Extension of Mango Shelf life by Aloe vera Coating and Gene Expression

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Abstract

The aloe vera based natural coatings can improve the quality and lengthen the shelf life of fruits and vegetables. In this study, the effect of mixtures of Aloe vera gel, pectin and olive oil on the improvement of quality and shelf life extension of six different cultivars of mango (Gopalbhog, Ranipochand, Khirsapat, Langra, Fazli and Aswina) have investigated. The coating of fruits (coating treated fruit) was found significantly delay the ripening process and softening of the fruit. The shelf life of coated fruits increased to 4 to 6 days compared to that of uncoated fruits. Average weight loss was 5-7% less in coated fruits compared to uncoated ones. The coated fruits showed less gene expression than uncoated one. All the above information of different mango cultivars showed variation in quality parameters.

Key words: Mango, Aloe vera gel, coating, shelf life, Caffeoyl- CoA 3-O methyltransferase

Introduction

Mango *(Mangifera indica)* is one of the best indigenous delicious and nutritious fruits of Bangladesh. It has excellent flavor, colour shades, taste and high nutritive value and also it is economic potentiality[1]. This is one of the most nutrition rich fruits and is an excellent source of vitamins and minerals[2,3]. Besides being consumed as fresh fruits, both ripe and green, mango can be used to prepare a range of food products such as jam, jelly, juice, pickle chutney, powder, nectar, squash etc[4-6]. Thus, mango is acknowledged as the king of fruits in Bangladesh[2-5].

In Bangladesh, total mango growth has increased over the decades. Figure 1 shows that mango was produced on 25055 acres of farmland and production was 26.43 tones/ha in 2004-05. Since then, the area coverage is continually being increased with high level of total growth[7]. Mango acreage stood at 37823 hectares in 2015-16. Figure 1 also shows that in 2004-05 to 2014-2015 the yield of mango increases at an accelerated rate[8].

In Bangladesh, the leading mango producing areas are Rajshahi, Dinjpur and Chapainawabgonj[8,9,10]. More than 500 varieties of sweet edible mangoes can be seen in Rajshahi and Chapainawabgonj district. It is estimated that around 85% people of the districts are directly or indirectly dependent on mango cultivation and business[7].

A vast amounts of mango (27 34%) is lost due to improper harvesting, transportation, packaging and storage, and post harvest diseases. Therefore, technology is to be developed for the promotion of the fruit industry. It is essential for increase of mango production and store[7,8,10].

The shelf life of mango comprises maturation and senescence. After harvest, fruits go through several physiological and biochemical transformation. The storage period of fruits could be extended appreciably by decreasing the pace of the process leading to maturation and reducing microbial count with the help of natural coatings like aloe vera gel[11-13]. Information on extension of storage life of mango through the treatment of natural coatings in Bangladesh is very scanty. This study aimed to extend the storage life and to reduce the degradation of mango without affecting the fruit quality[7,11-13].

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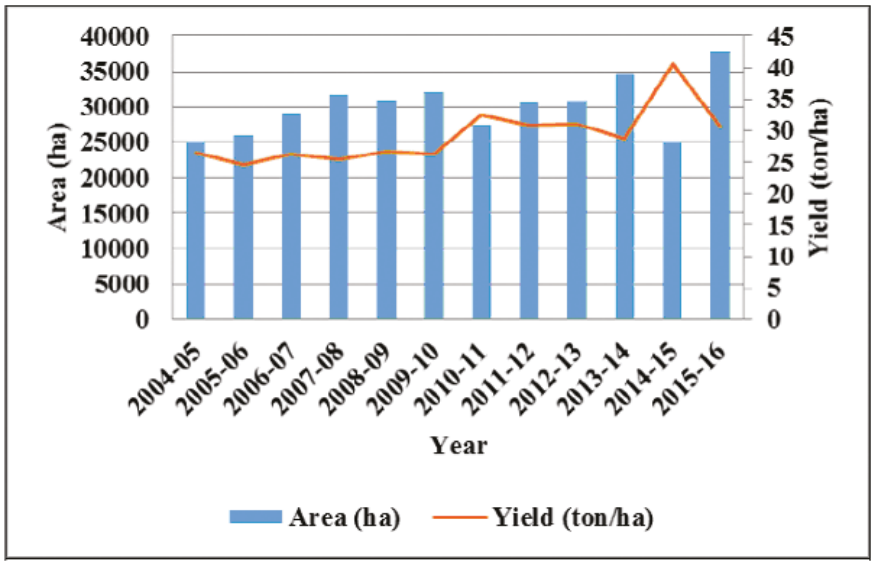

Figure 1: Area for mango production and Yield in Bangladesh from 2004-2016
Source: BBS

Plant hormones such as ethylene, auxins, brassinosteroids regulate the ripening cycle of fruits[14-16]. Ripening in climatic fruits including mango, banana, potato, tomato etc is characterized by a respiratory burst connected to ethylene formation[17-25]. Methyltransferases are ubiquitous enzymes, which helps transition of a methyl moeity from S- adenosyl- I-methionine to a receiver substrate, and forms O-, N-, S, C-methyl-derivatives and S-adenosylhomocysteine[26]. Methylation induces formation of different plant components like phenylpropenes, lignins, phenols, flavonoids, and alkaloids, which subsequently play a role in development and maturation, aroma formation, and plant protection [26,27]. Catechol-O-methyltransferase (COMT EC 2.1.1.6) helps the O-methylation of diols contributing to lignifications and can have other biochemical effects such as ripening as well as addition of flavor and tast[28]. In ripe mangoes, COMT are expressed in high levels than unripe ones.

Experiments on regulation of gene expression related to fruit shelf life and ripening is necessary to prove the efficiency of the preservatives by analysis of real time PCR data[29]. In this research, RT-PCR of COMT was done to observe the relative expression of the gene. Previous studies showed reliably directed rise in COMT protein expression behavior at full ripe stage relative to unripe condition[30,31]. This research aimed to assess the Aloe vera based coatings efficiency in extension of mango shelf life and analysis of related gene expression by real time PCR.

Materials and Methods

Preparation of Aloe vera gel

Aloe vera leaves were collected from the local market for processing and preparation of Aloe vera gel, which was used for coating the fruit. Undamaged, rot free and matured Aloe vera leaves were taken to have all active ingredients[32]. The leaves were washed with water and removed unexpected organ and peeled. The pulp were collected and homogenized by blending and the mixture was filtered, and was heated at low temperature for two hours[18,33,34].

Preparation of coating solution

The aloe vera gel was cooled and 3% pectin powder and 5% olive oil were added to the gel, which were mixed by over head stirrer in 3000 rpm for 30 minutes to one hour until homogenized. The cool homogenized mixture was used for coating[18,33].

Application of coating solution

Fresh and ripe mango samples (gopalbhog, ranipachand, khirsapat, langra, fazli and ashwina cultivars) were taken from from local market of Rajshahi, properly cleaned and air-dried. Fruits were kept in Aloe vera coating for 10 min. Uncoated mangoes were preserved in same condition at ambient temperature 28- 30ºC. Fruits were then stored for observation at the 28- 30ºC and relative humidity 70-72% for 20 days[11,12,27-29].

Physicochemical tests

Two sets of experiments were done for each analysis and fifteen fruits were in use in each set of analysis.

The firmness test was done by the firmness tester. Whole fruit firmness was obtained by a precision penetrometer (EFFGI, 48011 ALFONSINE, Italy). The deformation was observed using 0.5 kg force at the fruit equator for 5s [35]. The average firmness was taken for each set of coated and uncoated samples in every two days during the 14 days of storage or experiment period. Weight loss of the coated and uncoated mangoes was also measured. In each set, 15 mangoes were taken for each cultivar. Initial and end weight were measured. T-test was performed to observe the effect of coating.

The sugar content was determined spectrophotometrically by the Lane and Eynon method[36] and the reducing sugar was estimated by the DNSA method using 3,5-Dinitrosalicylic acid (DNSA)[37]. Vitamin C was estimated by spectrophotometric method using 10% Trichloroacetic acid and 10% Folin Ciocalteu's Reagent (FCR) solution. The absorbance was taken at 760 nm[38].

TSS was determined by refractometric method and pH was determined by a standard pH meter[39]. Acidity was estimated by titration with standard sodium hydroxide solution. The preserved tomato was analyzed periodically and the results were recorded in tables (Table 1 and 2). Protein was estimated by Kjeldahl method[40], phosphorus was measured spectrophotometrically[41]. Moisture and dry matter were measured by air-oven drying method[19], and ash % was determined with Presto's Muffle Furnace[5,19,35].

Another two sets of coated and uncoated mangoes were analyzed periodically for physical appearances, flavor and taste. Organoleptic tests of the uncoated and coated mangoes after 14 days of experiment started were evaluated by a panel of ten sensory experts. The mangoes were categorized depending on their scoring excellent 80% or above, good 70-80% and fair below 70% on the basis of general appearance, color, flavor, taste and texture[39].

Gene expression test:

The related gene expression of the uncoated and coated samples was done after 14 days. The forward primer GATTCAACAAGGT(C/T)TTCAA and the reverse primer CA (G/A) TTCTTCAA (G/A)AATTT (T/C)A (A/T)(G/A)CA were used for obtaining cDNA of the concerned gene[31]. Real Time PCR analysis was carried out in a Rotor Gene Q (QIAGEN). Twenty µl reaction volume consisting of 4 µl of master mix, 2.5 µl of primer and probe mix, 11 µl of ddH2O and 2.5 µl of DNA template containing 125 ng of total DNA was taken. The following cycling protocols were used: 95ºC for 5 minutes, followed by 30 cycles of 95ºC for 15 sec, 60 ºC for 30 sec per cycle and 72ºC for 15 sec with a final extension of 72 ºC for 10 min. Melting curve analysis was done finally[42].

3. Results and Discussions

3.1 Observations of physicochemical tests:

The Aloe vera based coatings applied for the extension of shelf life of mango cultivars (figure 1,2). Figure1 showed that the condition of the uncoated mangoes was not good and some of them were rotten. Figure 2 indicated better the coated mangoes.

Figure 1: Uncoated mangoes after 14 days experiment

Figure 2: Coated mangoes after 14 days of experiment

The Aloe vera coating retained the firmness of the mangoes and delay ripening. Firmness for the coated mangoes was found better than that for uncoated ones (figure 3). In uncoated fruits, firmness was observed to reduce from 7.8 to 3.6 N/mm on day 4 and then decreased in slow pace to 1.9 N/mm by day 6. In contrast, the parameter was decreased from 7.7 to 3.9 N/mm at day 10 and 19 N/mm at 14th day in the mangoes with Aloe-vera based coating.

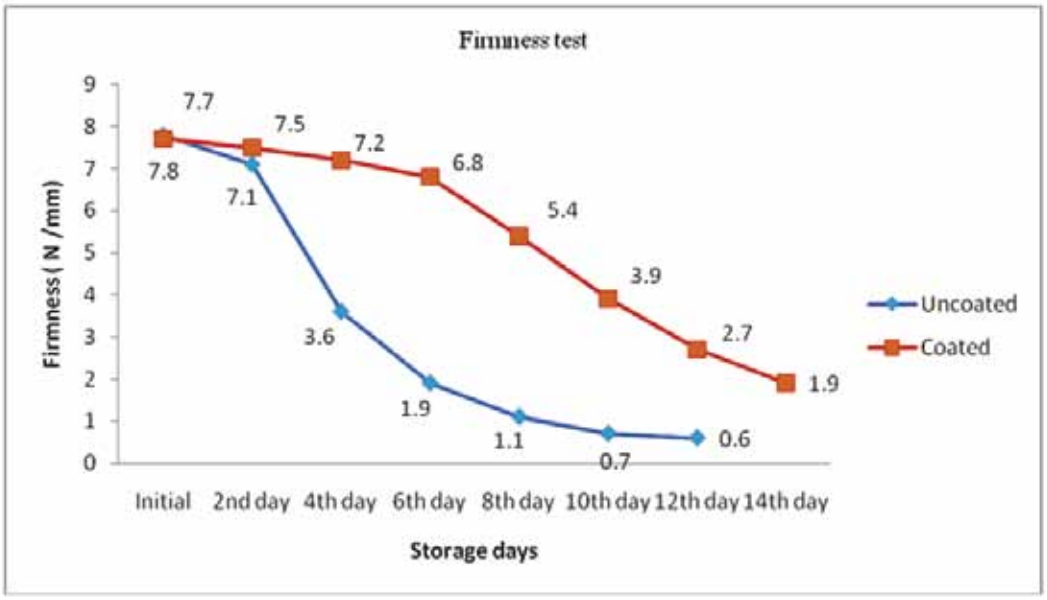

Figure 3: Firmness of mangoes during storage period

Figure 4 shows that the average weight loss in Aloe vera gel coated mangoes was significantly lower than that of uncoated ones. Table 1 showed whether the effect of coating is significantly affecting the average weight loss in mangoes. T test has been performed and all cultivars show that aloe vera based coating has significant effect on reducing average weight loss at 0.05 significance level.

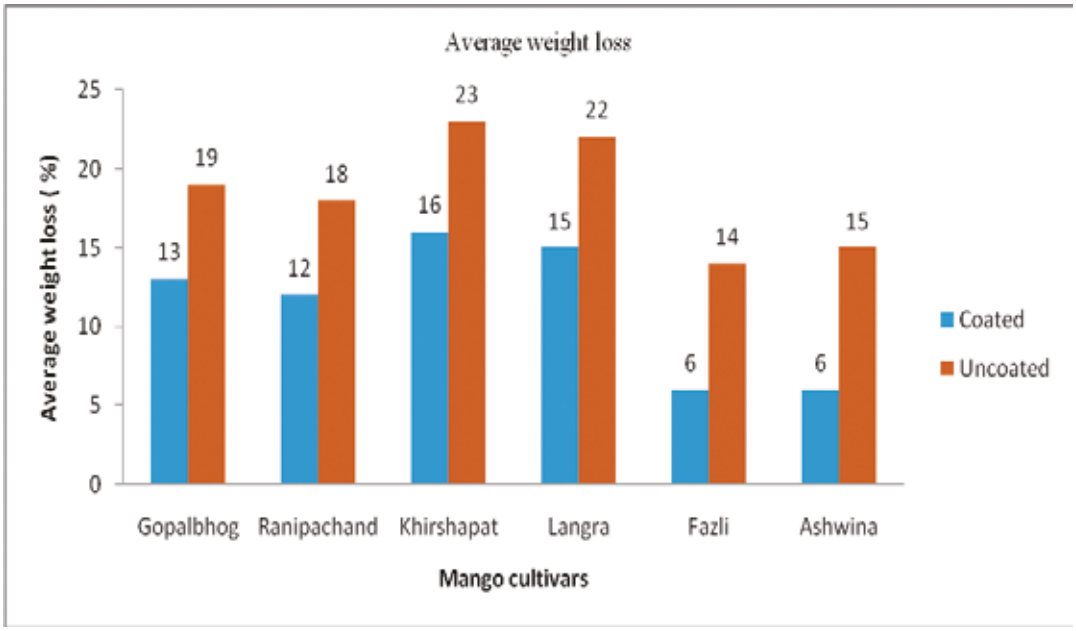

Figure 4 : Average weight loss of coated and uncoated mango during the storage period(14 days)

Table 1: Effect of Aloe vera gel coating on average weight loss of mangoes

Mango cultivars	Condition	Weight loss (%)	T test	P value	Result	Comment	Significance
Gopalbhog	Coated uncoated	13 ±1.16 19 ±1.72	T_{cal} (-11.201) belongs to critical or rejection region (T_{cr} value < -2.048 or >2.048)	0.0001	P value < 0.05	H_0 is rejected at 0.05	Significant decrease is observed in average weight loss (%) in coated mangoes
Ranipachand	Coated uncoated	12 ±1.62 18±1.40	T_{cal} (-10.853) belongs to critical or rejection region (T_{cr} value < -2.048 or >2.048)	0.0001	P value < 0.05	H_0 is rejected at 0.05 significance level	Significant decrease is observed in average weight loss (%) in coated mangoes
Khirsapat	Coated uncoated	16±1.06 23±1.50	T_{cal} (-14.760) belongs to critical or rejection region (T_{cr} value < -2.048 or >2.048)	0.0001	P value < 0.05	H_0 is rejected at 0.05 significance level	Significant decrease is observed in average weight loss (%) in coated mangoes
Langra	Coated uncoated	15± 1.13 22± 2.73	T_{cal} (-9.176) belongs to critical or rejection region (T_{cr} value < -2.048 or >2.048)	0.0001	P value < 0.05	H_0 is rejected at 0.05 significance level	Significant decrease is observed in average weight loss (%) in coated mangoes
Fazli	Coated uncoated	6 ± 0.88 14 ± 1.20	T_{cal} (-20.820) belongs to critical or rejection region (T_{cr} value < -2.048 or >2.048)	0.0001	P value < 0.05	H_0 is rejected at 0.05 significance level	Significant decrease is observed in average weight loss (%) in coated mangoes
Ashwina	Coated uncoated	6± 1.2 15±1.92	T_{cal} (-15.395) belongs to critical or rejection region (T_{cr} value < -2.048 or >2.048)	0.0001	P value < 0.05	H_0 is rejected at 0.05 significance level	Significant decrease is observed in average weight loss (%) in coated mangoes

Table 2 and 3 showed the nutritional quality of mango which was improved outstandingly after the application of Aloe vera coatings (after 14 days of experiment). Coated mangoes were found to have superior quality as the protein, reducing sugar, B carotene, vitamin C, total sugar, total soluble solids, non reducing sugar, carbohydrate, phosphorus etc contents of their pulp were better than those of uncoated ones.

Table 2: Physical parameters of mango pulp from uncoated and coated ripe mangoes during the storage

Mango cultivars	Condition	TSS %	pH	Moisture %	Dry matter %	Ash %	Acidity % as citric acid
Gopalbhog	Coated	12.50	4.32	79.37	20.63	0.607	0.421
	uncoated	18.00	5.40	78.13	21.87	0.694	0.033
Ranipachand	Coated	11.75	3.91	85.59	14.41	0.458	0.247
	uncoated	17.00	4.72	80.51	19.49	0.683	0.070
Khirsapat	Coated	14.50	4.25	83.09	16.91	0.419	0.220
	uncoated	20.50	4.90	77.32	22.68	0.335	0.075
Langra	Coated	13.25	4.57	85.21	14.79	0.529	0.423
	uncoated	16.00	5.26	81.47	18.53	0.791	0.068
Fazli	Coated	15.25	4.36	82.54	17.46	0.313	0.376
	uncoated	18.50	5.28	79.35	20.65	0.496	0.073
Ashwina	Coated	16.00	3.72	80.26	19.74	0.435	0.245
	uncoated	19.50	4.81	77.63	22.37	0.512	0.068

Table 3. Nutritional value of mango pulp from uncoated and coated ripe mango after 14 days of experiment

Mango cultivars	Condition	Sugar %	Reducing sugar %	NR sugar %	Protein %	Cho %	P %	Carotene µg /100g	Vitamin C mg/ 100g
.Gopalbhog	Coated	12.51	4.97	10.24	0.61	15.3	13.9	3721	15.81
	uncoated	11.03	3.62	07.41	0.60	13.2	11.0	3142	07.35
Ranipachand	Coated	13.65	4.35	9.30	0.54	12.6	17.2	3642	16.27
	uncoated	10.23	3.28	6.95	0.43	10.3	15.3	3262	15.02
Khirsapat	Coated	15.07	4.87	10.20	0.58	13.8	12.6	4260	13.09
	uncoated	12.53	4.21	8.32	0.41	11.2	10.3	4103	11.23
Langra	Coated	12.31	4.02	8.29	0.46	14.0	18.2	4452	17.21
	uncoated	11.63	3.65	7.98	0.38	11.4	15.9	4283	13.03
Fazli	Coated	15.23	4.76	10.47	0.82	15.7	22.4	4237	15.21
	uncoated	13.06	4.24	8.82	0.75	11.9	20.6	4065	11.33
Ashwina	Coated	14.03	4.26	9.77	0.63	13.6	16.3	3845	17.08
	uncoated	12.73	4.18	8.55	0.54	11.7	13.6	3278	12.55

NR: Cho: carbohydrate P: phosphoraous

The physical characters of coated and uncoated mangoes were compared by expert pannel on the basis of appearance, color, flavor, taste and texture. It can be concluded from their suggestion that the coated mangoes are quite superior to that of uncoated one (Table 4). Application of edible aloe vera based coatings on preservation of mangoes resulted in extended shelf life of the fruits, reduced average weight loss, microbial growth and acceptable in sensory characteristics.

Table 4. Quality grading of uncoated and coated of mango by expert panel

Qualities	Treatments	Scoring by expert panel (after 14 days)										Total score	Mean score	Rating
		01	02	03	04	05	06	07	08	09	10			
Appearance	Coated	84	81	86	85	82	78	85	83	72	86	822	82.2	Excellent
	uncoated	71	72	62	75	74	70	64	79	68	74	709	70.9	Good
Colour	Coated	85	79	80	81	78	85	84	80	80	83	815	81.5	Excellent
	uncoated	70	55	55	52	45	40	60	55	60	50	542	54.2	Fair
Flavour	Coated	85	79	82	86	75	78	85	75	75	81	801	80.1	Excellent
	uncoated	60	53	55	59	45	40	42	45	65	72	536	53.6	Fair
Taste	Coated	86	81	85	84	85	86	80	82	75	83	827	82.7	Excellent
	uncoated	65	56	52	66	75	76	84	86	75	82	717	71.7	Good
Texture	Coated	89	83	85	76	80	88	75	77	81	91	735	73.5	Excellent
	uncoated	55	61	71	80	84	78	69	73	75	69	715	71.5	Good

Coated: dipped in solution of aloe vera based coatings
Uncoated: dipped in water only,
Scoring value: excellent 80% or above, good 70-80% fair below 70%

Effect on gene expression

The Melt curve shows that the value of change in fluorescence level with respect to per unit change in temperature ? (dF/dT) is greater in uncoated mangoes (13) in comparison to coated mangoes (7.5). It means that the coated mangoes contained less amount of amplified COMT gene rather than the uncoated ones. This finding indicated that the aloe vera coating delayed the expression of COMT gene in mangoes[42,43].

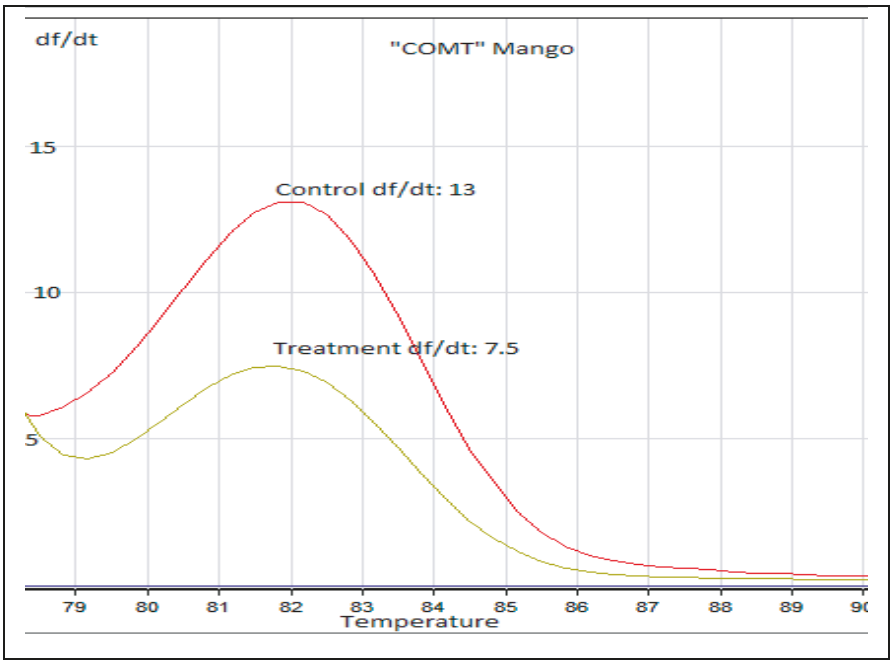

Figure 6: Comparative analysis of Melting Curve for COMT gene of Coated and control mangoes

Conclusion

Aloe vera coating can extend the shelf life of mango. This technique could help preservation of mango and other perishable fruits.

Acknowledgement

This research work was funded by Bangladesh Council of Scientific and Industrial Research (BCSIR), Bangladesh.

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