

A Study of Effectiveness of Natural Coatings on the Shelf Life Extension of Tomatoes by the Observation of TomloxC Gene Expression

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Tomato is a popular vegetable throughout the whole world. Use of harmful preservatives or high amount of preservatives can deteriorate the quality of food and cause harm. In this paper, we have studied the effect of natural natural coatings i.e. mixtures of Aloe vera gel, pectin and olive oil on the quality parameters and extension of shelf life of different tomato (Local, Bizli and Lovely) cultivars. TomloxC is a gene that increases in amount when the tomato ripens. We studied the expression of TomloxC gene and measured physical parameters and nutritional values of the coated and uncoated tomatoes. The expression of this gene is less in coated tomatoes than in uncoated tomatoes which indicated the efficiency of the coating. The physical parameters and nutritional characteristics were also found better in the coated ones. The shelf life of coated fruits increased to 4 to 6 days compared to that of uncoated ones. The average weight loss was 5-7% less in coated tomatoes than in uncoated one. So, the combined mixture of natural coating of Aloe vera gel, pectin and olive oil has high prospects to be used as a coating for vegetables.

Keywords: Aloe vera coating, Gene Expression, Pectin, Tomato, TomloxC, Physical parameters, nutritional value.

Tomato is very popular and widely consumed throughout the world. It can be eaten raw and cooked. They are used to prepare soup, juice ketchup, pickle, pasta and powder. Tomato is also popular because it is a promising source of vitamin C and adds variety of colors and flavors to the food.¹ Dried tomato juice is rich in vitamin C. It is also considered to be intestinal antiseptic, anti-

diabetic and also reduced heart disease. Depending on age groups, the requirement for vitamin C and vitamin A is about 30-40 mg/ 100mg and 2500-3000 mg /100mg thus daily consumption of 100g tomato, the requirement of vitamin C and vitamin A respectively will be met up. The perish ability of tomato is very high i.e. shelf life is very short. So extension of shelf life of tomato is required.

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The shelf life of any fruit consists of ripening and senescence.² After harvest, fruits undergo many physiological and biochemical changes. The shelf life of fruits could be prolonged significantly through slowing down the process leading to ripening and controlling the microbial decay.³⁻⁶ Information regarding the extension of storage life of tomato through the treatment of natural coatings in Bangladesh is very scanty. Artificial preservatives and chemicals are often very injurious to health in the long term. The present study has been undertaken to extend the storage life (Shelf life) and to reduce the spoilage of tomato caused by various factors without changing its quality. In this study, the aim is to observe the effect of natural coating of aloe vera, olive oil and pectin on delaying ripening of tomatoes and increasing the shelf life. Aloe vera has given good results in increasing shelf life of fruits previously⁷⁻¹⁰.

The ripening of fruits is influenced by auxins, ethylene, brassinosteroids and SBA. In climacteric fruits, including tomato, avocado, peach and apple, ripening is marked by a respiratory burst linked to the production of ethylene.^{11,12}

There are five lipoxygenases (TomloxA, TomloxB, TomloxC, TomloxD, and TomloxE) available in tomatoes (*Lycopersicon esculentum*). Lipoxygenases play vital role in disrupting chloroplast thylakoid membranes. This is required for the transformation from chloroplast to chromoplast during fruit ripening. The substrate for LOX is usually believed to be unesterified polyunsaturated fatty acids produced from complex lipids through the action of lipases.¹³ Chloroplasts have been suggested as the main site for hydroperoxide synthesis of fatty acids. Interruption of these thylakoid membranes may result in linoleic and linolenic acids. TomloxC is a primary lipoxygenase that primarily engages in the generation of short-chain flavor compounds derived from fatty acids C6 and is essential in the generation of fruit flavor and ripening properties.¹⁴ Experiments on the regulation of gene expression related to fruit shelf life and ripening is necessary to prove the efficiency of the preservatives by providing real time PCR data. TomloxC is a chloroplast-targeted lipoxygenase that is generated at significant concentrations during fruit maturation, and so it is clearly a worthy target.^{14,15}

Preservation of food, fruits and vegetables in a natural way is explored for a long time. The goal of this research is to evaluate the natural coatings efficiency in extension of shelf life of tomato and analysis of related gene expression by real time PCR.^{16,17}

MATERIALS AND METHODS

Freshly harvested Aloe vera leaves were collected from the local market for processing and preparation of Aloe vera gel finally used as natural coatings. The leaves should be sound, undamaged, mold/ rot free and matured in order to keep all the active ingredients in full concentration. The leaves were washed with water and removed unexpected organ and peeled. The pulp were collected and homogenized by blending and mixed and the mixture was filtered then it was heated at low temperature for two hours. Now the aloe vera gel was cooled and added to 3% pectin powder and 5% olive oil mixed with over head stirrer in 3000 rpm for two hours. Then the homogenized mixture was cooled and ready for use as natural coatings.^{17,18} Experimental samples were taken from sound ripe and disease free tomato (Local, bizli and lovely cultivars) from local market of Rajshahi and washed with distilled water and air dried.

Tomatoes were placed in 20 L container containing natural coatings for 10 min and exposed to different treatments. Control (uncoated) fruits were maintained in identical condition without treatment at ambient temperature. Tomatoes were air dried and stored for observation at an ambient temperature (28-30°C) and relative humidity (70-72%). For each parameter, three sets of independent experiments were carried out and fifteen Tomatoes were employed for each set of experiment and analytical work. Starting with day 0 (immediately after treatment) till day last edible stage (15 days), one tomato from each of the coated and uncoated were taken, analysis were performed. The experimental samples were taken from the uncoated and coated samples to analyze physical and chemical characteristics at two days intervals.

The sugar content was determined by spectrophotometrically by the Lane and Eynon method¹⁹, and the reducing sugar was estimated by the DNSA method using 3, 5-Dinitrosalicylic acid (DNSA)²⁰. Vitamin C was also estimated

by spectrophotometric method. 5 ml of 1gm/ml sample was prepared and centrifuged at 5000 rpm. Then 0.5 ml extract was taken and 0.8 ml 10% Trichloroacetic acid was added to it and frozen for 5 minutes. Then it was centrifuged again. The supernatant was taken. 3 ml dH₂O and 0.4 ml 10% Folin Ciocalteu's Reagent (FCR) solution was added. Then the absorbance was measured at 760 nm.²¹

TSS was determined by refractometric method and pH was determined by a standard pH meter. Acidity was estimated by acid-base titrimetric method using standard sodium hydroxide solution.²² The preserved tomato was analyzed periodically and the results were recorded in tables (Table 1 and 2).

The related gene expression of the uncoated and coated samples was done at the last edible stage; RNA was isolated from ripe tomato pulp tissue using Promega RNA isolation kit as per instructions provided therein. After RNA

extraction, quantification of RNA concentration was done by a micro spectrophotometer K2800 nucleic acid analyzer, Beijing Kaiiao Technology Development Co. Ltd; (China). Sample purity was evaluated by calculating absorbance ratio (A230:A280). The extracted RNA of the samples were equalized to 50 ng/ul to be used as template in reverse transcription PCR and then for real time PCR reaction by diluting with calculated volume of DNase / RNase free H₂O Amresco,(USA).^{23,24}

For amplification of tomato lipoxigenase(TomloxC), Forward primer: CACATTGGAGATAAATGCCTTAGC and reverse primer: CAGTTGTTGGCCTATTTGGAAAG were used (GenBank accession number: U37839).²⁵

TomloxC amount was measured by RT-PCR in 2 different conditions coated and uncoated). The following cycling protocols were used : 95°C for 5 minutes , followed by 30 cycles of 95°C for 15 sec, 60°C for 30 sec per cycle and 72°C for 15 sec with a final extension of 72°C for 10 min. Melt curve



Fig. 1. Uncoated Tomato at the initial stage (a) Tomato treated with natural coatings at the initial stage (b). Uncoated Tomato at the last edible stage (c). Tomato treated with natural coatings at the last edible stage (d)

analysis is done by instrument's default parameters. Real-Time PCR analysis was carried out in a Rotor Gene Q (QIAGEN) with 20 μ l reaction volume consisting of 4 μ l of master mix, 2.5 μ l of primer and probe mix, 11 μ l of ddH₂O and 2.5 μ l of DNA template containing 125 ng of total DNA. Required dilution was performed using DNase/ RNase free water.²⁶

RESULTS AND DISCUSSIONS

The natural coatings applied for the extension of shelf life of tomato cultivars extended the shelf life 4 to 6 days compared to uncoated one as shown in Figure 1 and Figure 2. The weight loss of the coated tomato was affected (Figure 3) showed that the weight loss in coated tomato

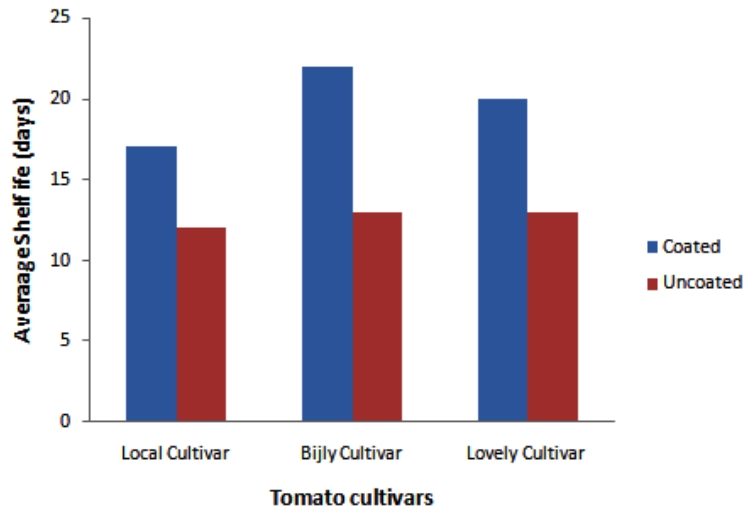


Fig. 2. Average shelf life of coated and uncoated tomato cultivars

Table 1. The Physical parameters of tomato pulp from uncoated and coated ripe sample tomatoes at the last edible stage during the storage period

Name of tomato cultivars	Treatment	Juice %	TSS %	pH	Dry mater %	Moisture %	Ash %	Acidity % as citric acid
Local	Coated	77.0	6.5	4.48	4.79	95.21	0.46	0.095
	uncoated	74.5	6.0	4.59	5.53	94.47	0.51	0.072
Bizli	Coated	75.0	6.5	4.47	5.03	94.97	0.34	0.103
	uncoated	72.5	6.0	4.53	5.04	94.96	0.50	0.043
Lovely.	Coated	70.0	7.0	4.02	6.09	93.91	0.52	0.163
	uncoated	75.0	6.5	4.22	6.40	93.60	0.55	0.127

Table 2. The nutritional value of tomato pulp from uncoated and coated ripe tomato at the last edible stage during the storage period

Name of tomato cultivars	Treatments	Total sugar %	Reducing sugar %	Non reducing sugar %	Protein %	B- carotene mg/100g	Phosphorous %	Vitamin C mg/ 100g	Carbohydrate %
Local	Coated	3.63	1.38	2.25	0.62	341	26.03	10.87	4.76
	uncoated	3.47	1.33	2.14	0.49	273	23.60	09.33	4.53
Bizli	Coated	4.38	1.42	2.96	0.58	380	19.35	13.60	5.42
	uncoated	3.95	1.26	2.69	0.51	326	18.09	18.43	4.91
Lovely	Coated	5.03	1.86	3.17	0.63	351	21.85	9.70	6.23
	uncoated	4.86	1.78	3.08	0.46	302	19.72	6.96	5.84

was significantly lower than that of uncoated Tomatoes. So, the natural coating was also found to have beneficial effects on firmness retention and delaying fruit softening

Table 1 and 2 showed the nutritional quality of tomato that was also affected remarkably after the treatment with natural coatings at the last edible stage.

Tomatoes from the coating treated (Coated) might have superior quality as the protein, *B* carotene, vitamin C, total sugar, reducing sugar, non reducing sugar, total soluble solids, carbohydrate, phosphorus etc content of its pulp were higher than those of uncoated Tomatoes. The amount of moisture, Total sugar, Reducing sugar,

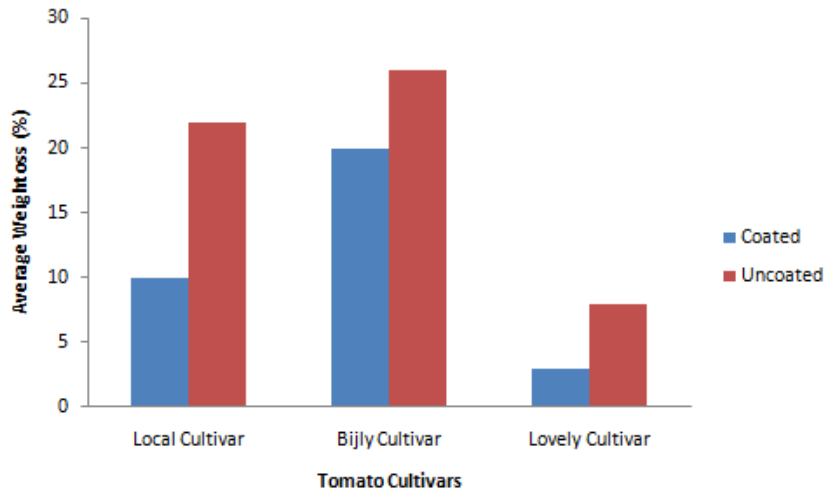


Fig. 3. Average weight loss (%) of coated and uncoated tomato cultivars

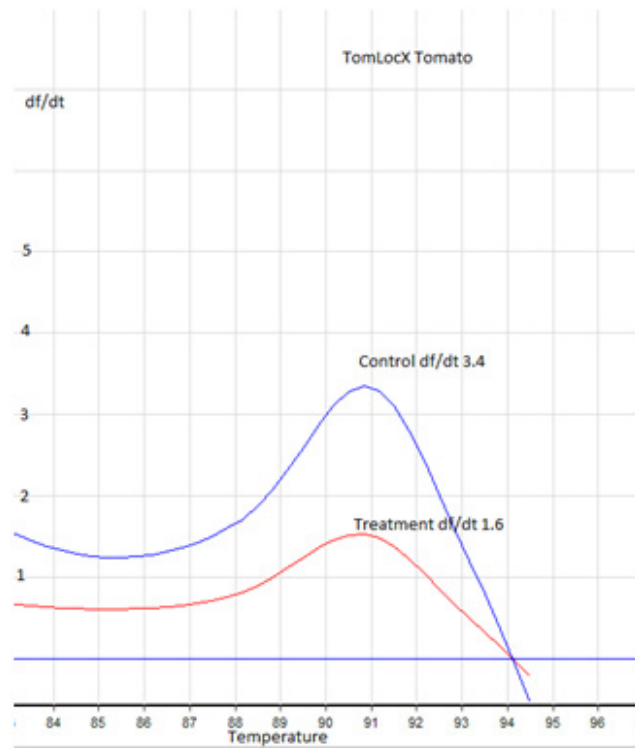


Fig. 4. Comparative Analysis data for TomloxC gene of Tomato

non reducing ,carbohydrate, phosphorus, vitamin C ,protein, *B*-carotene were higher while the amount of dry matter, ash, pH were lower as compared to the amount of those present in the pulp of uncoated tomato. Application of edible natural coatings on preservation of tomato resulted in extended shelf life, reduced average weight loss, and acceptable improvement in sensory characteristics.

Figure 4 indicates that the expression of TomloxC is less in treated tomatoes as compared to the control ones. So, it can be said that the coating has vital role in delaying ripening.

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 tomatoes (3.4) in comparison to coated tomatoes(1.6). It means that the coated tomatoes contained less amount of amplified TomloxC gene in comparison to coated ones. Previous studies show that TomloxC gene is expressed in higher amount in riper tomatoes. So, the findings indicate that the aloe vera gel coating has delayed the ripening of tomatoes.²⁸

The shelf life of coated fruits increased to 4 to 6 days compared to that of uncoated (control) ones. The average weight loss was 5-7% less in coated tomatoes than in uncoated one. So, it can be concluded that application of the pectin-based coating on tomatoes was effective in reducing the associated physiological changes and extending the storage life.

CONCLUSION

Nowadays, various toxic chemicals are often used to preserve fruits and vegetables or extend the shelf-life. Findings from the current work are effective for solving this issue. Usage of edible natural coatings on tomato resulted in increased shelf life, decreased average weight loss and reasonable sensory enhancement. Thus, it can be concluded that the application of aloe vera based natural coating has great potential in reducing the ripening-related physiological changes and prolonging the shelf life of tomatoes and other fruits and vegetables. The natural coatings might be used to eradicate the use of hazardous chemicals in order to conserve the nation and climate.

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