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ORIGINAL RESEARCH PAPER

EVALUATION OF NEEM LEAF EXTRACT AND ARABIC GUM AS AN EDIBLE COATTING ON THE SHELF-LIFE OF TOMATO FRUITS

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Abstract: Tomato (*Solanum lycopersicum* L.) is one of the most important crops in the world, covering an area of 5,167,388 hectares and producing 189,133,955.03 tons/year. Tomato postharvest losses can reach 30-40 % globally, but they are significantly greater in developing nations due to the lack of measures to stop decay brought on by fungi. Fruit coatings can reduce postharvest losses and play a critical role in extending fruit's shelf life. Evalua tion of neem leaf extract with or without Arabic gum as edible coating on shelf life of tomato fruits was performed. Tomato fruits were randomly distributed among four immersion treatments: 1st in distilled water only, 2nd in neem leaf extract only, 3rd in Arabic gum solution only and 4th in neem leaf extract followed by drying and reimmersion in Arabic gum solution. Arabic gum-coating treatments and those that combined it with neem leaf extract during storage resulted in improved weight loss, total soluble solids, titratable acidity, and colour parameters for tomatoes. The treatments containing neem leaf extract alone reduced the total number of plaques by about half by the end of the storage period. The coating treatment with a mixture of Arabic gum with neem leaf extract was the best treatment as it proved effective in maintaining the quality characteristics and increasing the shelf life of tomatoes.

***Keywords*:** *Arabic gum, edible coating, neem, shelf-life, tomato*

INTRODUCTION

The tomato (*Solanum lycopersicum* L.) one of the most important crops in the world, a member of the solanaceae family, covers 5,167,388 hectares and produce 189,133,955.03 metric tons [1]. Tomatoes are soft fruits due to their high moisture content, which makes them vulnerable to attack by microorganisms, as well as their high respiration rate, which reduces their quality and shelf life. Fresh tomatoes have 75 - 95 % moisture ones because they are living beings that continue to breathe and create heat and consumes starch or sugar that has been stored [2 – 3]. This is a topic of great concern in agricultural. In poorer nations, the incorrect storage of the fruit leads to losses of 20 - 50 % [4]. Due to their softness, tomatoes suffer a large percentage of damage during shipping and preservation due to mechanical injuries, which makes them vulnerable to attack by microorganisms, so they must be protected.

Bio-preservation using natural antibacterial compounds is a modern was to preserve fresh fruits, as it works to extend their shelf life [5]. In the past, chemical preservatives were used as antimicrobial agents to store food for a long period to satisfy consumers' needs, but due to their toxicity and health risks, consumers today have a lack of interest in this method [6]. When the surface of intact fruits is covered with a layer of edible coatings, a thin film is formed. It creates a modified atmosphere around them and acts as a barrier to prevent the escape of oxygen (O_2), carbon dioxide (CO_2), water vapor and aroma compounds during storage, which reduces their respiration rate and thus reduces waste production, retains water and flavour compounds and maintains their soft texture, which leads to a longer storage period with the highest possible quality [7, 8]. The coatings are also used to carry compounds such as antioxidants and antimicrobials [9]. Arabic gum, which is the dried resinous material secreted from the stems and branches of acacia trees, can be used as edible coating. It is one of the less viscous and more soluble hydrocolloids [10]. It is widely used in the industrial sector due to its emulsification, film formation and encapsulation capabilities [11]. It consists of a complex mixture of macromolecules of different size and composition, with a low percentage of proteins (3 %), and a large amount of carbohydrates (97 %) consisting mostly of D-galactose and L-arabinose units [12]. Neem (*Azadirctica indica*) leaf extract oil contains nimbidin, nimide and azadirachtin compounds that have antibacterial properties against pathogenic bacteria such as *Salmonella*, *Staphylococcus*, *Escherichia coli*, *Vibrio* etc. [13, 14]. Neem oil can be used as an edible and biodegradable coating to extend the shelf life of fruits [15]. The shelf life of apple fruits has been extended by coating the fruits with neem leaf extract oil, which has antifungal properties [16].

This study aims to extend the shelf-life of tomato fruits, by using Arabic gum fortified with neem leaf extract as an edible coating, as a partial barrier for gases such as oxygen (O_2), carbon dioxide (CO_2), water vapor and odour compounds from exiting, and hindering the growth of microorganisms.

MATERIALS AND METHODS

Materials

120 tomatoes fruits (*Solanum lycopersicum* L.) healthy, physically undamaged, and uniform-sized were harvested randomly of the black creamy variety were manually picked at the physiological maturity stage from farm in Irbid, Jordan. The fruits were selected based on their similar size, level of ripeness, and lack of outward signs of disease or defects. The fruits were then shifted to laboratory Arabic gum was obtained from Irbid local market, Jordan. Fresh leaves of Neem (*Azadirachta indica*) were collected from different locations at Irbid metropolis, Jordan the leaves were transported to the lab in pristine polythene bags. All raw materials obtained were temporarily stored at 4 °C then analyses were done on them the day after.

Methods

Preparation of neem extract
To eliminate surface dirt in the lab, the leaves were first thoroughly prewashed for one to two minutes under a mild stream of tap water. The next step was a 30-seconds washing in sterile distilled water that contained 1 % sodium hypochlorite. After that, the leaves were taken out and cleaned three times in sterile distilled water. Neem leaves extract was prepared 100 % (w/v) by weighing 100 g of neem leaves. The leaves were crushed using a mortar and pestle, transferred into 100 of sterile distilled water and allowed to soak for 1 to 2 hours after which sieving was done using a muslin cloth into separate beaker.

Preparation of Arabic gum solution
A 15 % (w/v) solution of Arabic gum powder was prepared in distilled water at 40 °C for 15 minutes. After that, the solutions were allowed to cool at 20 °C.

Application of the coating treatments
Tomato fruits were graded, cleaned with distilled water, and dried at room temperature. They were randomly distributed among four treatments: 1^{st} in distilled water only, 2^{nd} in neem leaf extract only, 3^{rd} in Arabic gum solution only and 4^{th} in neem leaf extract followed by drying and reimmersion in Arabic gum solution. The treatments were applied in triplicate on 10 fruits samples. Each replicate group of fruits was dipped for 1 h until the coating solution covered the surface uniformly. Then the fruits were air-dried on trays, packed in cardboard boxes and stored in a clean room under conditions in a controlled setting 20 °C and relative humidity (RH) 85 %, until signs of degradation were observed.

Estimate of losing weight
Before the beginning of the storage, three fruits from each sample were labelled and weighed separately. For the duration of the experiment, up until the tomatoes showed signs of degradation, fruits were afterwards weighed at each sample interval date to follow their weight changes. In order to calculate the overall weight changes, weight measurements were made, and their averages were calculated. The following equation (1) was used to compute the percentage weight change or loss:

$$W_l = \frac{W_f - W_i}{W_i} \times 100 \quad (1)$$

Where:
W_l - weight loss, [%]
W_i, W_f - initial and final weights respectively, [g]

Physico-chemical analyses

Determination of the total soluble solids (°Brix)

Three tomato fruits were chosen at random from each replicate that had undergone the various treatments, then they were homogenized using an electric blender. Physio-chemical parameters were then examined in the mixed solutions. Initial assessments were made on day 0 and then every five days.

Homogenized sample solutions were made by mixing tomato flesh from three different samples treated with different treatments and using a digital refractometer (range 0 - 32 %), the total soluble solids (Brix) were measured. On the refractometer's prism-plate, a drop of the particular solution was applied [17]. Total soluble solids (Brix) were directly recorded from the measurement obtained after setting the refractometer to the target [18]. The refractometer was calibrated anew using distilled water before use for the following sample after performing this procedure on three replicated sample solutions.

Estimation of titratable acidity

A pipette was used to transfer 10 mL of the various prepared tomatoes sample solutions into a conical flask measuring 250 mL. The flask was filled with distilled water in an equivalent volume. The solution was then mixed after the addition of the phenolphthalein indicator (3-4 drops). The contents were quickly titrated with 0.1 N NaOH solution, and the end point was established when a clear colour shift occurred [11]. The final burette reading was then recorded, and titratable acidity (expressed as citric acid) was calculated using the equation (2) [19]:

$$\%acid = \frac{[mL\ NaOH\ used \times Millieequivalent\ factor (0.064) \times 100]}{grams\ of\ sample} \quad (2)$$

Total plate count

After 20 days of storage, coated and uncoated tomato samples underwent microbiological examination using the pour plate method with plate count agar (PCA) (Merck, Darmstadt, Germany) as the medium. The plates were incubated for two days at 35 °C. The results of the microbiological analysis were reported as log 10 colony forming units per grammes (log 10 CFU·g^{-1}), which was done in triplicate [20].

Colour estimation

The Hunter colorimeter was used to measure the tomato's colour (Colour Quest XE, USA). The formula shown below was used to compute the chroma value (ΔC) using the average values of L*, a*, and b* as equation (3) [21]. L* denotes the intensity of light and darkness (from white to black), while a* denotes the variation in red and green colour and b* inculcate about yellow and blue colour [22].

$$\Delta C = \sqrt{(L^*)^2 + (a^*)^2 + (b^*)^2} \quad (3)$$

Statistical analysis

Randomised block was the experimental design used in the study. Data were subjected to statistical analysis using done SPSS program (IBM SPSS Statistics v.25.0) [23] to find the variation. ANOVA was applied to analyses the data, and Duncan's multiple range test was used to determine differences that were statistically significant ($p \leq 0.05$).

RESULTS AND DISCUSSION

Impact of several coating treatments on tomato weight loss

Table 1 shows the effect of edible coating on the percentage of loss in tomato fruits during storage. The results indicate an increase in the percentage of loss in fruit weight with an increase in the storage period in all treatments. However, this percentage differed from one treatment to another according to the type of edible coating used, as it was noted that the best treatments are the use of coating a mixture of Arabic gum with neem leaf extract, followed by coating Arabic gum alone. While it was found that the use of neem leaf extract as a coating alone had no significant effect on the percentage of loss compared to the control treatment.

Weight loss may be due to the effect of Arabic gum coating in hindering the evaporation of moisture from inside the fruit, and thus its effect on the percentage of weight loss of the fruit, as well as its shine due to the shrinkage of the outer shell cells.

***Table 1**. Impact of several coating treatments on tomato weight loss while being stored at room temperature (%)*

Treatments	**Storage time [day]**			
	5	**10**	**15**	**20**
Control	5.24 ± 0.06^{Ca}	9.59 ± 0.04^{Cb}	14.03 ± 0.05^{Cc}	18.58 ± 0.11^{Cd}
Neem	5.21 ± 0.01^{Ca}	9.35 ± 0.7^{Cb}	13.99 ± 0.12^{Cc}	18.62 ± 0.03^{Cd}
Gum	4.25 ± 0.04^{Ba}	6.12 ± 0.15^{Bb}	9.67 ± 0.23^{Bc}	13.30 ± 0.09^{Bd}
Gum + Neem	3.53 ± 0.08^{Aa}	4.04 ± 0.07^{Ab}	7.17 ± 0.12^{Ac}	11.45 ± 0.07^{Ad}

Capital letters indicate to significant difference between treatments in the same column; small letters indicate to significant difference between storage times in the same row ($p < 0.05$).

These results are consistent with what was stated by [24] that increasing the coating layer around the fruit surface led to a decrease in the phenomenon of fruit transpiration causing loss of its volume, as this was evaluated by measuring the difference between the water vapor pressure of the fruit and the water vapor pressure of the surrounding air. The cuticle layer and epidermal cell layer are beneficial for reducing transpiration [25], while fruit coating reduced transpiration because it totally or partially covered stomata, lenticels, and micropores on the fruit's surface, creating a semipermeable barrier to gas exchange and eventually causing transpiration to drop [3].

Impact of several coating treatments on tomato total soluble solids

Table 2 shows that the total dissolved solids (TSS) in the coated and uncoated tomato samples gradually increased with increasing storage period up to 20 days. On the other hand, it was found that uncoated tomatoes and those coated with neem leaf extract alone contained a high percentage of TSS. While increase in TSS was much lower in tomatoes coated with a mixture of gum Arabic and neem leaf extract, as well as tomatoes coated with Arabic gum alone.

TSS may be due to the reduction of ethylene production and slow respiration due to coating tomatoes with Arabic gum, which leads to a prolongation of the metabolism process, which reduces TSS, and the coating forms an excellent semi-permeable barrier surrounding the fruit, which changes its internal environment by reducing oxygen and increasing carbon dioxide production.

Table 2. *Impact of several coating treatments on tomato total soluble solids while being stored at room temperature (°Brix)*

Treatments	Storage time [day]				
	0	5	10	15	20
Control	4.12 ± 0.04^{Ae}	4.30 ± 0.07^{Ad}	4.45 ± 0.03^{Ac}	4.61 ± 0.06^{Ab}	4.73 ± 0.02^{Aa}
Neem	4.11 ± 0.06^{Ae}	4.28 ± 0.05^{Ad}	4.43 ± 0.02^{Ac}	4.62 ± 0.04^{Ab}	4.71 ± 0.07^{Aa}
Gum	4.13 ± 0.04^{Ae}	4.17 ± 0.07^{Bd}	4.25 ± 0.04^{Bc}	4.34 ± 0.08^{Bb}	4.42 ± 0.05^{Ba}
Gum + Neem	4.12 ± 0.03^{Ae}	4.16 ± 0.01^{Cd}	4.19 ± 0.02^{Cc}	4.26 ± 0.06^{Cb}	4.32 ± 0.07^{Ca}

Capital letters indicate to significant difference between treatments in the same column; small letters indicate to significant difference between storage times in the same row ($p < 0.05$).

These results are consistent with the results obtained by [26] in their study of the effect of coating with whey protein, xanthan gum, and clove oil on prolonging the shelf life of tomatoes.

Impact of several coating treatments on tomato titratable acidity

Titratable acidity is a metric used to determine how much acid is present in a solution and is recognized as a good indicator of fruit development. Table 3 shows that the titrated acidity of all treatments decreased with increasing storage time. The uncoated treatment (control treatment) and the treatment coated with neem leaf extract alone showed a significant decrease in titrated acidity higher than the treatments coated with Arabic gum alone or mixed with neem leaf extract.

Table 3. *Shows the impact of several coating treatments on tomato titratable acidity while being stored at room temperature (%)*

Treatments	Storage time [day]				
	0	5	10	15	20
Control	0.598 ± 0.001^{Aa}	0.500 ± 0.003^{Ab}	0.484 ± 0.005^{Ac}	0.475 ± 0.003^{Ad}	0.460 ± 0.003^{Ae}
Neem	0.596 ± 0.002^{Aa}	0.498 ± 0.004^{Ab}	0.485 ± 0.003^{Ac}	0.474 ± 0.002^{Ad}	0.462 ± 0.002^{Ae}
Gum	0.593 ± 0.004^{Aa}	0.462 ± 0.001^{Bb}	0.432 ± 0.002^{Bc}	0.421 ± 0.004^{Bd}	0.412 ± 0.003^{Be}
Gum + Neem	0.597 ± 0.003^{Aa}	0.440 ± 0.002^{Cb}	0.421 ± 0.00^{Cc}	0.402 ± 0.002^{Cd}	0.386 ± 0.002^{Ce}

Capital letters indicate to significant difference between treatments in the same column; small letters indicate to significant difference between storage times in the same row ($p < 0.05$).

Citric acid, which regulates fruit respiration and lessens acidity in strongly respiring fruits, may be to blame for these titratable acidity decreases with increasing storage time. In essence, the Arabic gum coating slows the rate of transpiration, which lowers the consumption of organic acids and, as a result, lowers the titrated acidity. These results are consistent with those obtained by [27].

Impact of several coating treatments on tomato total plate count

The results shown in Table 4 that the total plate count increased with the increase in the storage period of tomatoes in all treatments. The results also confirmed the presence of significant differences between the treatments, where it was found that the best (low of total plate count) treatments were the treatment that had a coating of neem leaf extract alone and the treatment that contained a mixture of Arabic gum with neem leaf extract. While the treatment containing Arabic gum alone showed plate count more. The control treatment had the most treatments on the plate count.

Table 4. *Shows the impact of several coating treatments on tomato total plate count while being stored at room temperature (log 10 CFU/g)*

Treatments	Storage time [day]				
	0	5	10	15	20
Control	1.97 ± 0.11^{Ae}	4.22 ± 0.10^{Ad}	5.87 ± 0.11^{Ac}	7.89 ± 0.13^{Ab}	9.33 ± 0.11^{Aa}
Neem	1.98 ± 0.12^{Ae}	2.71 ± 0.16^{Cd}	3.24 ± 0.13^{Cc}	4.35 ± 0.16^{Cb}	5.04 ± 0.13^{Ca}
Gum	1.97 ± 0.14^{Ae}	3.82 ± 0.14^{Bd}	4.81 ± 0.15^{Bc}	5.73 ± 0.14^{Bb}	6.26 ± 0.12^{Ba}
Gum + Neem	1.99 ± 0.15^{Ae}	2.70 ± 0.12^{Cd}	3.21 ± 0.14^{Cc}	4.31 ± 0.12^{Cb}	5.22 ± 0.15^{Ca}

Capital letters indicate to significant difference between treatments in the same column; small letters indicate to significant difference between storage times in the same row ($p < 0.05$).

Treatments containing neem leaf extract reduced the total number of platelets, which may be due to the presence of Nimbidin, Nimoid and Azadirachtin, which are active ingredients against the growth of microorganisms. While the coatings containing Arabic gum led to the formation of a thin film on the tomato fruit, which works to prevent the entry of microorganisms into the fruit, in addition to providing an anaerobic environment that prevents or hinders its growth. These findings concur with those of [13 – 28] who showed a smaller rise in the number of total plates in blueberries after covering them with chitosan and aloe vera liquid fraction.

Impact of several coating treatments on tomato L*, a*, b* colour values

Colour values is the first and most important characteristic to judge the quality a consumer perceives in a tomato, and is often seen as an indicator of other attributes such as flavour and taste… etc. It is also a good indicator of the ripeness of the fruit. L*, a*, b*, hue, and chroma values provide an excellent illustration of the differences in colour between coated and uncoated tomatoes Table 5. The value of L* and b* in all treatments decreased to the 20th day of the storage life, while the a* values were exactly the opposite. By comparing the values of L*, a*, b* between all treatments at the end of the storage period, it was found that the best treatments were the coating of Arabic gum with the neem leaves extract and the coating of Arabic gum alone, as there were moral differences between these treatments and control treatment. While treatment the coating of alone neem leaf extract showed non -moral differences with control treatment. Results of tomatoes colour indicate the ability to the coating of Arabic gum mix with the neem leave extract, as well as the coating of Arabic gum alone, to maintain the brighter and colour of tomatoes for the longest possible period during storage.

The higher L* values in results may be due to the ability of the tomato polish to prevent the evaporation process and thus not wrinkle and become shinier. While the lower b* values are probably due to the genesis of the carotenoid pigments. Also, the lower values of a* may be due to better of coatings retention to the colour. Similar outcomes were found by [26] for edible tomato coverings based on whey protein isolate, xanthan gum and clove oil.

Table 5. *Shows the impact of several coating treatments on tomato L*, a*, b* colour values while being stored at room temperature*

Treatments	Colour values	Storage time [day]				
		0	5	10	15	20
Control	*L**	52.26^{Aa}	41.48^{Bb}	37.13^{Bc}	32.45^{Bd}	30.23^{Be}
	*a**	21.72^{Ae}	24.09^{Bd}	26.62^{Ac}	28.03^{Bb}	27.12^{Ba}
	*b**	23.35^{Aa}	20.87^{Bb}	18.95^{Bc}	16.72^{Bd}	14.26^{Be}
	ΔC		11.31^{Ad}	16.50^{Ac}	21.82^{Ab}	24.43^{Aa}
Neem	*L**	52.63^{Aa}	$41,35^{Bb}$	37.12^{Bc}	32.25^{Bd}	30.16^{Be}
	*a**	21.22^{Ae}	24.12^{Bd}	26.42^{Bc}	28.36^{Bb}	27.93^{Ba}
	*b**	23.24^{Aa}	20.93^{Bb}	18.41^{Bc}	16.21^{Bd}	14.44^{Be}
	ΔC		11.87^{Ad}	17.05^{Ac}	22.71^{Ab}	25.04^{Aa}
Gum	*L**	52.92^{Aa}	50.64^{Ab}	48.33^{Ac}	46.14^{Ad}	44.03^{Ae}
	*a**	21.62^{Ae}	29.42^{Ad}	31.53^{Ac}	33.39^{Ab}	35.12^{Aa}
	*b**	23.61^{Aa}	22.15^{Ab}	21.92^{Ac}	20.01^{Ad}	18.09^{Ae}
	ΔC		8.83^{Bd}	11.05^{Bc}	14.05^{Bb}	17.08^{Aa}
Gum + Neem	*L**	52.14^{Aa}	50.42^{Ab}	48.92^{Ac}	46.91^{Ad}	44.92^{Ae}
	*a**	21.51^{Ae}	28.32^{Ad}	32.12^{Ac}	33.82^{Ab}	35.13^{Aa}
	*b**	23.22^{Aa}	22.73^{Ab}	21.82^{Ac}	20.94^{Ad}	18.57^{Ae}
	ΔC		7.03^{Bd}	11.17^{Bc}	13.56^{Bb}	16.11^{Aa}

Capital letters indicate to significant difference between treatments of the same colour values in the same column; small letters indicate to significant difference between storage times in the same row ($p < 0.05$).

CONCLUSIONS

The results showed that coating tomato fruits with gum Arabic alone, or mixed with neem leaf extract, as edible coatings, was effective in extending the shelf life of tomatoes and maintaining their quality during storage for 20 days at a temperature of 20 °C and 85 % relative humidity. The results also showed a slight loss in fruit weight, total soluble solids content, titratable acidity and colour parameters, while treatments containing neem leaf extract reduced the total platelet count by almost half by the end of the storage period. The results showed that the treatment of coating the Arabic gum mixture with neem leaf extract was the best treatment, as it proved effective in maintaining the qualitative characteristics and increasing the shelf life of tomatoes.

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