# THE EFFECT OF RICE HUSK BASED ETHYLENE SCAVENGERS ON THE POSTHARVEST QUALITY OF TOMATO FRUITS

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## ABSTRACT

This study was designed to investigate the effect of a novel active packaging pad that affectively scavenges ethylene evolved by climacteric fruits and thereby delayed their ripening process. These active packaging pads were developed by using rice husk and eggshell with the help of binder. Two different types of ethylene scavenging pads were formulated i.e. fine rice-husk (P1) and composite rice husk along with eggshell powder (P2). Green but matured tomatoes were stored in plastic container with P1, P2 and without pad as control. The delay in ripening tomato samples in both P1 and P2 was observed as compared to control in same conditions of temperature ( $30 \pm 2C^{\circ}$ ) and humidity ( $60 \pm 2\%$ ). During ripening process weight loss, pH, soluble solids, titratable acidity, phenolic, chlorophyll, carotinoids, betacarotene contents were evaluated and compared with the control. Adsorbing capacity of ethylene was found to be 0.377, 0.344, 0.306, 0.295 and 0.27 mmol/g in (P1) storage container and almost similar results were obtained for (P2) that showed eggshell has no role in adsorbing of ethylene gas. Tomatoes stored in (P1) as well as (P2) as ethylene scavenger significantly delayed ripening rate up to 40% and extended shelf life by 12 days.

Keywords: Tomato, Postharvest life, Ethylene scavenger, Rice husk, Eggshell, Storage

## INTRODUCTION

Pakistan is agro-based country; the area of cultivation is 27% of total land, in which different crops, vegetables and fruits are being grown to cover the market demand locally and internationally. Lack of proper handling and technological advancement for postharvest life of fruits and vegetables reduced its consumption as well as export considerably (Faostat, 2017) (Fatima *et al.*, 2009). Substantial losses of various fruits and vegetables occur due to poor postharvest handling as well as lack of appropriate storage and processing infrastructure. About 30-50% losses have been reported in various fruits and vegetables throughout the supply chain (Hasan *et al.*, 2019). However these postharvest losses could be minimized by implementing modern scientific techniques that eventually overcome local price fluctuation due to shortage and increase export as well.

Tomato (*Lycopersicon esculentum* L.) is one of the most consumable fruit worldwide and usually used both in uncooked and processed form as tomato powder, tomato juice, ketchup, puree and tomato sauces etc (Zapata *et al.* 2008). In 2017-2018, Pakistan produced 620100 tonnes of tomatoes and average production per hector was about 10 tonnes (Agriculture Statistics of Pakistan 2017-2018). Tomato contains various compounds particularly basic amino acids, mono unsaturated fatty acids (mostly oleic acid) and minerals (Cu, Fe, Zn and Mn). Additionally, it contains substantial amounts of bioactive compounds like cancer prevention agents (lycopene, ascorbic acid and tocopherols), folate and flavonoids. Freshness of tomato has been assured with its quality parameters such as color, flavors, appearance, firmness, dry matter, brix and titratible acidity which can be used to determine its market value (Liu *et al.*, 2009). One of the basic factor related to tomato postharvest shelf-life is exogenous ethylene gas especially in high temperature tropical and subtropical regions which brings about quicker fruit maturing and decline shelf-life of tomato fruit (Wills *et al.*, 2001).

Tomato is a type of climacteric fruit, so it has shorter postharvest life than non-climacteric fruits (Bailén *et al.*, 2006). Being climetric fruit, it is necessary to maintain quality of tomato fruit from farm to end user. In ripening process, exogenous ethylene evolves and water losses occur through various types of biochemical and physiochemical reactions. The ethylene gas and moisture content are primarily responsible factors to accelerate the ripening process by increasing the rate of respiration resulted in rapid spoilage of tomatoes (Fagundes *et al.*, 2014).

There are several factors which could affect the postharvest life of tomato such as humidity, temperature and mainly exogenous ethylene levels. By controlling these parameters in storage may increase the shelf life of tomato fruits and quality as well. Different techniques are being used commercially like low temperature storage that reduces the respiration rate and thermal decomposition resulted in enhanced the postharvest life of tomato fruits. However, due to the chilling injury the quality of fruit may be deteriorated over longer storage at low temperature (Zapata *et al.*, 2008). Hypobaric storage could be another option to extend the postharvest life of tomato but

commercially it is not economical because of the higher cost (Artés and Gómez, 2006). Ethylene scavenging is one of the preferred active packaging considered for the storing of fruits and vegetables (Srithammaraj and Magaraphan, 2012). The use of ethylene adsorber widely accepted because there is no oxidation or reduction of ethylene taken place inside the package and it has been practiced to enhance the shelf life of various fruits by slowing down the respiration rate and water losses (Martínez-Romero *et al.*, 2006).

Different types of commercial ethylene scavenging products were patented for enhancing postharvest life of vegetables and fruits, mainly activated carbon which was formed from various botanical sources by high temperature degradation (Kruijf *et al.*, 2002) and impregnation of potassium permanganate on activated carbon. These scavengers require ventilation because oxidation of ethylene by potassium permanganate may produce some undesirable gases that may interrupt the condition of controlled atmosphere. These ethylene scavengers are available in the form of polymeric sachets that are usually kept inside the package before sealing. However, the toxicity of the chemicals limits the application of such sachets in food packaging. Currently, montmorillonite, cloister, zeolite, clay etc belong from natural porous material being used in pure and modified form as ethylene scavenger. Halloysite nano-tubes are new innovative addition (Bodbodak and Rafiee, 2016) which is low price, high performance and environmental friendly material made from natural rocks deposits (Yuan and He, 2015) but purification of material limits its applications.

The objective of the current study was to develop an innovative, low cost and recyclable ethylene scavenging material by utilizing different food processing waste like eggshell and rice husk that can effectively retard the metabolic changes of fresh fruits and vegetables (Baldwin *et al.*, 1998). Utilization of these processing waste for developing ethylene scavenging active packaging would not only manage the environmental solid-waste burden but also would reduce the dependency on chemicals that eventually would be a positive impact on economy. Although, various food processing waste materials have been used as an original source to make activated carbon with large surface area for capturing ethylene gas (Wang and Yoshio, 2010) but no study has been published so far to utilized rice husk in its raw form as active ethylene scavenger. The present study is therefore aimed to investigate the effect of rice husk based active packaging on the postharvest quality and shelf life of the tomato fruit.

#### MATERIALS AND METHODS

#### **Materials:**

Rice husk and eggshell were collected as waste from the Matco Rice Mills, Karachi and Young Food Industries, Karachi respectively. All chemicals used were of analytical grade and purchased from Sigma-Aldrich Company, Germany. Purified Ethylene gas was used and purchased from BOC Pakistan Limited, Karachi.

#### Preparation of rice husk & eggshell:

To remove impurities and other foreign material and dust, the rice husk was cleaned and rinsed several times with distilled water and dried in an oven at 45 °C for two days and then ground and sieved (40 mesh) to obtain rice husk powder. Eggshells were washed several times with distilled water and dried in an oven at  $110^{\circ}$  C for 3 days. The residual eggshell membrane was removed manually and the dried shells ground to get the fine powder (325 mesh). The eggshell powder (100 g) was submerged in 2500 ml of 5% acetic acid solution at room temperature for 2 h. Treated egg shells powder then washed with distilled water five times and dried at  $110^{\circ}$ C.

## **Preparation of ethylene scavengers:**

Powder rice husk (100gm) and eggshell powder (1gm) were mixed thoroughly. Polyvinyl alcohol (PVA) solution (20 % w/w) was prepared by dissolving PVA powder in distilled water at 90°C with continuous stirring for 2 h. PVA solution (30 ml) was then added to dry mixture of rice husk powder and eggshell powder to make a dough like matrix. It was then loaded into a rectangular mold (7.5 x 13 x 0.5cm), mechanically pressed and dried in an oven at 60°C for 2 days. The resultant rectangular pad is named as P1. Another active pad was prepared by using rice husk and PVA only and it was named as P2.

#### Ethylene adsorption by active storage containers:

This procedure was designed to check injected ethylene gas concentration in sealed chamber as control, and measured the adsorbing capacity of P1 & P2 separately at room temperature. Pure ethylene gas (1mL) was injected in a sealed desiccator (2.5L) through syringe via rubber septum containing ethylene gas detector (Oldham-MX 2100-France). Ethylene absorption rate was monitored at every 24h for 5 days.

Green but matured tomatoes (20) were stored in an air tight plastic container. Active pads P1& P2 were adjusted with the lid inside the containers and tested after every four days interval for 20 days of storage period. The plastic container without any active pad was also used as control. All the observations were taken at the controlled conditions of temperature ( $30 \pm 2$ °) and humidity ( $60 \pm 2$ %).

## Physicochemical properties of tomatoes during storage

#### Weight loss:

% Weight loss was determined according to the method describe previously (Bassetto *et al.*, 2005). The difference between the initial and final weights were taken as weight loss at every four days interval.

#### **Total soluble solids:**

The total soluble solids of tomato was determined by using a hand refractometer Maser-10 $\alpha$  (Atago co. Ltd., Japan).Tomato homogenate was prepared by crushing and blending the tomato fruit and then 10 ml slurry was taken and centrifuged at 5000 rpm. The clear supernatant (2ml) was taken and filtered using a syringe fitted with a 0.45 $\mu$ m pore diameter filter and two drops of the filtrate were then carefully applied on the refractometer using plastic dropper and reading was obtained directly as percentage soluble solids concentration (Teka, 2013).

#### Total titratable acidity:

Tomato homogenate (1ml) of each sample was diluted with 4 ml of distilled water. The resultant homogenate was titrated against standardized 0.1 N NaOH using phenolphthalein as an indicator. The volume of NaOH was expressed as gm citric acid/100 ml of homogenate. Titratable acidity was calculated by the method described previously (Tiwari *et al.*, 2008b).

#### **Firmness:**

Firmness of tomatoes was determined by simple compression test using Universal Testing Machine (Zwick GmbH &Co, Ulm Germany) by following the method described by (Gomez *et al.*, 2008)

## **Total phenolic content:**

Total phenolic content in the each sample was determined by Folin-Ciocalteu (FC) method (HOrVátHOV *et al.*, 2007) using a Lambda 25 UV/VIS spectrophotometer (PerkinElmer, Inc, USA). An aliquot of sample solution (0.02 ml) was mixed with 1.58 ml distilled water, followed by the addition of 100µl FC reagent. Mixture was homogenized completely and incubated for 8 minutes. Subsequently,  $300\mu$ l aqueous sodium carbonate solution (15%) was added and incubated again for minimum 2h with continuous shaking, then absorbance were measured against 765 nm. Gallic acid was used as standard and calibration curve was plotted at the same 765 nm wavelength. The data for total phenolic contents were expressed as mg of gallic acid equivalent weight (GAE) / gm of sample.

#### Determination of β-Carotene, Lycopene and Chlorophyll:

One gram ground tomato was dispersed in 10 ml of the mixed solvents of acetone and hexane (4:6 v/v) and then filtered (Bhumsaidon and Chamchong, 2016). The absorption value of filtrate was measured at 453, 505, 663 and 645 nm wavelength. Determination of chlorophyll,  $\beta$ -carotene and lycopene was calculated by using the following formulae:(Nagata and Yamashita 1992).

Lycopene (mg/100ml) = - 0.0458A<sub>663</sub> +0.204A <sub>645</sub>+0.372A<sub>505</sub> -0.0806A<sub>453</sub>  $\beta$ - Carotene (mg/100ml) = - 0.216A<sub>663</sub> +1.22A <sub>645</sub> - 0.304A<sub>505</sub> + 0.452A<sub>453</sub> Chlorophyll<sup>a</sup> (mg/100ml) = 0.999A<sub>663</sub> - 0.0989A<sub>645</sub> Chlorophyll<sup>b</sup> (mg/100ml) = -0.328A<sub>663</sub> -1.77A<sub>645</sub>

#### **Determination of pH:**

The pH of tomato homogenate obtained from control and treated samples were measured by following the previous reported method (Tiwari *et al.*, 2008a) using a digital pH meter (Mettler Toledo MP220-USA)

#### **Statistical Analysis:**

All measurements for each sample were made in triplicate. Sources of variation were the time of storage and treatments.

## **RESULTS AND DISCUSSIONS**

## Ethylene adsorbing capacity of active pads:

This experiment was designed to check adsorbing capacity of active pads for ethylene gas in isolated environment. (Fig. 1) showed that there was no significant change observed in ethylene concentration in control system which assured that it was perfectly sealed. Figure1 also showed the concentration of ethylene adsorbed by active pad P1 was found to be gradually reduced as 0.377, 0.344, 0.306, 0.295 and 0.27mmol/gm.h in 5 days. This trend is a clear indication of an effective adosrber of ethylene gas for P1 package. Almost similar results were obtained for P2, suggested that eggshell was not participating in adsorbing the ethylene gas. Reproducibility of the results was checked by repeating the experiment in identical conditions and confirming that a good replication was obtained. This result meant that the rice husk contained efficient adsorption sites to ethylene that could be possibly its microfibrous cellulose. It can be suggested that rice husk are effective ethylene adsorbing material that could be effectively used as a natural and non-toxic material to enhance the postharvest life of tomatoes and other climacteric fruits.

#### Weight loss:

Weight loss of fruits at different stages during postharvest period is referred as transpiration (Ayranci and Tunc, 2003). During storage the increase in weight loss was observed by many researches (Bassetto *et al.*, 2005; García-García *et al.*, 2013). The process of respiration involves the oxidation of carbohydrate and organic acid to carbon dioxide and water with the release of energy and formation of some intermediate compounds (Kader, 2002). The observed weight loss for tomatoes during 20 days of storage at room temperature was shown in (**Fig. 2**). It was observed in (Fig. 2) that weight loss in tomato was drastically reduced by active pad of both P1 & P2 as compared to the control. Weight loss was directly proportional to the storage time and was found to be greatest for control sample. The control samples were lost 11.51% moisture whereas only 6.4% and 6.5% reduction in moisture affects the rate of weight loss (Kumar and Nussinov, 1999). Diffusive ethylene from fruits increases the respiration rate and causes the reduction of shelf life of fruits (Emadpour *et al.*, 2015). Therefore, the adsorption of ethylene by rice husk based active pad present inside the containers would also be effective to slow down the production of moisture and carbon dioxide.

#### **Total soluble solid:**

Total soluble solid of fruit is a quality parameter correlated to the texture and composition (Kamiloglu, 2011) of the fruit. Soluble sugars, organic acids and dry matter constituents, such as pectic fragments are the major components of the soluble solids. The change in total soluble solids of tomato fruit during storage were shown in (Fig. **3**). It was observed that the increase in TSS of tamatoe samples was delayed if stored in active packages P1 & P2 which ultimately inverse the storage period of tomatoes in normal atmosphere condition (Farahana and Supri, 2015). This is due to the unavailability of active ethylene gas in P1 & P2 packages. It was reported previously that ethylene in the atmosphere can have a direct effect on fruit tissues by raising the internal concentration of fruit to an active level (Gelly *et al.*, 2003).

#### **Titratable acidity**

The amount of organic acid usually decreases with time as organic acids are the substrate of respiration (Wills and Golding, 2016). The titratible acidity of all samples were represented in (Fig. 4) indicated the progressively reduction in acidity during storage period. The samples preserved in active package P1 were found to decrease the acidity level more than that of the sample in P2 package, whereas drastic decrease was noticed in the control sample. Maximum acidity i.e. 0.4125% was observed in matured green tomatoes which found to be decrease gradually with progression in maturation. As respiration process was retarded in P1 package due to adsorption of ethylene gas from surrounding so the quantity of organic acid particularly maleic and citric acid will not consumed so fast as in control sample. As a matter of fact, rise in pH value and reduction in titratable acidity goes simultaneously due to the loss of citric acid concentration during the tomato ripening stage (El-Anany and Hassan, 2009).

#### Firmness

Firmness of fruit is a major and important factor for consumers as an indicator of high quality and often it is considered as the last test before selecting a tomato for purchase. Firmness usually demonstrates the fruit's biochemical constituents, water content and cell wall composition of fruit. The most noticeable textural change in fruits and vegetables is the loss of firmness during storage (Martínez-Romero *et al.*, 2006). During postharvest period, any external or internal factor affecting these characters that eventually deteriorate the texture and also lead

to undesirable changes in product quality (Fagundes *et al.*, 2014). Result showed in (Fig. **5**) that the firmness of the control tomatoes was significantly lower after 20 days of storage at room temperature. However, the firmness of tomatoes was higher when they were packaged with P1 active pad (20 days storage) compared to P2 (12 days storage). These results demonstrated the positive effect of low concentrations of ethylene and water vapors on the firmness during storage. The difference of shelf life between P1 & P2 is depending on adsorbing capacity of ethylene of active packaging and water vapors in package's environment. In the presence of moisture content, adsorbing capacity of active packaging was found to be decrease. This result was in agreement of the similar type of finding reported by (Abreu *et al.*, 2019).



Fig. 1. Ethylene adsorption rate of tomatoes during storage.



Fig. 3. Total soluble solids (<sup>o</sup>Brix) of tomatoes during storage.





Fig. 4. Total titrable acidity of tomatoes during storage.

Fig. 5. Firmness of tomatoes during storage.



Fig. 6. pH values of tomatoes during storage.



Fig. 9. Total Chlorophyll content of tomatoes during storage.

pH:

Amount of organic acid in tomato fruit gradually decrease during storage because of the process of respiration that moves the pH towards neutral side. In the present study, change in acidity presented in (Fig. 6) showing the progression of ripening process of tomatoes during storage. In general, the change in pH during storage of tomatoes was found to be low and usually ranging from 4.0 to 5.3 in different packaging systems. Tomatoes stored in P1

package showed less variation in pH (between 4.0 and 4.9) than those of the tomatoes stored in P2 package (between 4.2 and 5.1). P1 active pad adsorbed ethylene along with moisture from package's surrounding that may be resulted in slow respiration which retarded the conversion of organic acid into sugar compared to the sample stored in P2 package. As mentioned earlier, P2 was made without eggshell as moisture absorber so it decreases the adsorbing capacity of active packaging and eventually relatively less effective to retard ripening process during storage. This trend confirmed the previous studies on the pH values of tomato fruit (Mohammed *et al.*, 1999) that reported the pH of ripe tomatoes may exceed 4.6.

## Lycopene, β-carotene, total chlorophyll (a&b):

Ripening of tomatoes is a combination of processes including the breakdown of chlorophyll and build-up of carotenes. during the chlorophyll degradation chloroplasts ( a green color pigment) are converted into chromoplasts (a yellow to red pigment) (Radzevičius *et al.*, 2009) and at the same time carotenoids are converted from a colorless precursor (phytoene) to red lycopene (López Camelo and Gómez, 2004).

Lycopene,  $\beta$ -carotene, total chlorophyll (a, b) contents of tomatoes were determined in control as well as test samples. Tested tomatoes showed in (Fig. **7,8,9**) significant increase in carotenoid and lycopene content but decrease in chlorophyll content during their storage at controlled conditions of temperature and humidity. Noteworthy, such a significant increase in lycopene and  $\beta$ -carotene content was recorded at each successive ripening stage of tomato fruit. As a matter of fact, red color of tomato is usually developed due to the replacement of degraded chlorophyll by carotenoid indicating gradual ripening (Moneruzzaman *et al.*, 2008). Results showed that the total chlorophyll of tomatoes was completely faded in control, P1 and P2 in 12, 16 and 20 days respectively. Lycopene and  $\beta$ -carotene increased optimally at 11, 15 and 20 days in all above samples respectively. In comparison among these samples, P1 showed relatively delay ripening of tomatoes stored in P2 package is due to the higher moisture content in surrounding of package that eventually reduced the adsorbing capacity of active packaging (Abreu *et al.*, 2019). Somewhat similar findings were also reported by (Toor and Savage, 2006). These results confirmed that there was a significant positive influence of P1 contributing to delay maturation and ultimately prolonged the shelf life of tomatoes.



Fig. 10. Total phenolic compounds of tomatoes during storage.

#### **Total phenolic compounds:**

Tomato consists of a natural reservoir of vitamin C, folic acid, carotenoids and phenolic compounds (Gougoulias *et al.*, 2012). These phenolic compounds affect the color as well as taste in many fruits, while exercising potent antioxidant properties (Campos *et al.*, 2013). According to some authors, phenolic acid content decreases whereas flavonoid phenol content increases during ripening of the fruits (Silberberg *et al.*, 2005). The ripening stage affects the phenolic content of the fruits. It was observed that in mature red tomatoes the polyphenol content was highest while it was lowest in green stage of tomatoes. In addition, from the mature red color to over ripening stage color showed a significant reduction in total phenolic content of the fruits (Raffo *et al.*, 2006). (Fig. **10**) showed that highest phenolic content in control, P1 and P2 was observed on 8, 16 and 11 days respectively. P1 package was found to be effective to increases the shelf life of tomatoes up to more eight days than that of the

sample stored in P2. Obviously, one of the great reason of such delayed in ripening was the adsorption of ethylene from package surrounding which decelerate the chlorophyll loss, reduce ripening or destimulate the phenyl propanoid metabolism (Terry *et al.*, 2007). On contrary, P2 active packaging was found to be less effective to control the ripening rate as it comprised without moisture absorber (eggshell powder) and possibly it may resist or reduce the adsorbing power of ethylene in package environment (Abreu *et al.*, 2019).

## CONCLUSION

Rice husk containing active packaging material can provide an alternative ethylene scavenging material in replacement of chemical and polymer based material to prolong the postharvest life of tomatoes. It was found that the highest strength of packaging material is most likely depending on the amount of cellulose fiber. It was also observed that an increase in the eggshell powder slightly enhanced the moisture absorption as well as ethylene adsorption capacity that eventually improves the effectiveness of active packages. PVA is an important component that not only facilitates the interaction of eggshell and the rice husk but it also increased the mechanical properties of the packaging material. A major benefit of such type of material is that it is easily reusable by removing the adsorbed ethylene in a simple drying process. Therefore, rice husk based packing material is an innovative environmentally friendly material act as an ethylene scavenger and could have potential applications in making food and agricultural commodities containers for extension of postharvest life of fruits and vegetables during storage period.

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