintain the firmness of fruits during storage and marketing (Ribeiro *et al*., 2007, Adetunji *et al*., 2012) [10, 11].

Coating of custard apple fruits with calcium chloride and wax, individually and in combination, lowered the respiratory rate throughout the storage period (Table 1). Calcium treatments can maintain fruit quality by reducing respiration rate and

ethylene production. It has been proposed that calcium delays ethylene production by pre-venting solubilization of calcium binding sites in cell walls which activate the ethylene generation system located in cell wall plasmamembrane complex in turn reducing the respiration rate (Agusti *et al*., 2004) [12]. Calcium is hypothesized to delay ripening by reducing disintegration of tissues and maintaining membrane integrity (Torre *et al.*, 1999) [13]. The suppression of respiration was likely due to the modification of the internal atmosphere of the fruit (decreasing oxygen, and increasing CO_2) caused by the semi-permeable characteristics of the coating like wax to these gases (Banks, 1984) [14].

There was increase in total soluble solids along the storage period in all the treatments (Table 2). The calcium chloride and wax treated fruits had significantly lower TSS (22.25°B) as compared to untreated fruits at the end of the storage period of 12 days. Calcium treatments decrease TSS, probably due to slowing down respiration and as a result slower change from carbohydrates to sugars (Rohani *et al*., 1997) [15]. This may be also due to the fact that low temperature conditions prevailing in cold chamber might have resulted in lower respiration rate which made starch-sugar conversion at a slower rate as compared to room temperature stored fruits.

There was increase in the titratable acidity along the storage period in all the treatments (Table 2). The increased titratable acidity might be due to the synthesis of ascorbic acid in the fruits. The highest TA values found in untreated fruits may be related to higher fruits water losses, resulting in a higher concentration of organic acids present in the cell sap. Least titratable acidity (0.27%) was observed in treatment 4 and it may be due slow metabolic rates.

Changes in the colour of custard apple were monitored by measuring lightness value, *a** value, *b** value, chroma value and hue angle during 12 days of storage at 14°C. Parameter *L** related to luminosity is an excellent index for evaluation of color in fruits (Saucedo-Pompa *et al*., 2007) [16]. Treated and untreated fruit samples showed a significant decrease in the peel *L**, *b**, *C* value and h^o and increase in the *a** value during the storage period of twelve days (Table 3). The least decrease in the peel *L**, *b**, *C* value and h^o was observed in the treatment T_4 as compared all other treatment indicating less darkening of the fruits. Similarly pulp *L**, *a** value, *b** value, chroma value and hue angle was studied during 12 days of storage at 10±1°C (Table 4). There was decrease in the pulp *L**, *b**, *C* value and h^o and increase in the *a** value was observed indicating change in the pulp colour. Combined treatment of calcium chloride and wax darkened only slightly as evidenced by decreasing *L** values where as rest of the treatments resulted in more change in *L** value of both the peel and pulp and same thing might have happened with respect to all the other instrumental colour values of peel and pulp.

Treated fruits obtained good sensory scores throughout storage period as compared to non-treated fruits (Table 5, Figure 1, 2 and 3). On the 8th day of storage, highest scores (4.69) were obtained by the combination treatment of wax and calcium chloride treated fruits depicting the peak ripe stage of fruits and there was decrease in the scores thereafter.

Table 1: Influence of $CaCl_2$ and wax treatments on physiological loss in weight, firmness and respiration rate of custard apple fruits under cold storage (10±1°C). Similar alphabets within the column represents non-significant differences at ($p<0.05$)

	Storage days			
	Initial	**4**	**8**	**12**
Physiological loss in weight (%)				
T_1	0.0	8.14±0.92^a	16.53±3.50^a	28.68±2.58^a
T_2		6.13±0.32^b	13.19±1.15^b	19.45±0.86^b
T_3		6.13±0.97^b	13.65±0.91^{ab}	21.40±1.01^b
T_4		3.89±0.80^c	9.69±1.04^c	16.57±1.81^b
Firmness (N)				
T_1	7.37	3.415±0.38^b	2.42±0.17^b	1.35±0.17^c
T_2		3.73±0.21^b	2.65±0.35^b	2.00±0.03^b
T_3		3.99±0.15^b	3.37±0.45^a	2.40±0.36^{ab}
T_4		5.00±0.61^a	3.675±0.34^a	2.49±0.44^a
Respiration rate (ml CO_2/Kg/h)				
T_1	15.23	26.32±3.17^a	34.19±2.74^a	37.28±2.53^a
T_2		22.35±1.37^b	23.70±1.64^c	25.86±2.64^b
T_3		22.41±2.65^b	26.54±1.08^b	27.24±4.43^b
T_4		18.26±2.60^c	21.59±1.19^c	24.24±0.79^b

T_1: Control, T_2: Wax (1:10), T_3: $CaCl_2$ (4%), T_4: Wax (1:10) and $CaCl_2$ (4%)

Table 2: Influence of $CaCl_2$ and wax treatments on TSS and titratable acidity of custard apple fruits under cold storage (10±1°C). Similar alphabets within the column represents non-significant differences at ($p<0.05$) probability level according into Duncan's multiple range test.

	Storage days			
	Initial	**4**	**8**	**12**
TSS (°B)				
T_1	10.6	21.25±0.50^a	25.75±0.96^a	28.50±0.58^a
T_2		17.25±0.50^c	21.25±0.96^{bc}	23.25±0.96^c
T_3		18.50±0.58^b	22.50±0.58^b	25.75±0.96^b
T_4		16.75±1.00^c	20.00±0.82^c	22.25±0.96^c
Titratable acidity (%)				
T_1	0.19	0.27±0.02^a	0.30±0.01^a	0.37±0.02^a
T_2		0.24±0.01^b	0.27±0.01^{bc}	0.29±0.01^c
T_3		0.25±0.02^{ab}	0.29±0.01^{ab}	0.33±0.02^b
T_4		0.23±0.02^b	0.26±0.02^c	0.27±0.01^c

T_1: Control, T_2: Wax (1:10), T_3: $CaCl_2$ (4%), T_4: Wax (1:10) and $CaCl_2$ (4%)

Table 3: Influence of $CaCl_2$ and wax treatments on instrumental peel color values of custard apple fruits under cold storage (10±1ºC). Similar alphabets within the column represents non-significant differences at ($p<0.05$) probability level according into Duncan's multiple range test.

	Storage days			
	Initial	**4**	**8**	**12**
L* values				
T_1	63.04	49.92±2.61^c	42.76±0.71^d	40.87±0.46^d
T_2		55.36±0.39^b	53.20±0.87^c	49.38±0.44^b
T_3		51.95±1.78^c	48.00±1.18^b	45.83±0.73^c
T_4		59.13±2.20^a	54.87±1.12^a	52.51±1.11^a
a* value				
T_1	-5.65	2.29±0.48^a	2.77±0.50^a	4.27±0.79^a
T_2		-2.15±0.54^c	-0.71±0.56^c	-0.66±0.15^c
T_3		0.02±0.19^b	0.59±0.21^b	1.58±0.36^b
T_4		-3.82±0.35^d	-2.73±0.59^d	-2.02±0.62^d
b* value				
T_1	40.90	23.31±1.04^d	20.67±1.46^d	18.04±2.29^c
T_2		29.28±1.12^b	26.80±1.14^b	23.93±1.07^b
T_3		26.65±1.76^c	24.11±0.83^c	22.15±0.76^b
T_4		34.12±1.50^a	31.45±0.93^a	30.12±0.44^a
C* values				
T_1	37.86	23.20±0.64^d	20.92±1.23^d	17.94±2.10^d
T_2		30.49±0.06^b	28.08±0.52^b	25.49±0.90^b
T_3		25.16±0.51^c	23.73±0.72^c	22.38±0.52^c
T_4		34.22±1.65^a	31.61±1.19^a	29.24±0.73^a
h° values				
T_1	103.78	87.36±1.28^d	83.57±0.74^d	81.28±1.64^d
T_2		94.31±1.91^b	91.57±0.92^b	88.03±1.73^b
T_3		91.31±1.83^c	88.30±2.01^c	85.12±0.56^c
T_4		98.58±1.59^a	97.02±0.44^a	93.93±0.59^a

T_1: Control, T_2: Wax (1:10), T_3: $CaCl_2$ (4%), T_4: Wax (1:10) and $CaCl_2$ (4%)

Table 4: Influence of $CaCl_2$ and wax treatments on instrumental pulp color values of custard apple fruits under cold storage (10±1ºC). Similar alphabets within the column represents non-significant differences at ($p<0.05$) probability level according into Duncan's multiple range test.

	Storage days			
	Initial	**4**	**8**	**12**
L* values				
T_1	92.56	62.53±1.57^a	36.03±1.55^d	10.29±3.01^d
T_2		85.71±0.47 ^{b}	47.71±0.63^b	27.73±5.79^b
T_3		81.28±1.37^b	40.98±1.87^c	19.39±7.16^c
T_4		88.05±1.41^b	50.17±0.68^a	35.77±1.24^a
a* value				
T_1	-4.74	-0.71±0.14^a	-0.21±0.09^a	0.96±0.44^a
T_2		-1.04±0.21^b	-0.74±0.05^c	0.16±0.49^b
T_3		-0.85±0.07^{ab}	-0.43±0.05^b	0.57±0.37^{ab}
T_4		-1.27±0.06^c	-1.18±0.01^d	-0.95±0.15^c
b* value				
T_1	19.54	9.85±0.70^c	7.76±0.90^d	6.34±0.63^c
T_2		11.54±0.38^b	10.23±0.49^b	8.34±0.29^b
T_3		10.60±0.37^{bc}	9.36±0.29^c	7.87±0.70^b
T_4		14.67±1.55^a	13.00±0.32^a	10.68±0.77^a
C* values				
T_1	18.24	10.05±0.58^c	7.53±0.38^d	6.44±0.57^d
T_2		13.03±0.30^b	10.58±0.31^b	8.94±0.61^b
T_3		10.97±0.15^c	9.27±0.25^c	7.90±0.67^c
T_4		14.73±1.53^a	11.76±0.27^a	9.84±0.07^a
h° values				
T_1	101.45	94.96±0.61^c	87.91±1.58^c	80.32±1.69^c
T_2		96.61±0.11^b	94.11±0.81^{ab}	91.26±0.50^a
T_3		96.27±0.12^b	93.44±0.34^b	84.76±1.02^b
T_4		97.16±0.25^a	95.50±0.29^a	92.41±0.29^a

T_1: Control, T_2: Wax (1:10), T_3: $CaCl_2$ (4%), T_4: Wax (1:10) and $CaCl_2$ (4%)

Table 5: Influence of $CaCl_2$ and wax treatments on sensory qualities (5 point hedonic scale) of custard apple fruits under cold storage (10±1ºC). Similar alphabets within the column represents non-significant differences at ($p<0.05$) probability level according into Duncan's multiple range test.

	Storage days		
	4	**8**	**12**
Appearance scores			
T_1	4.07±0.13^c	2.75±0.29^c	1.82±0.24^c

T_2	4.82±0.13^{ab}	4.63±0.14^{ab}	4.19±0.13^{b}
T_3	4.63±0.14^{b}	4.50±0.41^{b}	4.00±0.20^{b}
T_4	4.88±0.14^{a}	4.94±0.13^{a}	4.50±0.20^{a}
Texture scores			
T_1	4.50±0.41^{a}	3.94±0.31^{b}	1.88±0.75^{c}
T_2	4.38±0.14^{ab}	4.07±0.13^{b}	3.38±0.48^{b}
T_3	3.63±0.32^{c}	4.69±0.24^{a}	4.32±0.24^{a}
T_4	3.88±0.48^{bc}	4.88±0.14^{a}	4.69±0.24^{a}
Taste and flavour scores			
T_1	4.69±0.13^{a}	3.94±0.43^{c}	1.69±0.38^{b}
T_2	3.69±0.47^{b}	4.32±0.38^{bc}	4.50±0.35^{a}
T_3	3.07±0.13^{b}	4.50±0.20^{ab}	4.25±0.46^{a}
T_4	3.25±0.65^{b}	4.94±0.13^{a}	4.69±0.24^{a}

T_1: Control, T_2: Wax (1:10), T_3: $CaCl_2$ (4%), T_4: Wax (1:10) and $CaCl_2$ (4%)

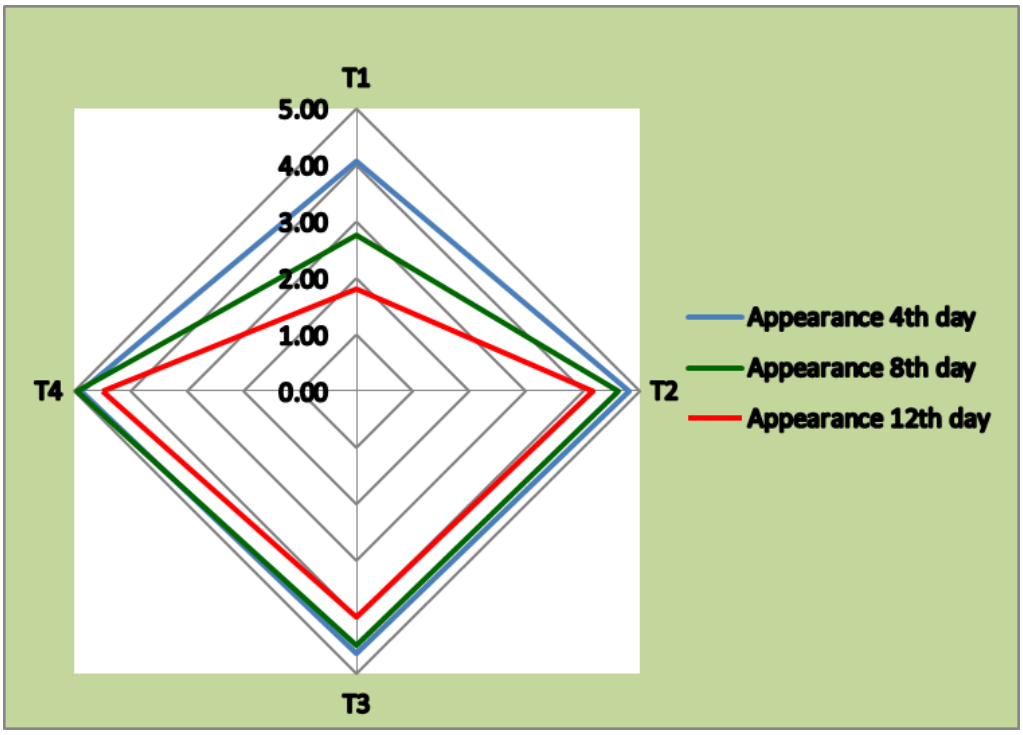

Fig 1: Effect of wax and $CaCl_2$ on sensory qualities: appearance

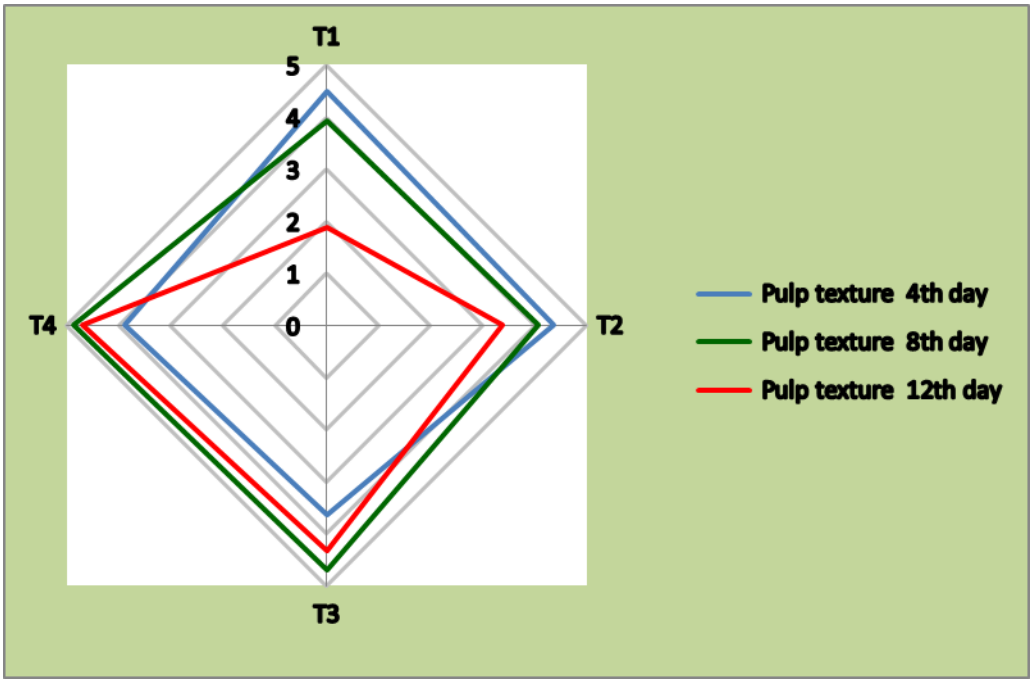

Fig 2: Effect of wax and $CaCl_2$ on sensory qualities: Pulp texture

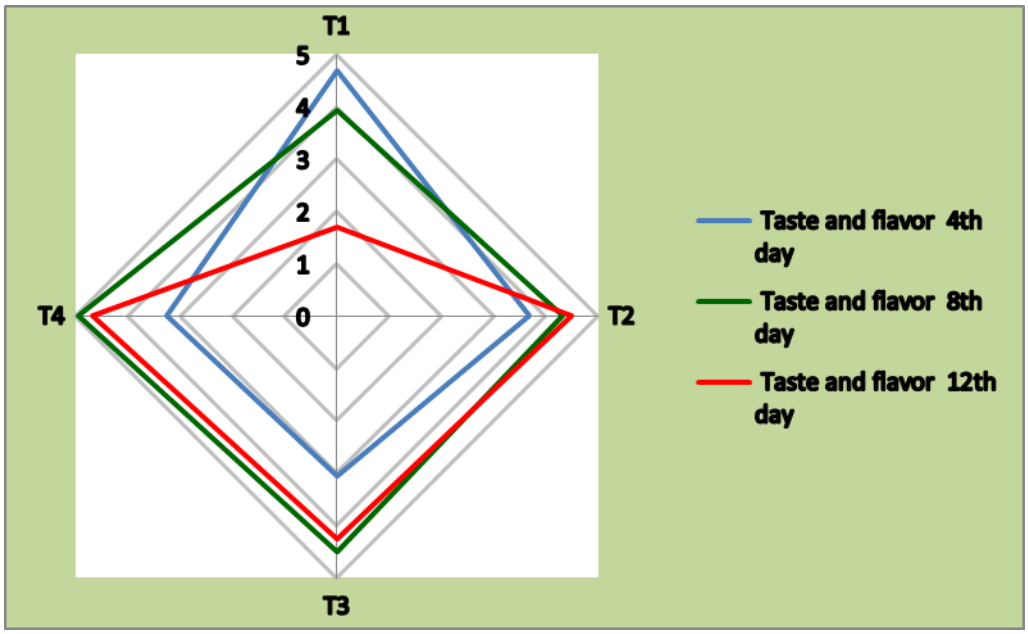

Fig 3: Effect of wax and $CaCl_2$ on sensory qualities: taste and flavor

4. Acknowledgements
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