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STUDY OF BIO-PRESERVATIVE POTENTIAL OF TURMERIC ON TOMATO

Mudiganti Ram Krishna Rao[1*] and Lakshmanan V[1].

[1]Department of Industrial Biotechnology, Bharath Institute of Higher Education and Research, Selaiyur, Chennai.

Running Title: Bio-preservative potential of Turmeric on Tomato

Abstract:

Preservation of fruits and vegetables is a challenge for farmers, sellers and processors. Many chemical preservatives are used to increase the shelf life, to reduce the spoilage, to protect from microorganisms. The present study is to address this problem with an age old traditional preservative, turmeric, as bio preservative. Aqueous solution of turmeric at 5, 10 and 25 % (w/v) concentrations were used to preserve the tomatoes and the study was undertaken for 30 days. The change in physio-chemical characteristics such as weight, pH, lycopene content, dry matter content, total phenolic content were studied. The antimicrobial role of turmeric was also studied on E. coli, Lactobacillus and Aspergillus Niger. It was observed that there was a perceptible improvement in each of the parameters studied. Thus turmeric is suggested as a cheap and affordable preservative for harvested tomatoes. Further qualitative aspects must be studied to prove the bio preservative role of turmeric on tomatoes.

Keywords: *Shelf life, Bio preservative, Turmeric, Tomato, Spoilage, Antimicrobial*

Corresponding author:

Dr. Mudiganti Ram Krishna Rao,

Professor,

Department of Industrial Biotechnology,

Bharath Institute of Higher Education and Research,

Chennai-600073.

E mail: mrkrao1455@gmail.com

QR code

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INTRODUCTION:

Increasing the shelf life of vegetables, uncooked food and cooked food is a challenge to food technologists and scientists. There is an urgent need to develop strategies in this direction since control of losses due to spoilage of food can change the world food scenario. In India alone it is estimated there is wastage of food of about 67 million tons per year which turns out to be Rs. 92,000 crores. About 1million tons of onions, 2.2 million tons of tomatoes and more than 5 millions eggs never reach the market. Lack of facilities like storage, transport, preservative techniques, knowledge base among farmers and other players are some of the attributes in this regard. There is also a lack of focus on this major issue by the governmental agencies around the world. The use of chemical and synthetic preservatives have improved the situation to some extent but there is always a risk of the short and long term side effects of these preservatives. Thus there is an urgent need to change this scenario for which both government and non governmental agencies, scientists, agriculturists and others should work towards this issue. It is imperative to develop low cost, natural, plant based, affordable preservatives to protect the food materials from spoilage and its related health hazards.

The present study is one step in this direction. The use of turmeric powder to improve the shelf life of tomato and its antimicrobial activity was studied.

Tomato, *Lycopersicon esculentum* (Synonym: *Solanum lycopersicum*), is the second most important member of Solanaceae next to potato. In 2017, the worldwide production of tomatoes was 170.8 million tons. China, India and the United States are the largest producers of tomatoes in that order. Tomatoes are a valuable source of several health-promoting compounds due to the balanced mixture of minerals, micronutrients and antioxidants, including vitamin C and E, carotenoids, potassium, folate, tocopherol, and flavonoids such as quercetin. They are well known for being a cheap source of vitamins, with high vitamin C content and also significant amounts of vitamin A and B. It also contains a large amount of water and minerals, such as potassium, magnesium, phosphorous and calcium (Yeung & Laquatra, 1998) with great importance in human metabolic activities [1].

Tomatoes are a major dietary source of lycopene, the red pigment in tomato fruit that is associated with dietary health benefits. Many of the carotenoids, such as lycopene and β-carotene and flavonoids seem to protect from cardiovascular diseases and different types of cancer (Basu and Imrhan, 2000) [2].

The concept of tomato quality refers to all attributes that consumer consciously or unconsciously believes that fruit must have such as color, firmness, flavor etc. Important colr changes occur at various stages of tomato development in terms of chlorophyll (green colour), β-carotene (orange color) and lycopene (red color) contents. The most visible changes are associated with chlorophyll loss (green color) and gradual accumulation of lycopene (red color), where plastids such as chloroplasts present in the green fruits is transformed into chromoplasts. The characteristic flavour of a fresh tomato is the result of complex interactions between organic acids, soluble sugars and over 400 volatile compounds, that are synthesized during the ripening process in the intact fruit (primary aroma compounds) and upon tissue disruption (secondary aroma compounds) (Baldwin *et al*, 2000; Antunes *et al*, 2013; Arah *et al*, 2015; García-Alonso *et al*, 2009) [3-6].

Turmeric (*Curcuma longa* L.) is a rhizomatous perennial plant related to Zingiberaceae family, commonly known as Curcuma, Curcum, Haridra and Indian saffron. In South Asian countries, turmeric is being used since ancient times as a spice, food preservative, coloring agent and cosmetic in traditional systems of medicine (Ayurveda, Siddha, Unani and Tibetian). Turmeric oil has many applications in cosmetic sector, perfumes and soap industries. It is an antacid and in small doses acts as a carminative, stomachic, appetizer and tonic. It is also found to be effective against bronchial asthma in clinical trial. Furthermore, exhibits therapeutic properties which are responsible for anticancer, anti-oxidant, antimicrobial, antiparasitic, antimutagenic, immunomodulatory, anti-inflammatory, anti-protease and apoptosis inducing properties. (Zorofchian *et al*, 2014; Gul *et al*, 2015; Belloso *et al,* 2008)[7-9].

There are some encouraging reports on the use of natural preservatives to increase the shelf life of vegetables and other food products. The antimicrobial activity of concentrated volatile oil of eight varieties of turmeric was studied on food microorganisms *Escherichia coli* and *Pseudomonas aeruginosa*. (Kaur *et al*, 2006; Salas *et al,* 2013; Mohanka *et al*, 2014)

The preservative effect of whey permeates at 0.5%, 1.5 % and 3% concentrations on fresh cut carrot and lettuce indicted promising results. (Martin-Diana *et al,* 2006). Rao *et al*, 1998, have reported the use of edible coating of methyl cellulose and palm oil on the ripening of guava fruit.

MATERIAL AND METHODS:

1. PLANT MATERIALS AND STORAGE CONDITIONS

Tomatoes (*Solanum lycopersicum*) were procured from the local market Koyembedu, Chennai, India. Tomato fruits were collected in separate bags and immediately transported to the laboratory for the experiment. Fruits were harvested at mature pink and light red stage (Half ripe) and their classification was performed through external color according to USDA standard tomato color classification (USDA, 1991). Tomatoes with uniform color, weight, size and maturity were selected and also without major bruises or signs of infection.

Upon arrival to the laboratory, fruits were gently washed under running tap water, paper dried and divided into five groups of 30 fruits each, placed in plastic trays, and stored at 29 ºC (± 0.5 ºC). Room temperature was measured daily during storage with help of a hand-held thermometer.

Fresh turmeric powder to be used as bio-preservative was purchased from Erode Market, Tamilnadu.

2. SAMPLING AND TREATMENT DESIGN

The procured tomato fruits were numbered, weighed and kept in normal conditions with constant room temperature (28±0.5 ◦C) in plastic trays for 30 days in a randomized manner for further evaluation. The surface sanitized tomatoes were divided into five groups, I group was kept as control (untreated) whereas group II, III, IV were treated with 5%, 10%, 25% (w/v) aqueous turmeric solution respectively.

The group V was kept at 5°C storage condition without any addition of preservative.

Turmeric solution of different concentrations (5%, 10%, 25% (w/v)) were prepared by dissolving turmeric powder in distilled water. Each treatment was carried out in different baskets by immersing the tomato fruit in aqueous solution of turmeric for 1 min with agitation and excess coating material was allowed to drip off. Then it was paper dried.

Samples of fruits were taken on 0, 3, 6, 9, 12, 15, 21, 24, 27, 30 days of storage for the analysis of percentage weight loss, pH, dry matter content, lycopene and total phenolic content. Each data recording was of two replicates of a tomato fruit.

3. WEIGHT LOSS

Weight loss was measured according to the method of Diaz *et al,* 2016. A batch constituted of three fruits per treatment and on particular day of analysis, was weighed. After weighing, tomatoes were put back to original storage conditions. The weight loss was calculated relative to the weight taken at day zero (t=0).

(%) Weight loss= W_0-W_t/ W_0 x 100

Where, W_0 is the average weight of the first batch at day 0 and W_t is the average weight of the same batch at day t (i.e. 0, 3, 6, 9….30). Two replicates for each treatment was carried out.

4. TOTAL PHENOLIC CONTENT

Total phenolic content was determined using the Folin-Ciocalteau reagent method (Singleton & Rossi, 1965). Tomato fruits were chopped and blended for 2 min until attaining uniform size. Samples (10 g) were homogenized in 70 % aqueous methanol (10 ml) using a homogenizer and vortex mixer and centrifuged at 19,000 rpm for 20 min at 4 ºC and the supernatant was collected. One hundred microliter of supernatant was mixed with 5 ml of Folin-Ciocalteau (1/10, v/v) and 4 ml of Na_2CO_3 (7.5 %, w/v). The mixture was placed in a water-bath (45 °C for 15 min) and the absorbance was measured at 765 nm in UV/VIS spectrophotometer, using Gallic acid as standard. Results were expressed as milligram Gallic acid equivalents (mg GAE.100 g-1) of fresh fruit weight. Two replicates for each treatment was carried out.

5. LYCOPENE CONTENT ESTIMATION

Lycopene extraction was based on the method of Davis *et al*, 2003, with slight modifications. Tomato fruits were finely ground for 1 min to puree using a stainless-steel blender. Ground tissues were kept on ice and out of light after preparation until assayed. Approximately 1 g of the puree (without seeds) was put in 50 ml aluminium sheet wrapped test tubes while they were on ice. Lycopene extraction solution (39 ml) consisting of hexane, 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone and 95% ethanol in a ratio of 1:1:1 was added to the tubes and shaken for 10 min at 180 rpm using tabletop shaker while they were still in ice. To each tube 6 ml of cold double distilled water was added and agitated for an additional 5 min for better separation of polar and non polar compounds. Tubes were then removed from shaking and left for 15 min at room temperature for separation of polar and non-polar layers. Supernatants were put into fresh 15ml aluminium foil wrapped test tubes and kept at - 80°C for further analysis. The absorbance of supernatant (hexane layer) containing lycopene was read three times using spectrophotometer at the wavelength of 503 nm. Absolute hexane was used as blank. The amounts of lycopene in tissues were then estimated by the following formula:

Lycopene (mg/kg) = $(x/y) \times A_{503} \times 3.12$

Where x is the amount of hexane (ml), y the weight of fruit tissue (g), A_{503} the absorbance at 503 nm and 3.12 is the extinction coefficient.

6. CHANGE IN pH RANGE

Ten-gram sample of ground tomato tissue was blended for 2 min in 20 mL of deionised water. The pH of the slurry was measured at room temperature using digital pH-meter between 18 and 20 ^{0}C.

7. DRY MATTER CONTENT

Known weigh of ground tomato tissue was kept at 100 ^{0} C for 24 h in a hot air oven. Dry matter was calculated by using the weight after heating as a percentage of the initial weight.

8. MICROBIOLOGICAL STUDY

Total plate counts (TPC), yeast and mould counts were carried out for microbiological analysis of coated and uncoated tomato samples during 16 days of storage following the method of Balouiri *et al,* 2016 [18]. Total plate counts were determined using the pour plate method and Plate Count Agar (PCA) as medium. The plates were incubated at 35 ^{0} C for 2 days. Yeast and mould counts were determined using the spread plate method. All microbiological analysis was carried out in duplicate and the results were expressed as log10 colony forming units per gram (log10 CFU/g).

9. ANTIMICROBIAL ACTIVITY OF TURMERIC

The antibacterial activity of different solvent extracted samples of turmeric was carried out by agar disc diffusion assay and agar well diffusion assay. For disc diffusion assay, Mueller Hinton Agar (MHA) was used as medium. Filter paper discs (Whatman no. 1) of 8 mm diameter were prepared and sterilized. Using sterile forceps, these discs were aseptically placed over MHA agar plates seeded with the respective test microorganisms. Three different concentrations of turmeric (5%, 10%, and 25%) were aseptically transferred to these discs. The plates were incubated in an upright position at 37 °C for 24 h. The diameters of inhibition zones (in mm) were measured. Likewise, antimicrobial activity of turmeric against food spoilage microorganisms was tested by taking swab from the surface of affected control sample.

RESULTS AND DISCUSSION:

1. Weight loss

At the harvest time fresh fruits contain between 70 to 95 % of water. Fresh-cut fruits are highly susceptible to weight loss. Therefore, evaluating weight loss is very important for fresh-cut fruit during storage. It has been shown that storage duration, storage temperature and treatment have significant effects on weight loss. According to Javanmardi & Kubota (2006) the main cause for weight losses in stored tomato at room temperature can be attributed to increased transpiration rate which results in shrinkage, weight loss and changes in texture (softening) and appearance (fading) of tomatoes [19]. Appropriate formulations of an edible coating may provide an excellent barrier against gaseous exchange and water loss which are unfavourable to postharvest quality.

From the present study, the percentage weight loss was found to be 3.66 to 28.36 %, 4.6 to 28.96 % and 4.16 to 27.92 % in the samples treated with 5%, 10% and 25% (w/v) aqueous solution of turmeric respectively as shown in the Figure 1a and b, Table 1. Also, it has been shown that the percentage loss in weight was retarded in a concentration dependent manner. The weight loss of every sample treated with different concentration of turmeric solution was found to be lower than that of the untreated group which was kept as control.

Figure 1 a.

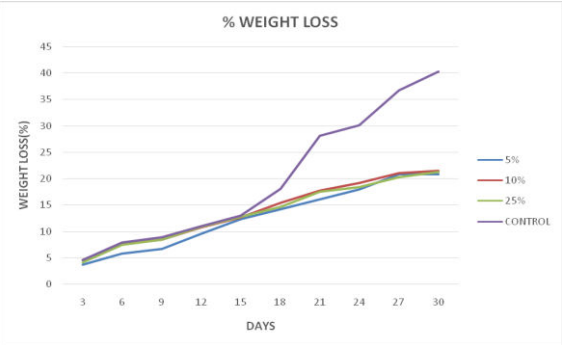


Figure 1b

Figure1a, b: Effect of treatments on tomato weight loss (%) during storage period

Table1 – Changes in weight loss (%) of tomato fruits treated with different concentrations of turmeric

Days of Analysis	Turmeric Conc. 5%	Turmeric Conc. 10%	Turmeric Conc. 25%	Control
3	3.666698	4.63324	4.163505	4.602934
6	5.740409	7.613742	7.530349	7.941325
9	6.683005	8.542247	8.478756	8.952959
12	9.510793	10.86351	10.84977	10.97623
15	12.33858	12.72052	12.74659	12.99949
18	14.22377	15.50604	14.6434	18.05766
21	16.10896	17.8273	17.48862	28.174
24	17.99416	19.22006	18.43703	30.19727
27	20.82194	21.07707	20.33384	36.77289
30	20.82194	21.54132	21.28225	40.31361
33	22.70714	22.46982	22.23065	47.09155
36	23.64973	23.86258	23.17906	58.52301
39	24.59233	25.16249	24.60167	41.14
42	26.47752	26.6481	26.02428	46.86938
45	28.36271	28.96936	27.92109	40.76584

2. pH measurement

The pH of fresh tomatoes treated with 5%, 10% and 25% (w/v) solution of turmeric were found to be ranging from 4.30 to 4.6, 4.12 to 4.41 and 4.03 to 4.51 respectively. The normal range of pH for tomato was maintained in the treated samples during the evaluation period and considerable difference was not observed in the treated and untreated (control) samples except during the last 5 days of 30-day evaluation period in which control group exhibited slight increase from the initial pH recorded [Table 2].

The increase in pH throughout storage suggests that the amount of organic acids, and in turn the pH, could be affected by the microbial deterioration of cut fruits.

Table 2: Effect of turmeric treatment on pH of tomato during the storage period (30 days)

DAY OF ANALYSIS	5%	10%	25%	CONTROL
0	4.21	4.12	4.03	4.35
3	4.19	4.12	4.15	4.48
6	4.32	4.02	4.21	4.09
9	4.48	4.14	4.18	4.41
12	4.42	4.23	4.42	4.57
15	4.39	4.21	4.2	4.48
18	4.37	4.27	4.44	4.49
21	4.53	4.22	4.48	4.52
24	4.49	4.41	4.39	4.57
27	4.57	4.49	4.45	4.59
30	4.59	4.41	4.43	4.63

3. ESTIMATION OF LYCOPENE

The results of lycopene estimation indicated that the concentration of lycopene in the tomatoes treated with 5%, 10%, 25% (w/v) solution of turmeric increased slightly but continuously during the 15 days of storage period initially from 43.19 to 82.96 mg/kg, 47.25 to 63.45 mg/kg, 34.39 to 66.65 mg/kg of fresh weight, respectively followed by a gradual decrease during the later period of storage (Table 3, Figure 4 a, b). The results showed that the treated group retained the lycopene content to some extent in a stabilized manner during the storage period of 30 days whereas the control group underwent

considerable increase in the concentration of lycopene from day 0 to 30.

Lycopene is an isoprenoid compound that gives the red colour to fruits and vegetables. In addition, it has been demonstrated that a large number of bacteria and moulds can synthesize carotenoids during the second growth phase from their precursor mevalonic acid pyrophosphate. Thus high levels of aerobic mesophilic micro-organisms or yeasts and moulds in fresh-cut tomatoes might enhance their carotenoid content. On the other hand, lycopene is susceptible to oxidation in the presence of light, oxygen and low pH.

Table 3: Effect of turmeric treatment on lycopene content of tomato during the storage period (30 days)

DAY OF ANALYSIS	5.00%	10.00%	25.00%	Control
0	43.1964	47.2524	34.39488	31.27176
3	46.15728	52.1196	50.27412	42.42576
6	67.00512	55.40496	63.69948	85.45992
9	95.15376	72.82548	95.43768	105.2126
12	68.85871	91.40196	95.03208	73.94088
15	75.99322	95.23488	95.23488	80.08572
18	79.26235	54.5532	64.77432	90.63132
21	88.70472	63.09108	65.66664	95.53908
24	92.31456	64.40522	67.57296	99.50179
27	79.99243	64.57963	67.20792	96.09881
30	82.9632	63.4587	66.6547	97.6211

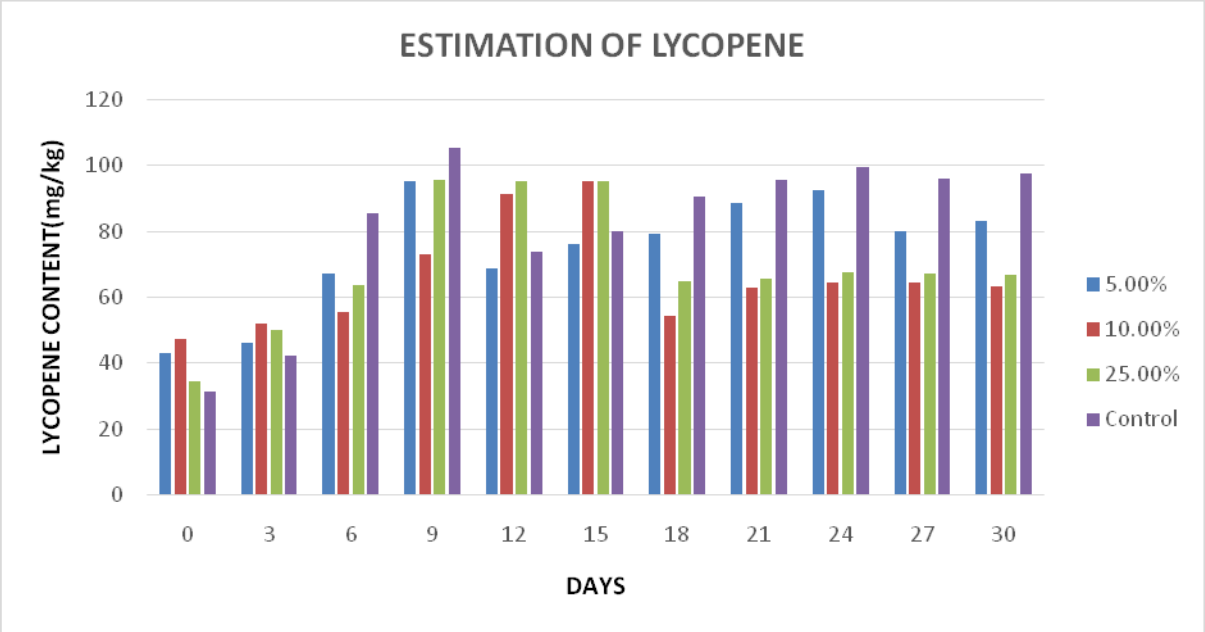


Figure 4(a): Effect of treatments on tomato lycopene content during storage period

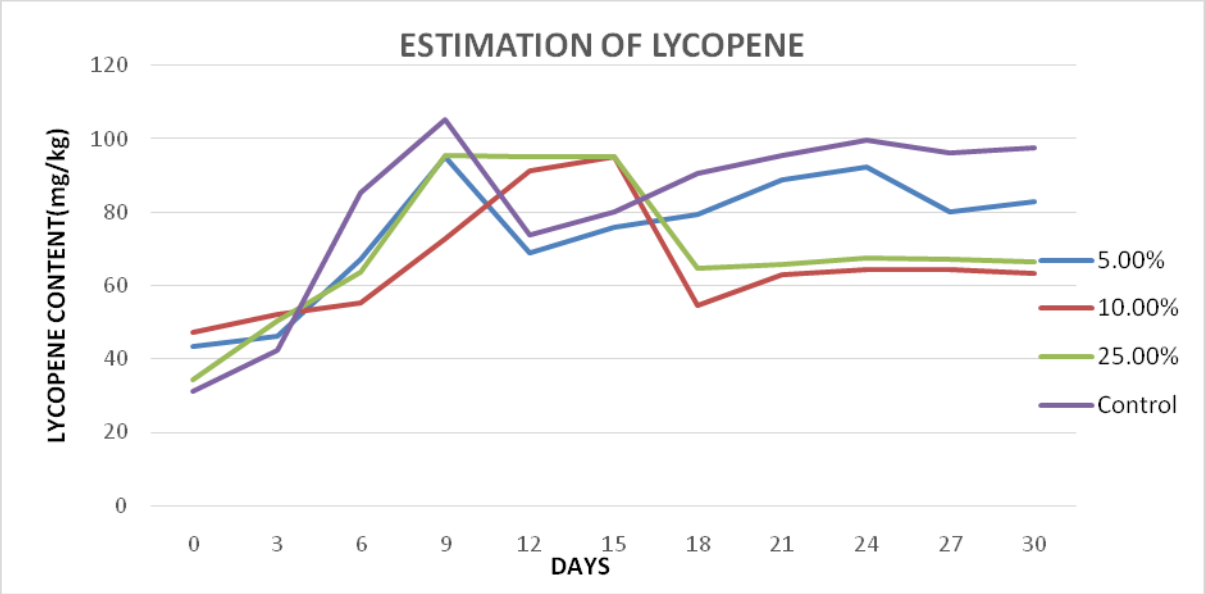


Figure 4 (b): Effect of treatments on lycopene content of tomato during storage period

4. DRY MATTER CONTENT

The dry matter content of tomatoes was measured by placing the homogenised seedless samples in an oven at 105º C for 24 h. The dry matter content of tomatoes during the study period ranged from 4.8% to 5.4% and was not affected.

The loss of water is a natural process of the catabolism of fresh vegetables and is attributed to the respiration and other senescence-related metabolic processes during storage.

5. TOTAL PHENOLIC CONTENT

The total phenolic content of treated tomatoes was found to be increasing during the 15 days of storage period initially and decrease in the content was observed in the later period of storage after 15 days which was in accordance to the data reported by Toor and Savage (2006), noticing an increase on tomato phenolic content until the 8th storage day, followed by a slight decrease until the end of storage time (10 days) [20]. Also, it has been shown that the group treated with highest concentration (i.e., 25% w/v) of turmeric solution retained the phenolic content without any drastic changes when compared to that of the control [Table 4, Figure 5a; Table 5, Figure 5b].

In general, the increase of phenolic content could be associated with ripening development, especially with the increase of phenylalanine ammonia-lyase (PAL) enzyme activity, which plays an important role in synthesis of phenolic compounds. The control samples had increased phenolic content due to the early ripening of tomato fruit compared to the treated samples. The increase in phenolics is due to the conversion of flavonoids to secondary phenolic compounds.

Table 4:

CONCENTRATION	ABSORBANCE
0	0
50	0.06
100	0.123
150	0.179
250	0.284
500	0.546

Absorption values of standard Gallic acid solution at different concentration

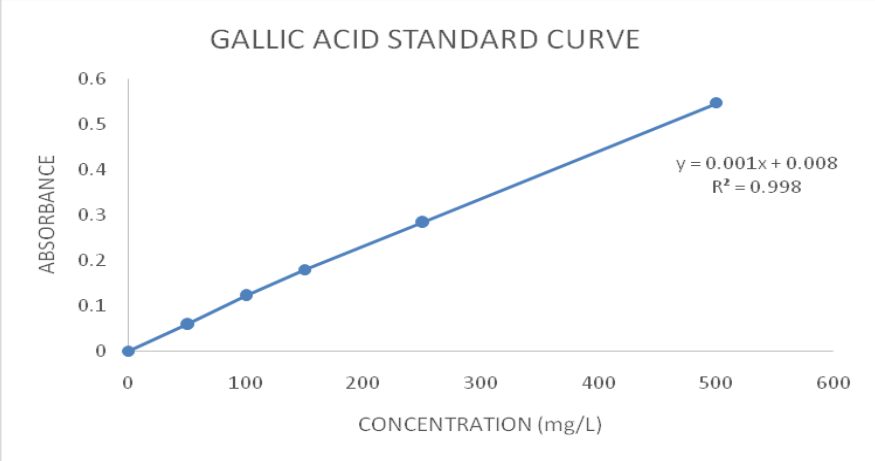


Figure 5 a. Absorption values of standard Gallic acid solution at different concentration

Table 5: Effect on the retention of phenolic content in tomatoes treated with turmeric

DAY OF ANALYSIS	5%	10%	25%	Control
0	35.73636	35.37273	31.37273	34.28182
3	37.28182	49.91818	31.64545	46.19091
6	36.28182	40.91818	43.37273	51.73636
9	40.73636	43.46364	54.82727	36.1
12	44.28182	57.55455	58.19091	62.73636
15	59.91818	54.82727	58.55455	58.00909
18	51.64545	45.00909	51.28182	49.00909
21	49.00909	47.19091	49.1	51.46364
24	47.00909	46.55455	48.46364	51.82727
27	45.37273	45.46364	48.82727	52.28182
30	44.82727	44.46364	46.46364	53.37273

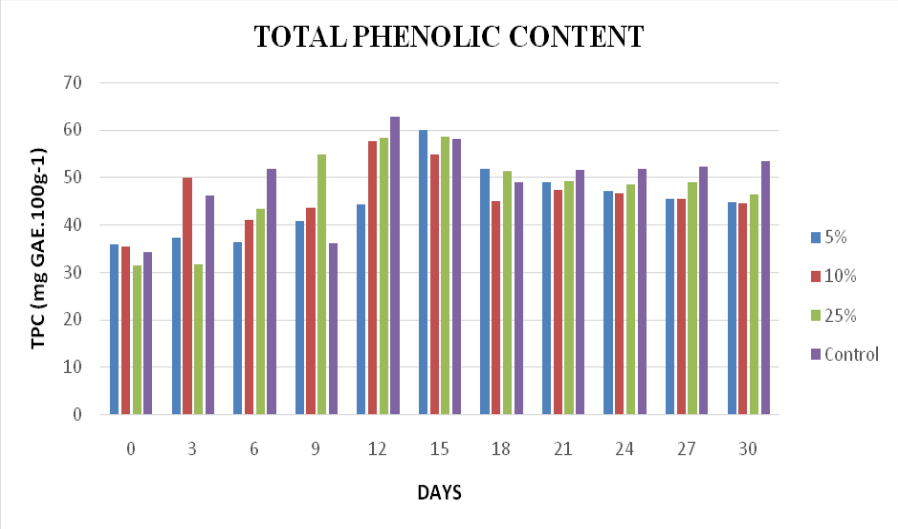
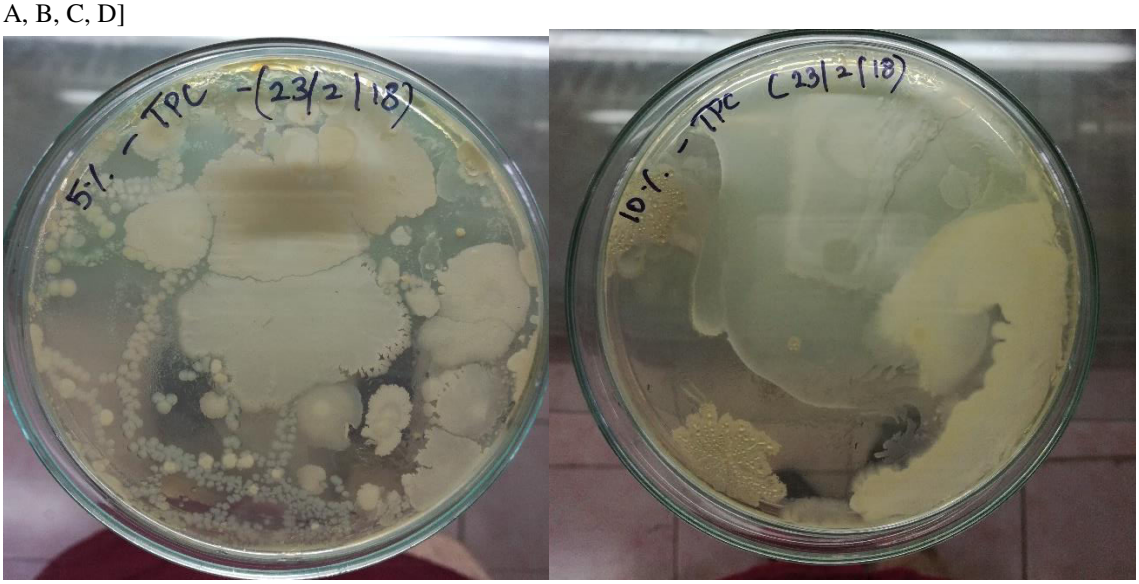


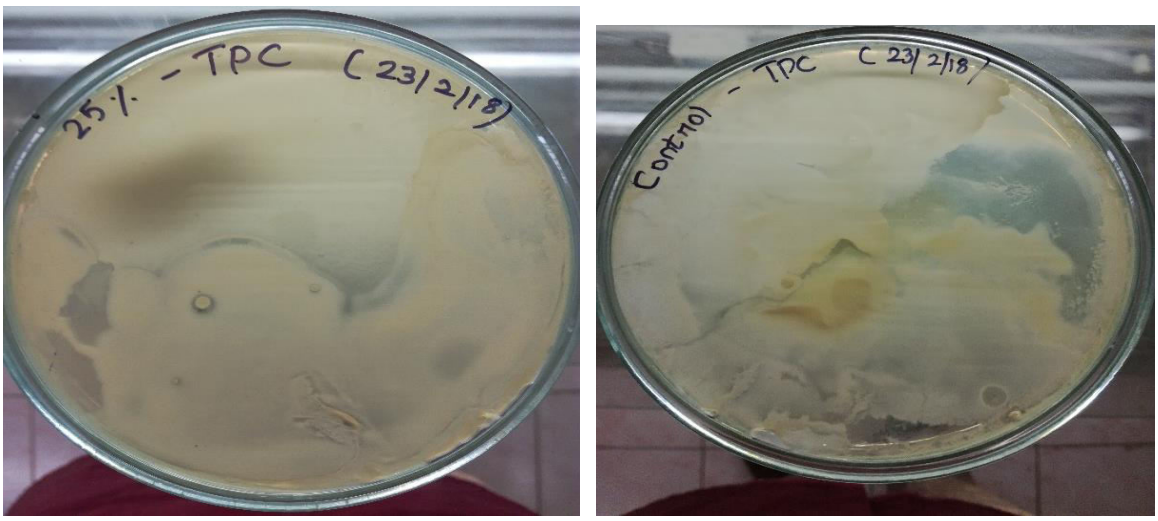
Figure 5 b. Effect on the retention of phenolic content in tomatoes treated with turmeric.

6. TOTAL PLATE COUNT

Total plate counts and yeast and mould counts were carried out for microbiological analysis of treated and untreated (control) tomato fruits during 30 days of storage at 15 days interval. The results obtained showed that the total plate counts showed increasing trend in every samples in which it was found to be higher in the control samples [Figure 6 A, B, C, D]



A B

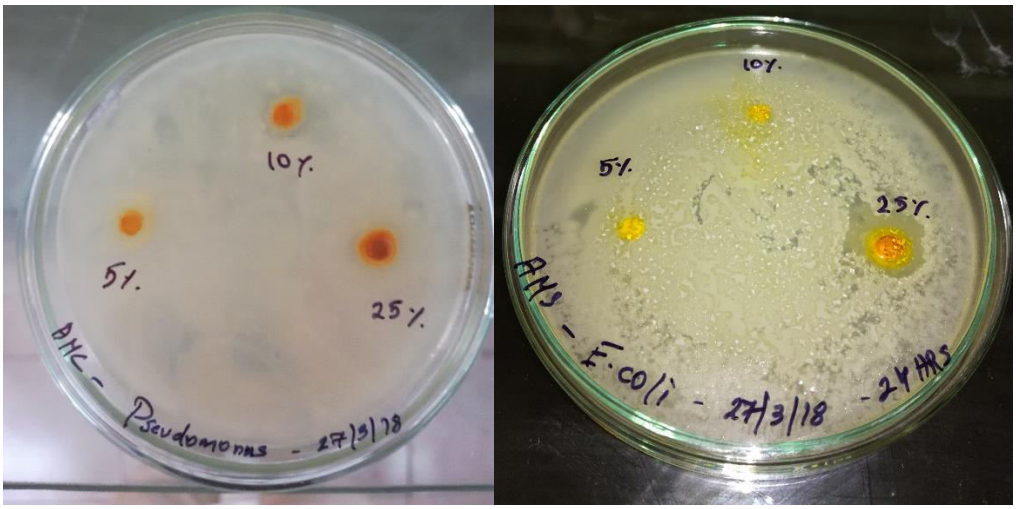


C D

Figure 6: Total plate count of tomato samples at day 15
A- 5% Turmeric solution B- 10% Turmeric solution C- 25% Turmeric solution
D – Control (Untreated)

ANTIMICROBIAL ACTIVITY OF TURMERIC

The antimicrobial activity of different concentrations of turmeric (5, 10, and 25%) against *Escherichia coli, Pseudomonas aeruginosa, Aspergillus niger* and against spoilage microorganisms was carried out by disc diffusion and agar well diffusion method. Among the pathogenic microorganisms tested, *E. coli* was found to be more susceptible to 25% (w/v) aqueous turmeric solution when compared to *Pseudomonas aeruginosa* having lesser zone of inhibition (Figure 7 A, B, C, D). Evaluation of turmeric against spoilage microorganisms showed inhibitory activity in a concentration dependent manner (Figure. 8 A, B, C, D)



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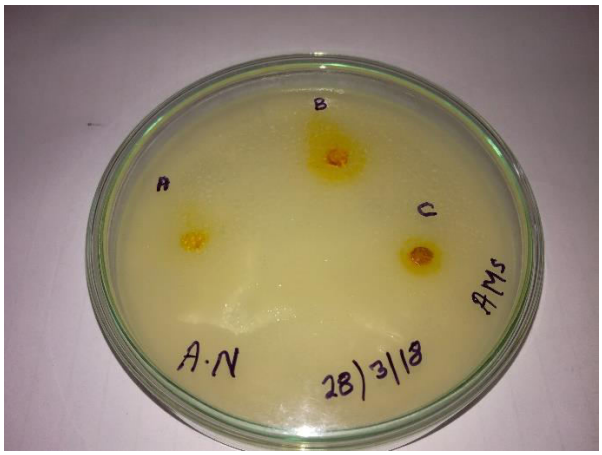
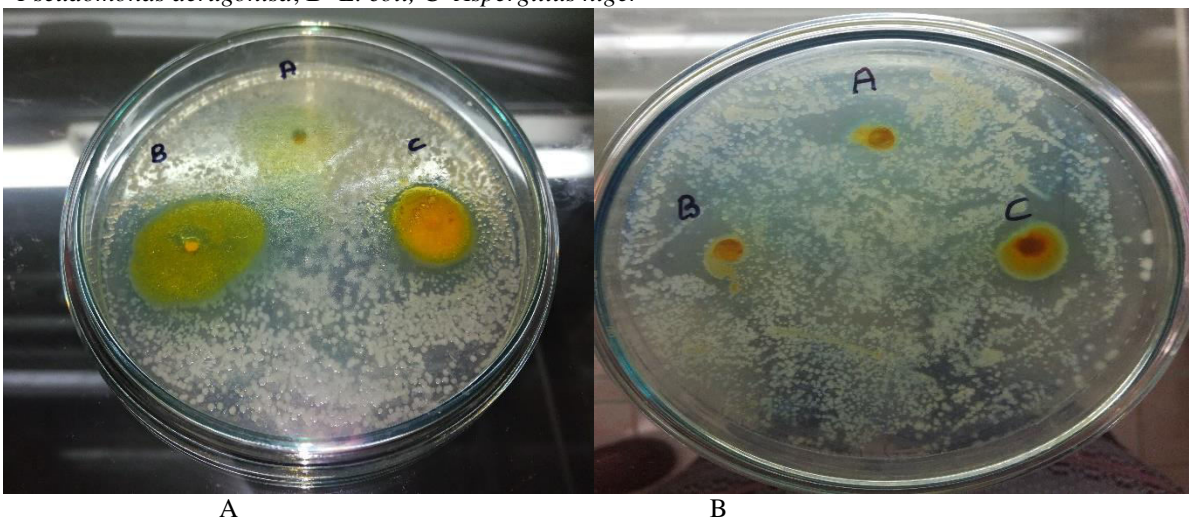


C

Figure 7: Antimicrobial activity of turmeric against food borne pathogens
A – *Pseudomonas aerugonisa*, B- *E. coli,* C- *Aspergillus niger*



A B

Figure 8: Antimicrobial activity of turmeric against food spoilage microorganisms
A- Agar well diffusion method B- Disk diffusion method

The present study was undertaken to investigate the bio-preservative potential of turmeric in extending the shelf life of harvested tomato fruits and antimicrobial activity of turmeric against food borne pathogens and food spoilage microorganisms.

CONCLUSION:

From the present study it has been demonstrated that turmeric could be exploited as promising agent for preventing postharvest losses of tomatoes by extending their shelf life. Physio-chemical characteristics defining the quality of fresh harvested tomatoes and antimicrobial activity were evaluated during the storage period of 30 days, which showed considerable stability in all the qualities of tomatoes in treated samples when compared to control group. Thus, it has been shown that turmeric could be a cheap and affordable alternative to other forms of preservative techniques whether using cold storage or by using other chemicals in preserving harvested tomatoes. However, further studies are required to identify the bioactive compound of turmeric, molecular changes due to action of turmeric in the ripening process to reduce postharvest losses in tomato.

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COMPETING INTERESTS

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