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Studies on Biochemical Changes in Immature Mangoes (*Mangifera indica* L.) cv Konsam Heinou of Manipur using Different Skin Coatings During Storage

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ABSTRACT

The present investigation on "Studies on Biochemical Changes in Immature Mangoes (*Mangifera indica* L.) cv Konsam Heinou of Manipur Using Different Skin Coatings during storage" was carried out during month of May-June of the year 2021 at Post Graduate Laboratory, Department of Horticulture, College of Agriculture, Central Agricultural University, Manipur. Freshly collected uniform sized mango fruits were picked, washed, cleaned and treated in the laboratory with various coatings, including castor oil (T_1), palm oil (T_2), coconut oil (T_3), mustard oil (T_4), aloevera gel (T_5), paraffin wax (T_6), cling film (T_7), and no coating (T_8). The experiment was framed in CRD (Completely Randomized Design) and repeated 3 times with 7 treatments and an uncoated treatment. The results obtained from the current investigation on biochemical changes in quality parameters the fruits treatments with cling film (T_7) had minimum TSS, TSS : Acid ratio, reducing sugar and total sugar content with maximum acidity content while fruits coating with paraffin wax (T_6) and cling film(T_7) had maintaining of chlorophyll content up to 15th days of storage under ambient condition.

Keywords Biochemical CRD, Immature, Coating, Storage, Experiment.

INTRODUCTION

Mango (*Mangifera indica* L.) is a member of the Anacardiaceae family and originated in the Indo-Burma region. Mango is one of the top tropical fruits in the world market because of its superb flavor, attractive scent, gorgeous color, and exquisite flavor and health-giving properties; hence it is popularly called as "King of fruits." Mango fruits are very delicious with an excellent flavor, attractive fragrance and rich in vitamin 'A' (beta carotene), vitamin B1, vitamin B2, and vitamin C (ascorbic acid). Mango consumption, because it is high in bioactive chemicals, can provide a good source of antioxidants that assist to lower the risk of certain types of cancer, delay the ageing process, enhance lung function, and lessen difficulties linked with cancer with diabetes Alam *et al.* (2016). Uttar Pradesh, Bihar, Andhra Pradesh, Orissa, West Bengal, Maharashtra, Gujarat, Karnataka, Kerala, and Tamil Nadu are the major mango producing states. Mango postharvest losses are still very significant. They are primarily caused by poor

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fruit maturity, mechanical damage during harvesting or improper field handling, sap burn, spongy tissue, lenticels discoloration, fruit softening, chilling injury, and disease and pest damage, among other things. According to Nanda *et al.* (2012), 5.8–18.1% of fruits were lost during harvesting, postharvest operations, handling, and storage. Quality losses are common as a result of tight fruit packing, poor transportation, and insufficient field treatment. Fruit losses during export can vary dramatically depending on post-harvest handling and export conditions, especially concerning rates of decay, pests and physiological breakdown. Despite a variety of uses, unfortunately, the post-harvest treatments of mango crops are minimal and there is very less processed product in some parts of North East India, especially in Manipur. In Manipur, despite its abundance during the peak season, most of the native types are prone to insect infestation and do not fully ripen. As a result, in order to reduce and minimize loss, it is required to maintain the immature mango using various post-harvest handling and processing procedures such as correct processing and value addition, as well as extending the shelf life of immature mangoes by using different skin coatings and storage at optimum temperature and relative humidity, packaging treatments. Cling film the plastic packaging film has revolutionized the food industry by protecting and preserving the food. Cling film is a thin polyethylene film that attaches to the surface of fruit and acts as an additional covering. By slowing respiration and transpirational losses, the film improved the physico-chemical properties of guava, such as appearance, weight loss, total soluble solids, titrable acidity, ascorbic acid concentration and total sugars. Pure coconut oil as an edible coating for fruits is gaining popularity due to its anti-senescence property of reducing respiration rate, transpiration rate, and ethylene biosynthesis process binding. Coconut oil and liquid paraffin coating blocked stomata and lenticels, lowering transpiration and respiration rates as well as microbial activity (Bisen *et al.* 2012).

MATERIALS AND METHODS

TSS

Juice was squeezed by hand through muslin cloth and the juice of fruits was immediately used for determining the total soluble solids by using hand refractometer of 0-32 °Brix at 20°C. Three readings were taken in each treatment and their average value was calculated and the mean value was expressed as per cent Brix.

Total sugar

The titrametric method of Lane and Eynon described by Ranganna (1979) was adopted for estimation of reducing sugar, which is given as under :

For estimation of total sugars, the filtrate obtained in the estimation of reducing sugar was used. The total sugars of the filtrate were estimated by standardized Fehling's solution A and B using methylene blue indicator as per the procedure described below.

Procedure

An aliquot of 25 ml from the filtrate was taken and one-fifth of its volume of 5 ml hydrochloric acid (1:1) added and the inversion was carried out at room temperature for 24 hrs. Subsequently, the contents were cooled and neutralized with 40% sodium hydroxide using phenolphthalein as indicator and the final volume was made up to 100 ml. The solution was filtered through Whatman's No. 1 filter paper and then titrated against boiling Fehling's mixture as described earlier. The percentage of total sugars was expressed as invert sugar according to the following formula.

$$\text{Total sugar (\%)} = \frac{\text{Glucose eq. of Fehling solutions 0.05}}{\text{Titer taken}} \times \frac{\text{Total volume made up}}{\text{Weight of pulp}} \times \frac{\text{Volume made up after inversion}}{\text{Aliquot take for inversion}} \times 100$$

Reducing sugar

The titrametric method of Lane and Eynon described by Ranganna (1979) was adopted for estimation of reducing sugar, which is given as under:

Principle of the method

Invert sugar or reducing sugar reduces copper in the Fehling's solution to red insoluble cuprous oxide.

The sugar in a sample was estimated by determining the volume of unknown sugar solution required to completely reduce a measured volume of Fehling's solution. In advance, the mixture of Fehling' s solution A and B (5 ml of A and 5 ml of B) was standardized against standard glucose for obtaining glucose equivalents and to arrive at a conversion factor.

Procedure

25 g of the homogenized pulp was taken in 250 ml volumetric flask and 2 ml of 45% basic lead acetate solution added for clarification. After 10 minutes, the solution was de-leaded by adding potassium oxalate crystals in excess and the volume was made up with distilled water up to 250 ml. It was then filtered through Whatman's No.1 filter paper. The filtrate was taken in a burette and titrated against boiling Fehling's mixture (5 ml of Fehling's solution A and 5 ml of Fehling's solution B) till the blue color faded. Then 2-3 drop methylene blue indicator (1 %) was added and then titration continued till the contents attained a brick red color. At this stage the titrate value was noted and percentage reducing sugar calculated by following formula :

$$\text{Reducing sugar (\%)} = \frac{\text{Glucose equivalent (0.05)} \times \text{Total volume made up}}{\text{Titer} \times \text{Weight of pulp}} \times 100$$

Acidity

The acidity level was obtained through titration. For estimation, 2 ml mango juice was titrated against 0.1 N NaOH, phenolphthalein used as an indicator (AOAC 1990). The acidity level was expressed in terms of percentage.

$$\text{Acidity (\%)} = \frac{0.0064 \times \text{Volume of N/10 NaOH used (ml)}}{\text{Volume of the mango juice (ml)}} \times 100$$

TSS : Acid ratio

The ratio for fruit juice under each treatment was calculated by dividing TSS value by titratable acidity content of fruit.

Chlorophyll

The chlorophyll content is determined using the method as described by Sadasivam and Manickam (1991). Chlorophyll is extracted in 80% acetone and the absorption at 663 nm and 645 nm are read in a spectrophotometer. Using the absorption coefficient, the amount of chlorophyll is calculated.

Procedure

1. Weigh 1 g of finely cut and well mixed representative sample of leaf or fruit tissue into a clean mortar, 2. Grind the tissue to a fine pulp with the addition of 20 ml of 80% acetone, 3. Centrifuge (5000 rpm for 5 min) and transfer the supernatant to a 100 ml volumetric flask, 4. Grind the residue with 20 ml of 80% acetone, centrifuge and transfer the supernatant to the same volumetric flask, 5. Repeat this procedure until the residue is colorless. Wash the mortar and pestle thoroughly with 80% acetone and collect the clear washings in the volumetric flask, 6. Make up the volume to 100 ml with 80% acetone,7. Read the absorbance of the solution at 645, 663 and 652 nm against the solvent (80% acetone) blank.

$$\text{Total chlorophyll (mg)} = 20.2\,(A645) + 8.02\,(A663) \times \frac{V}{1000 \times W}$$

Where
A = Absorbance at specific wavelengths.
V = Final volume of chlorophyll extract in 80% acetone.
W= Fresh weight of tissue extracted.

RESULTS AND DISCUSSION

Observations recorded on total soluble solids (ºBrix), acidity (%), TSS: Acid ratio, total sugar (%), reducing sugar (%) and chlorophyll (mg) during storage period are described below :

Effect on TSS (ºBrix)

Conversion of starch and formation of sugars are the main causes of changes in TSS content of fruits

Table 1. Effect of coating treatments on total soluble solids of local mango during different days of storage.

Treatment		Total soluble solid (ºBrix) Storage period (days)			
		0 day	5 days	10 days	15 days
T_1	(Castor oil)	6.00	6.20	7.16	7.90
T_2	(Palm oil)	6.00	7.30	8.03	8.93
T_3	(Coconut oil)	6.00	6.30	7.26	8.03
T_4	(Mustard oil)	6.00	6.33	7.36	8.10
T_5	(Aloevera gel)	6.00	6.46	7.90	8.23
T_6	(Paraffin wax)	6.00	6.16	7.06	7.70
T_7	(Cling film)	6.00	6.06	6.63	7.70
T_8	(No coating)	6.00	7.40	8.20	9.00
SEm±		0	0.099	0.119	0.22
CD 5%		0	0.296	0.357	0.68

during the storage period. The increased activity of enzymes responsible for the hydrolysis of starch to soluble sugars and the conversion of starch to sugar is closely associated to the increase in total soluble solids in fruits, indicating that the fruits are ripening. Table 1 shows that different coating treatments had a significant impact on TSS content of mango fruits at the beginning, 0 day, 5th, 10th and 15th days of storage. At the 0 day TSS was found in fruits (6.00 ºBrix), at the 5th day after storage the minimum total soluble solid was recorded on fruits covering with T_7 (cling film) and T_6 (paraffin wax) i.e. 6.06 and 6.16 ºBrix respectively and the maximum TSS was found T_8 (no coating) i.e. 7.40 ºBrix at 10th day after storage. At the 15th day after storage, the minimum total soluble solid was recorded fruits coated with T_1 (castor oil), T_6 (paraffin wax) and T_7 (cling film) i.e. 7.70 ºBrix. Reduced respiration rates in fruits covered with paraffin wax, castor oil, or cling film may have resulted in reduced PLW and delayed ripening due to delayed conversion of starch to sugars upon ripening, shown by lower TSS when compared to controls. The maximum TSS was recorded in control (T_8) i.e. 7.40, 8.20, and 9.00 (ºBrix) at the 5th, 10th and 15th days of storage, respectively. When compared to coated fruits, uncoated fruits experienced continuous gaseous exchange and regular ripening processes, which could be reflected in higher TSS in fruits. Similar findings were observed in some fruits treated with edible coatings, as those reported previously for mango, guava and apples (Sabir *et al.* 2004).

Table 2. Effect of coating treatments on acidity of local mango during different days of storage.

Treatment		Acidity (%) Storage period (days)			
		0 day	5 days	10 days	15 days
T_1	(Castor oil)	2.88	2.08	1.8	1.3
T_2	(Palm oil)	2.88	2	1.61	1.25
T_3	(Coconut oil)	2.88	2.04	1.73	1.29
T_4	(Mustard oil)	2.88	2.02	1.68	1.28
T_5	(Aloevera gel)	2.88	2.01	1.66	1.26
T_6	(Paraffin wax)	2.88	2.4	2.01	1.48
T_7	(Cling film)	2.88	2.4	2.01	1.48
T_8	(No coating)	2.88	1.98	1.48	1.21
SEm±		0.06	0.06	0.09	0.05
CD 5%		NS	0.2	0.27	0.17

Table 3. Effect of coating treatments on TSS: Acidity ratio of local mango during different days of storage.

Treatment		TSS: Acidity ratio Storage period (days)			
		0 day	5 days	10 days	15 days
T_1	(Castor oil)	2.08	2.96	4.24	6.30
T_2	(Palm oil)	2.08	3.61	5.13	6.95
T_3	(Coconut oil)	2.09	3.09	4.26	6.33
T_4	(Mustard oil)	2.08	3.17	4.48	6.52
T_5	(Aloevera gel)	2.08	3.22	4.73	6.67
T_6	(Paraffin wax)	2.08	2.64	3.51	5.22
T_7	(Cling film)	2.08	2.52	3.30	4.86
T_8	(No coating)	2.08	3.62	5.55	7.01
SEm±		0.04	0.10	0.33	0.42
CD 5%		NS	0.32	1.00	1.26

Effect on acidity (%)

Table 2 show the acidity of mango fruits after treatment with paraffin wax, aloevera gel, castor oil, coconut oil, mustard oil, and cling film coatings. The acidity was decreasing due to increase of soluble sugars during ripening. Organic acids, such as malic or citric acid, are primary substrates for respiration; a reduction in acidity is expected in highly respiring fruit (Al-Juhaimi *et al.* 2013). At the beginning, 0 day, 5th, 10th, and 15th days of storage, the results demonstrated that different coating treatments had a substantial impact on the acidity of mango fruits. The first day of storage revealed acidity, i.e. (2.88 %). At the 5th, 10th and 15th days of storage, paraffin wax (T_6) and cling film (T_7) had the maximum acidity accumulation, i.e. 2.40, 2.01, and 1.48%, respectively. The lowest acidity accumulation, 1.98, 1.48 and 1.21%,

Table 4. Effect of coating treatments on total sugar of local mango during different days of storage.

Treatment		Total sugar (%) Storage period (days)			
		0 day	5 days	10 days	15 days
T_1	(Castor oil)	5.50	10.11	10.99	10.91
T_2	(Palm oil)	5.53	12.18	15.46	16.47
T_3	(Coconut oil)	5.43	10.18	11.19	11.62
T_4	(Mustard oil)	5.35	10.44	13.36	15.08
T_5	(Aloevera gel)	5.45	11.72	15.52	15.76
T_6	(Paraffin wax)	5.50	8.42	9.00	9.18
T_7	(Cling film)	5.35	7.58	8.44	9.08
T_8	(No coating)	5.50	13.13	15.97	16.96
SEm±		0.12	0.19	0.15	0.11
CD 5%		NS	0.59	0.47	0.33

Table 5. Effect of coating treatments on reducing sugar of local mango during different days of storage.

Treatment		Reducing sugar (%) Storage period (days)			
		0 Day	5 Days	10 Days	15 Days
T1	(Castor oil)	2.29	5.56	6.01	2.48
T_2	(Palm oil)	2.31	6.61	6.82	3.44
T_3	(Coconut oil)	2.32	5.62	6.03	2.54
T_4	(Mustard oil)	2.28	5.64	6.11	3.24
T_5	(Aloe vera gel)	2.27	5.93	6.29	3.42
T_6	(Paraffin wax)	2.32	4.46	5.99	2.31
T_7	(Cling film)	2.33	4.17	5.80	2.18
T_8	(No coating)	2.33	6.70	6.78	3.52
SEm±		0.029	0.107	0.093	0.064
CD 5%		NS	0.319	0.279	0.191

was recorded with no coating (T_8) on the 5^{th}, 10^{th}, and 15^{th} days of storage, respectively. It is considered that coating reduces the rate of respiration and may therefore delay the utilization of organic acids which resulted in higher acidity in pulp of 6% waxol coated fruits. Uncoated fruits exhibited a greater PLW due to a faster rate of respiration, which resulted in early ripening and decreased acidity in the pulp throughout the storage period.

Effect on TSS : Acid ratio

At the beginning, 0 day, 5^{th}, 10^{th} and 15^{th} days of storage, the results demonstrated that different coating treatments had a substantial impact on the TSS : Acidity ratio of mango fruits shows in the Table 3. The first day of storage revealed TSS : Acidity ratio, i.e. (2.08). At the 5^{th}, 10^{th} and 15^{th} days of storage, cling film (T_7) had the gradually increased TSS : Acidity ratio, i.e. 2.52, 3.30 and 4.86 respectively was recorded minimum as compared to other treatment. While the highest TSS: Acid ratio was recorded with no coating (T_8) fruits at the 0 day, 5^{th}, 10^{th} and 15^{th} day after storage, i.e. 2.08,3.62,5.55 and 7.01 respectively. Uncoated fruits (T_8) exhibited a greater PLW due to a faster rate of respiration, which resulted in early ripening increased total soluble solid and decreased acidity in the pulp throughout the storage period. Mandal *et al.* (2018) observed that TSS and TSS: Acid ratio was increased during storage as the fruits gained TSS during the period, while acidity markedly dropped. It was found that TSS which ranged between 8.00 to 9.87 °Brix at 4 DAS was increased and ranged between 9.60 to 13.20 ^{o}Brix, whereas acidity that ranged between 1.92 to 3.16% at 4 DAS dropped and ranged between 0.45 to 1.00% at 12 DAS. At 12 DAS, it was found that fruits at control got maximum TSS: Acid ratio (29.33) compared with the wax coated fruits (9.60).

Effect on total sugar (%)

Total sugars in mango fruits rise during storage time due to two mechanisms: Amylase activity, which converts starch to simple sugars (sucrose, fructose, and galactose), and sucrose biosynthesis. In mature mango fruits, sucrose is the most prevalent sugar. Table 4 shows that different coating treatments had a significant impact on the buildup of total sugar in mango fruits on the 0^{th}, 5^{th}, 10^{th} and 15^{th} days of storage. At 5^{th}, 10^{th} and 15^{th} day after storage the data were recorded as minimum total sugar the fruits were treated with (T_7) i. e. 7.58, 8.44 and 9.08% respectively. Whereas the maximum total sugar percentage was noted with uncoated fruits (T_8) at 5^{th}, 10^{th}, and 15^{th} day after storage, i. e. 13.13, 15.97 and 16.96% respectively. It's probable that cling film packing hindered the conversion of starch to sugars and the production of sucrose as a result of lowered respiration rates and modified gaseous exchange, resulting in lower total sugars content during ripening. The continued breakdown of starch into sugars and production of sucrose in uncoated fruits resulted in increased total sugars in the fruits at ripening. Hubbard *et al.* (1991) suggested that soluble sugar increased during fruit ripening due to action of sucrose phosphate synthase, a key enzyme

Table 6. Effect of coating treatments on chlorophyll (mg) of local mango during different days of storage.

Treatment	Chlorophyll Storage period (days)			
	0 day	5 days	10 days	15 days
T_1 (Castor oil)	1.23	1.26	1.16	0.97
T_2 (Palm oil)	1.36	1.20	0.99	0.95
T_3 (Coconut oil)	1.33	1.23	1.09	0.97
T_4 (Mustard oil)	1.30	1.23	1.06	0.96
T_5 (Aloevera gel)	1.26	1.23	1.06	0.95
T_6 (Paraffin wax)	1.40	1.33	1.23	0.98
T_7 (Cling film)	1.36	1.36	1.30	1.06
T_8 (No coating)	1.26	1.13	0.94	0.89
SEm±	0.05	0.04	0.04	0.01
CD 5%	NS	NS	0.14	0.05

in sucrose biosynthesis.

Effect on reducing sugar (%)

The results found in Table 5 that different coating treatments had a substantial impact on the accumulation of reducing sugar in mango fruits on the 0 day, 5^{th}, 10^{th} and 15^{th} days of storage. At 5^{th},10^{th} and 15^{th} day after storage the data were recorded as minimum reducing sugar the fruits were treated with T_7 (cling film) i.e. 4.17, 5.80 and 2.18% respectively. Whereas the maximum reducing sugar percentage was noted with uncoated fruits (T_8) at 5^{th},10^{th}, and 15^{th} day after storage, i.e. 6.70, 6.78 and 3.52 respectively. The decrease in reducing sugars percentage in coated mango fruits as compared to control treatment might be due to its slow ripening process in coated fruits (Youssef *et al.* 2002).

Effect on chlorophyll (mg)

Table 6 shows the results that different coating treatments had a substantial impact on the degradation of chlorophyll (green color) in mango fruits on the 0 day, 5^{th}, 10^{th}, and 15^{th} days of storage. During different periods of storage, there was a considerable difference in chlorophyll degradation due to various skin coatings treatments. At the 0 day, 5^{th}, 10^{th}, and 15^{th} days of storage, fruits coated with cling film (T_7) had recorded significantly the least chlorophyll content (mg) degrade, i.e. 1.36, 1.36, 1.30, and 1.06 (mg), respectively. However, at 0 day, 5^{th}, 10^{th} and 15^{th} days of storage, fruits in the no-coating condition (T_8) had the highest degradation of chlorophyll content (mg), with 1.26, 1.13, 0.94 and 0.89 (mg), significantly. When compared to no coating, all treatments observed a reduction in physiological weight loss (T_8). Chlorophyll content decreased while carotenoid content increased during ripening as reported in guava fruits cv Banarsi Surkha. In the present study, total chlorophyll content of skin decreased as the fruit ripened and attained an edible appeal. In case of control fruits 231.18 ± 6.46 mg/g, fresh weight of total chlorophyll value reduced rapidly to 71.77 ± 3.77 mg/g, fresh weight on day 3 itself and then it showed insignificant gradual decrease. Coated fruit on other hand showed slow decrease in total chlorophyll content which reduced from 201.17 ± 8.60 mg/g, fresh weight on day 1 to 80.30 ± 1.37 mg/g, fresh weight on day 9. Results indicate that the edible coating can delay the degradation of chlorophyll demonstrated by Chitravathi *et al.* (2014).

CONCLUSION

The application of cling film and paraffin wax was found to be good quality in terms of TSS, acidity, TSS: Acid ratio, reducing sugar, total sugar, and chlorophyll at the same time.

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