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# Saussurea costus phenolic extract as a novel material with gum arabic and chitosan for enhancement of shelf life and tomato quality during the cold storage process

**Mohamed El-Kholy**
Agricultural Research Center
**Reham Kamel**
Agricultural Research Center
**Mohamed Abdin**
Agricultural Research Center
**Enas El fadly**
Kafrelsheikh University
**Mohamed Abdelbaset Salama** ( mohamedabdelbastsalama@gmail.com )
Agricultural Research Center
**Mohamed Saleh**
Agricultural Research Center

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# Abstract

In this study, aqueous solutions of gum arabic (G) and chitosan (CH) with Saussurea costus extract (SCE) were used as a novel edible coating for tomato surface during the storage process at 4°C. The stored tomatoes were evaluated in terms of physicochemical quality and antioxidant properties during storage (15 days). Coating revealed considerable variations between fruits which appeared in delaying ripening factors that were characterized by a reduction in weight loss (WL) and changes in total soluble solids (TSS), titratable acidity (TA), ascorbic acid content (AAC), and firmness (FM) compared to uncoated fruits. Also, the total phenolic content (TPC) of coated samples was higher than the uncoated ones during the storage period. Using SCE in the edible coating of tomatoes showed a significant delay in WL, TPC, TA, TSS and AAC compared to uncoated tomatoes during storage period at 4°C.


# Introduction

Recently, many studies have utilized edible coatings on fruit and vegetables as new technology. These are considered an environmentally friendly way to make the fruit last longer by reducing moisture loss and respiration rates, and making the fruit look better (Ncama et al., 2018).

A thin covering of components used to enhance the shelf life of food is known as an edible coating (Salama et al., 2019). In addition to keeping things fresh, edible coatings can be made with many ingredients, such as antioxidants and flavours, to make active packaging (Rambabu et al., 2019). In manufacturing edible coatings, polysaccharides, proteins, lipids, and their composites are commonly employed as biodegradable polymers (Salama and Aziz, 2020). Tomato (*Solanum lycopersicum* L.) is a versatile climacteric fruit, consumed both in raw and processed form. It is an essential component in sauces, puree, ketchup, etc. and in many traditional dishes. Essential amino acids, monounsaturated fatty acids (primarily oleic acid), and minerals abound in tomato (Fe, Mn, Zn, and Cu), making it an excellent food source. It also includes significant concentrations of beneficial substances like antioxidants (tocopherols, lycopene and ascorbic acid), folate, and flavonoids. Due to the growing demand for fresh tomatoes, it is important to keep them fresh and make them last longer so that they can be shipped long distances. Despite this, tomatoes have a shorter post-harvest life since they are climacteric. Several biochemical reactions such as ethylene production and high rate of respiration accelerate the ripening process that not only affects the flavor and nutritional value but also results in the rapid spoilage of fruit (Fagundes et al., 2014). Fresh tomatoes' market value is influenced by many quality variables, including their colour, taste, texture, and appearance (Liu et al., 2009). In tropical and subtropical areas where the temperature is high, climacteric fruits have a relatively high respiration rate, which makes them ripen faster and lose quality, which shortens their shelf life after being picked (Bailén et al., 2006). Tomatoes' shelf life can be prolonged by adjusting factors such as ambient gas, temperature, and humidity. By lowering the rate of respiration and thermal degradation, low-temperature storage enhances freshness and extends shelf life. However, the quality of fruit may be affected by chilling injury during prolonged storage at low temperatures (Zapata et al., 2008).

Gum Arabic (G), also called gum acacia, is a sticky substance obtained from Acacia species stems or branches and dries out. Its emulsification, film-forming, and encapsulating capabilities make it one of the most widely utilized hydrocolloid materials in the industry. G has also successfully delayed the ripening of cold-stored apples when applied as an edible coating (Motlagh et al., 2006). Furthermore, it was discovered that G preserved the post-harvest quality of mature green tomatoes throughout storage (20 days) at a temperature of 20°C (Ali et al., 2010).

Chitosan (CH) is a natural carbohydrate that comes from the shells of crabs, shrimp, and other seafood. Also, because CH comes from nature, it is non-toxic and biodegradable (Lizardi-Mendoza et al., 2016). Due to its ability to stop respiration and dehydration has been used as a coating on many fruits to improve their quality and lengthen their shelf life (Li et al., 2021).

Costus is the common name for Saussurea costus, one of the most well-known species in this genus. Root oil and roots of Saussurea costus have become an essential drug on the world market (Panigrahi et al., 2017). It is called "Al-Kost Al-Hindi" in Arab countries, and traditional healers have been using it since the Islamic civilization.

It is used as an antiseptic, stimulant, sedative, carminative, astringent agent and bronchodilator (Wani et al., 2011) and it contains chemicals known as "complement-inhibitors," which are useful in the treatment of disorders caused by an overactive complement system., like rheumatoid arthritis and respiratory distress (Fan et al., 2014).

To the best of our knowledge, incorporating SCE to G and CH to form multifunctional edible coatings was not reported before. Therefore, this study aims to assess the potential of using G, CH and SCE as an edible coating for delaying ripening whilst maintaining the antioxidant properties of tomatoes during storage.

# Materials And Methods

### Experimental materials:

Freshly harvested tomato (*Solanum lycopersicum*) samples were procured from Kafrelsheikh, Egypt. Fresh tomatoes for the experiment were selected of equal size, disease-free, absence of blemishes and uniform colour. The tomatoes were transported to the laboratory within one hour. Saussurea costus powder (SCP) was purchased from the local market in Cairo, Egypt.

G, CH and glycerol were supplied from Oxford Laboratories (Maharashtra, India), Lanxess (France) and Sigma-Aldrich (Germany) for edible coating preparation.

### Preparation of SCE

SCP (20 g) were macerated in 70% ethanol for 2 days at room temperature. The obtained mixture was filtrated, and the residue was re-extracted 3 times in the same manner. Resultant filtrates were collected

and evaporated under pressure using a rotary evaporator at 40 °C. The remained residues were frozen at -80 °C. Finally, the lyophilization process was done to get SCE lyophilized powder, and the powder was stored at -20 °C till use.

### Preparation of coating solutions

Four solutions were used in this work as follows:

1. Gum Arabic (G).
2. Chitosan (CH).
3. Gum Arabic and chitosan (G+CH).
4. Gum arabic, chitosan and SCE (X).

Solution A was prepared as follows: 5 g of G was dissolved in distilled water (100 mL) (900 rpm - 70 °C) for 1 h. Then 1 g of glycerol was added (900 rpm - 70 °C) for 30 min. Solution B was done by dissolving 2 g of CH in 100 mL water containing 0.2% glacial acetic acid, followed by homogenization (900 rpm - 70 °C) for 1 h. After that, 1 g of glycerol was added (900 rpm - 70 °C) for 30 min. Solution C was prepared as follows: 5 g of G was dissolved in distilled water (100 mL) (900 rpm - 70 °C) for 1 h. Then, 2 g of CH in 100 mL water containing 0.2% glacial acetic acid, followed by homogenization (900 rpm - 70 °C) for 1 h. After that, 1 g of glycerol was added (900 rpm - 70 °C) for 30 min. Solution D was prepared like solution (C), but finally, 0.15 g of SCX was added (900 rpm - 40 °C) for 30 min. Tomato samples were dipped in each solution for 60 sec then kept in a refrigerator at 4 °C until analysis.

### Treating tomatoes with edible coatings

All tomato samples were sorted into five groups, and each group was given a different treatment: The first group was referred to as the control group (C) without dipping in any solutions and groups 2, 3, 4 and 5 were dipped separately for 60 sec solutions of G, CH, G-CH and X, respectively. They were left to dry at room temperature for 60 sec. Each treatment in the experiment was conducted in triplicates. All samples were subjected to the analysis at zero time, 3, 9 and 15 days. Tomatoes were stored for 15 days at 4 °C.

### Determination of weight loss (WL):

WL was determined in each set by the following equation:

$$WL\ (g) = W_0 - W_1$$

where $W_0$ is the initial weight of fruit samples and $W_1$ is the weight of fruit samples at each storage period.

### Determination of firmness (FM):

An eight mm plunger tip digital Force Gauge penetrometer was used to measure FM of tomatoes in each treatment group, near the fruit equator, where rid had previously been removed. Newtons (N) were used to express the results. Three readings were taken for each tomato.

### Determination of titratable acidity (TA):

TA was measured by NaOH (0.1 N) and expressed as g of malic acid equivalent per 100 g fresh weight (Siddiq et al., 2011).

### Determination of total soluble solid (TSS):

TSS was measured according to AOAC (2000) method (932.14) using an Abbe 3-L refractometer and reported as °Brix.

### Determination of total soluble solid/titratable acidity (TSS/TA):

The ratio between TSS and TA was determined by dividing the TSS by that of TA to have a sugar-acid balance of samples for each treatment. The amount of TSS/TA was measured as follows:

$$\text{TSS/TA} = \frac{TSS\ value}{TA\ value}$$

### Determination of ascorbic acid (AAC):

2,6 dichloroindophenol titrimetric was used to measure AAC in the sample. As a first step, 3% metaphosphoric acid ($HPO_3$) was used to macerate the fruit and extract the juices. Filtering and 2,6-dichloroindophenol titration of the resulting extract were performed until the rose pink color remained for 15-20 seconds (Ali et al., 2011). The amount of AAC was given in mg per 100 g of fresh weight.

### Determination of total phenolic content (TPC):

TPC was measured using the Folin-Ciocalteu method as mentioned by Attrad (2013) with some modifications.

Briefly, the procedure consisted of mixing 10 µL of sample/standard with 100 µL of Folin-Ciocalteu reagent (Diluted 1: 10). Then, 80 µL of 1M $Na_2CO_3$ was added and incubated at room temperature (25 °C) for 20 min in the dark. The resulting blue complex color was measured at 630 nm by the end of incubation time. Data are represented as means ± SD.

### Statistical analysis:

SPSS 16.0 package was used for data analysis. The Duncan test and one-way analysis of variance (ANOVA) were used for multiple comparisons. Statistically, differences were considered significant if $p \leq 0.05$.

# Results And Discussion

### Weight loss:

Tomatoes from control and treated samples showed a mass loss during the storage period (Table 1). Uncoated samples had statistically higher mass loss compared to others. All samples showed a drastic increase in mass loss until the $9^{th}$ day of storage, concerning uncoated samples reported the highest mass loss compared to the other samples. By 15 days, the total major WL appeared in the uncoated samples (4.10 g) then samples coated with G, CH, G+CH and finally X (3.22, 2.91, 2.85 and 2.12 g, respectively). On the $15^{th}$, no significant differences were reported in the samples coated with CH, G+CH and X.

Weight loss changes the texture and shrinks the surface of climacteric fruits and vegetables, which makes them go bad faster. The edible coating considers as a moisture barrier between the fruit and the surrounding air, reducing these negative effects and preventing water loss (Nawab et al., 2017). Because edible coating plays as a semi-permeable barrier to the exchange of gases, solute movement, and moisture, it likely slowed respiration, oxidation reaction rates, and moisture transfer (Baysal et al., 2010).

Similar results of reduction in WL of uncoated samples were observed by Ali et al. (2010) when tomatoes were coated with gum arabic. Another study also reported that corn zein edible coating reduced the water loss of apricots and thereby extended its postharvest storage life through modification of the internal atmosphere (Baysal et al., 2010).

Our results were supported by Ali et al. (2010), where water loss in papaya could be reduced by covering with chitosan (1.5 and 2%) compared to uncoated fruits.

### Firmness (FM):

Their texture heavily influences consumers' acceptance of vegetables and fruits. The fruit's molecular contents, cellular organelles, moisture level, and cell wall composition all play a significant role. When these characters are exposed to external or internal factors, the texture and quality of the product can be adversely affected (Fagundes et al., 2014). The loss of FM in vegetables and fruits is the most evident textural change after storage (Ali et al., 2010). The initial value of FM for control and coated tomatoes was 188.33 N (Fig. 1A). A slight decrease occurred after $3^{rd}$ day of storage in all samples, but on the $9^{th}$ day, the decrease in FM values was very fast. The coating showed a statistical difference in FM when compared to uncoated (44.00 N) during storage, particularly by coatings containing G (88.33 N) followed by G+CH (80.00 N) and CH (78.33 N) and finally with X (65.00 N). The samples treated with CH and G+CH did not show a significant variation in their FM, by the end of storage period. Fruits that had been coated with different materials were more resistant to softening than those had been left uncoated.

The results were in line with the findings of Chaturvedi et al. (2019) for tomatoes coated with pectin, cornflour. Also, many fruits were said to be more firm when coated with chitosan (Ruelas-Chacon et al.,

2017).

### Titratable acidity (TA):

TA of tomato is very important for the taste of fruits. When respiration activity goes up, the organic acids in the fruit, especially citric acid, are broken down to make intermediates for the tricarboxylic acid cycle. This makes harvested tomatoes very sensitive to respiration activity (Khatri et al., 2020). To sustain the activity of organic acids in crops such as tomatoes, an edible coating can be applied (Li et al., 2017). TA of stored fruit, both coated and uncoated, was reduced over time in this study (Fig. 1B) and the decrease was higher in uncoated samples compared to the coated samples.

At the end of the 15$^{th}$ day, the control sample recorded the lowest value of TA (0.25%) and the highest was reported to X samples (0.46%), while G, CH and G+CH did not show any significant differences between them (0.38, 0.35 and 0.38 %, respectively).

In this study, the slower rate at which the acidity drops in the coated fruits compared to the uncoated samples shows the important role of the coating materials, which slow the rate of respiration and keep the pH stable by putting a semi-permeable layer over the tomato fruit's skin (Baysal et al., 2010).

Similar studies found that the acidity contents in Aleo vera chitosan-coated tomatoes decreased with storage time, although at a slower rate than in control (Khatri et al., 2020).

### Total soluble solid (TSS):

TSS indicates the total sugar content in fruits, providing information about the fruit maturation state. TSS includes the water-soluble compounds of sugars, acids, vitamin C, and some pectins.

The effect of the edible coating of tomatoes on TSS with different materials is shown in Fig. 1C. At first, the TSS level in all tomato fruits was low, but after 15 days, the highest level of sugar was found in uncoated samples (4.77%) compared with the coated fruits (range, 3.97 - 4.29%). A gradual increase in TSS was observed in all tested samples after the 3$^{rd}$ day of the storage period. In turn, edible coatings slowed respiration rates. This delayed the production and use of metabolites, which led to a lower concentration of soluble solids (Gol et al., 2015). As a result, TSS started to increase from the 3$^{rd}$ day of storage until it reached the highest increment by the end of the storage period.

TSS was recorded on the 3$^{rd}$ day of storage at 3.95, 3.83, 3.80, 3.13 and 3.09% for the uncoated, G, CH, G+CH and X, respectively. After 15 days, the TSS level was increased in the coated fruits with a concomitant decrease in the acidity value. On the 15$^{th}$ day of storage, the lowest TSS value was recorded in G, CH and X (3.97, 3.99 and 4.00%) without any significant differences between them and the highest TSS value (4.77%) was recorded for C. Samples covered with G, CH, and X showed that the coatings made a good semi-permeable barrier around the fruit, changing the internal atmosphere by lowering or raising $CO_2$ production. This was in line with what Dong et al. (2004) found. They said that chitosan's

filmogenic property makes an excellent semi-permeable film around the fruit, which changes the atmosphere inside the fruit. Polysaccharides (hemicellulose and pectin) undergo hydrolytic alterations with ripening in postharvest storage, linked to variations in TSS. Oligosaccharins are released due to the decomposition of tomato cell wall polysaccharides during storage, which can have an impact on fruit ripening (Das et al., 2013). Khatri et al. (2020) and Ullah et al. (2017) showed near results of coated tomatoes and bell pepper with different materials.

### Total soluble solid/acidity (TSS/TA):

The TSS/TA ratio is an important quality criterion for tomato fruits because it is recognized that sweetness and sourness are important requirements for the flavor of tomato products (Stevens et al., 1979). Generally, TSS/TA ratio increased significantly along with increased storage time in both uncoated and coated fruits (Fig. 1D). This was in harmony with Zekrehiwotet al. (2017), who reported that TSS/TA ratio of tomato samples coated with different materials increased during the storage period. TSS/TA for zero time was 5.39 and subsequently reached 19.08, 11.29, 11.34, 10.50 and 8.70 by the end of the storage period for C, G, CH, G+CH and X. Generally, coated samples revealed relatively small ratio changes in comparison with the control sample.

### Ascorbic acid (AAC):

AAC found in tomato fruits has numerous health benefits for humans and plays a critical part in various plant processes. After three days of storage, AAC increased in all treatments and reached the maximum values in CH and G+CH treatments (38.53 and 38.08 mg/100g) (Fig. 1E). After the 3$^{rd}$ day, AAC content was maintained with X at 9 and 15 days (22.24 and 18.21 mg/100g, respectively) compared with other treatments, especially with the control sample (15.48 12.04 mg/100g, respectively). There was no noticeable difference at the end of the storage duration between CH (14.96 mg/100g) and G (14.05 mg/100g), and all were higher than C (12.04 mg/100g). As shown in Fig. 1E, by the end of the storage period, X maintained AAC (18.21 mg/100g), almost close to the initial amount of AAC in fresh samples (20.85 mg/100g) followed by G+CH (16.57 mg/100g).

This increase in AAC then decreases, agreed with the results obtained by Ali et al. (2011) who reported that AAC in papaya increases during ripening but decreases during senescence. At the end of storage, tomatoes treated with Aloe vera and chitosan showed similar delays in AAC decrease (Khatri et al., 2020). The quantities of AAC we found were in agreement with those found in previous investigations on capsicum (Patel et al., 2019).

### Total phenolic content (TPC):

Figure 1F represents the changes in TPC in uncoated and coated samples during storage using different coating materials. The concentration of TPC in all samples (except the control) increased for the first three days of storage, then decreased until the conclusion of the storage period. X coated sample had the highest concentration of TPC and reached the peak after 3$^{rd}$ day (60.97 mg GAE/100g) followed by G

(53.82 mg GAE/100g), G+CH (51.14 mg GAE/100g) and CH (39.68 mg GAE/100g) then decreased sharply during the complete storage period and reached 24.29, 17.24, 17.89 and 13.04 mg GAE/100g, respectively.

Overall, the coated samples kept their overall TPC longer than their counterparts in the uncoated group. TPC in fruits can differ during storage depending on many factors, such as the fruit species and cultivar, and temperature, climatic, and environmental conditions throughout the growth period. In the same line with our results, Toor and Savage (2006) reported that TPC showed a small increase during storage at 7 °C and 15 °C, for the first eight days; and showed some decline toward the end of the storage period of tomatoes samples.

However, uncoated samples started to decrease from 3$^{rd}$ day to the end of the study. As shown in Fig. 1F, X caused the major increase in TPC during the storage period reaching about 60.97, 41.55 and 24.29 mg GAE/100g on the 3$^{rd}$, 9$^{th}$ and 15$^{th}$ day of storage, respectively. TPC values dropped more slowly in coated fruit than in uncoated fruit, probably because oxygen couldn't get in as easily and enzymes didn't work as well (Wang and Gao, 2013). It could also be due to senescence and the breaking down of cell structure during storage, as was seen in a study using gum arabic on tomatoes (Ali et al., 2010). Similar decreases were observed in total phenolic content during the post-harvest storage of tomato samples (coated with whey protein isolate, xanthan gum) (Panigrahi et al., 2018).

By the end of this study, the post-harvest survivability of fruits, with intact nutrients for a more extended period, boosts farmers. In tomatoes especially, the peels are very susceptible to deterioration. The present study demonstrated an acceptable efficiency of G, CH and SCE as edible coatings in enhancing the post-harvest survivability period of tomato, with increases in TSS and TPC and gradual decreases in TA and AAC. Moreover, our results indicated that the combination of SCE and G and CH exert more superior effects than the individual coatings throughout the storage period, especially with TA, AAC and TPC.

# Declarations

**Ethics approval and consent to participate:** Not applicable

**Consent for publication:** All authors agree for publication.

**Availability of data and materials:** The data that support the findings of this study are available from the corresponding author upon reason-able request.

**Competing interests:** The authors declare that they have no conflict of interest.

**Funding:** Not applicable

**Authors' contributions:**

First author: Conceived and designed the analysis – Review the final manuscript.

Second author: Conceived and designed the analysis – Collected the data - Contributed data or analysis tools - Review the final manuscript.

Third author: Conceived and designed the analysis – Collected the data - Contributed data or analysis tools.

Fourth author: Conceived and designed the analysis – Collected the data - Contributed data or analysis tools - Performed the analysis.

Fifth author: Conceived and designed the analysis – Collected the data - Contributed data or analysis tools - Performed the analysis - Wrote the paper - Review the final manuscript.

Sixth author: Conceived and designed the analysis – Collected the data - Contributed data or analysis tools.

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# Tables

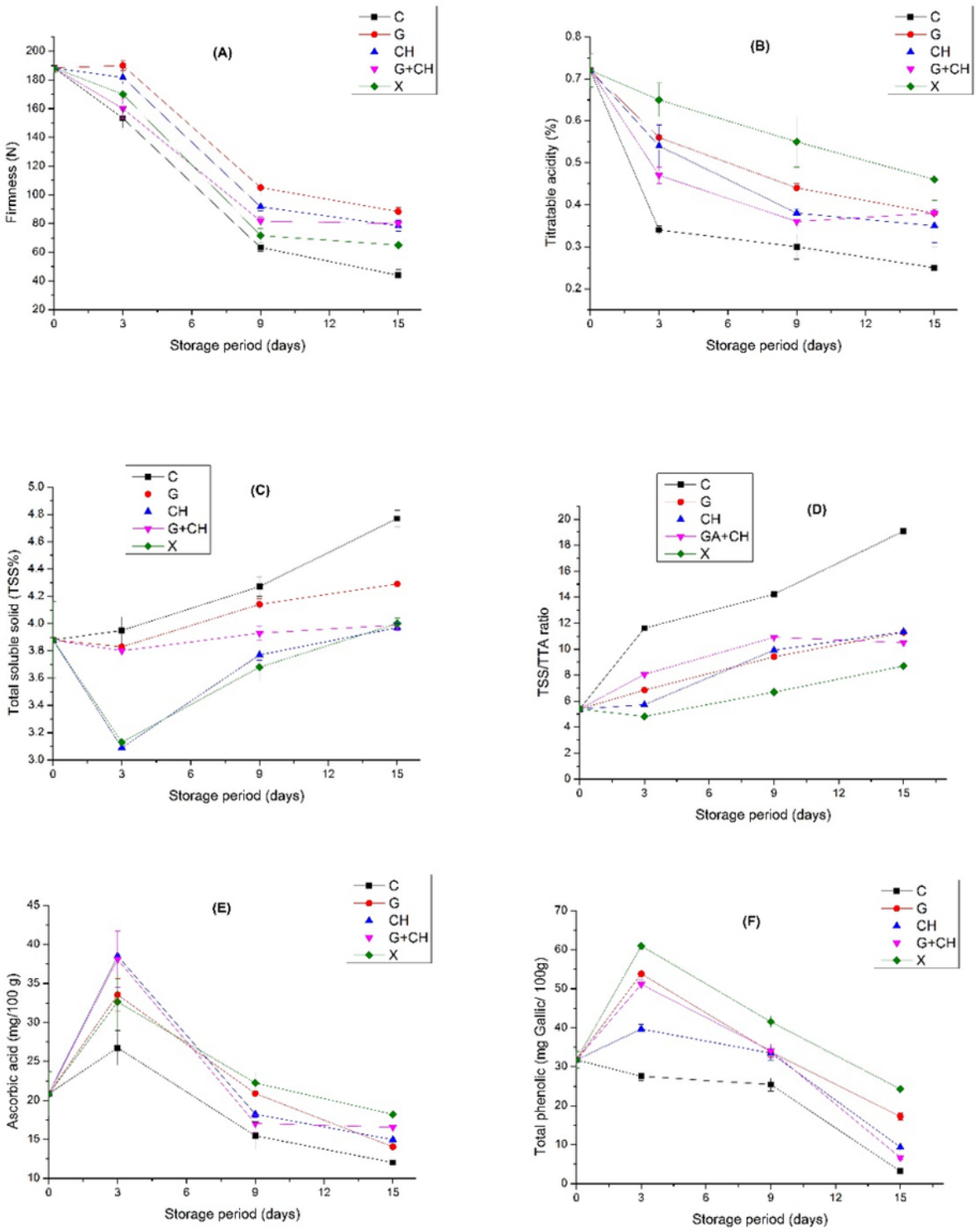
**Table 1 Weight loss of uncoated and coated tomato samples during storage for 15 days at 4 °C**

| Treatment | Storage period | | | Total loss (g) |
|---|---|---|---|---|
| | 3rd | 9th | 15th | |
| Control | 1.01±0.10$^{a}$ | 2.10±0.22$^{a}$ | 0.99±0.13$^{a}$ | 4.10 |
| G | 0.89±0.19$^{a}$ | 1.50±0.11$^{b}$ | 0.83±0.09$^{ab}$ | 3.22 |
| CH | 0.75±0.09$^{a}$ | 1.47±0.23$^{b}$ | 0.69±0.06$^{b}$ | 2.91 |
| G+CH | 0.38±0.08$^{b}$ | 1.82±0.35$^{ab}$ | 0.65±0.04$^{b}$ | 2.85 |
| X | 0.20±0.05$^{b}$ | 1.47±0.11$^{b}$ | 0.68±0.03$^{b}$ | 2.35 |

Values are means± SD. Means having the different case letter(s) within a column are significantly different at $p \leq 0.05$.

C=control, G=gum Arabic, CH=chitosan, G+CH=gum Arabic and chitosan, X=gum Arabic, chitosan and Saussurea costus extract.

# Figures




## Figure 1

A= Firmness, B= Titratable acidity, C= Total soluble solid , D= TSS/TA, E= Ascorbic acid and F= Total phenolic content of uncoated and coated tomato samples during storage for 15 days at 4 °C. C=control, G=gum Arabic, CH=chitosan, G+CH=gum Arabic and chitosan, X=gum Arabic, chitosan and Saussurea costus extract.

# Supplementary Files

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- GraphicalAbstract.png