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# Quality Attributes of Pork Patties Wrapped with Polyphenol Nano solutions Incorporated Sodium Alginate Films at Refrigerated Storage (4±1 °C)

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## Abstract

*The study was aimed at evaluating the efficacy of edible film wraps prepared with Quercetin and Tannic acid nanosolution loaded sodium alginate on the quality and shelf-life of pork patties at refrigerated storage (4±1 0C). The films with Tannic acid had higher physical strength and quality than the films with Quercetin in terms of tensile strength, elongation at break, water vapour permeability, water sorption, light transmission, antioxidant activity, antimicrobial activity and total phenol content than films with Quercetin. The quality of pork patties wrapped with the above films was compared throughout the storage period. The patties wrapped with Tannic acid nanosolution incorporated film had lower cooking loss, pH, TBARS, Total Plate Count and Yeast and Mould Count compared to their counterparts. The results revealed that the use of natural polyphenols in nanosolution form in edible packaging offers a great advantage to maintain the quality of the product.*

**Keywords:** Active Edible Films, Nano Solutions, Pork Patties, Quercetin and Tannic Acid

## Introduction

Polyphenols are a structural class of natural, synthetic or semi synthetic, organic chemicals characterized by the presence of large multiples of phenol structural units. The most abundant polyphenols are tannins (condensed form), found in all families of plants virtually. Polyphenols are widely considered as a major group of highly effective antioxidants, since they exhibit potent free radical scavenging activity and protection against oxidation of transition metals and lipid peroxidation (Zhou and Elias, 2012). Among polyphenols, tannins received most attention due to their wide spectrum and higher antimicrobial activity against bacteria like *Staphylococcus aureus, Escherichia coli* in comparison with other polyphenols and to the fact that most of them are able to suppress a number of microbial virulence factors (i.e., inhibition of bio-film formation, reduction of host ligands adhesion, and neutralization of bacterial toxins) and show synergism with antibiotics (Rodriguez *et al*., 2010).

Quercetin has a variety of actions besides antioxidant activity, highlighted by antimutagenic (Gupta *et al*., 2010) and antitumorigenic (Wang *et al.*, 2012) properties. Quercetin also proved to be very effective against wide range of microbes including both Gram positive and Gram-negative bacteria. Tannic acid (TA) is a glucoside of gallic acid polymer with multiple phenolic hydroxyl groups that are found in many plants (Gao *et al.*, 2014). This is an attractive molecule known to have antioxidant, antitumor and antibacterial activity (Zhou and Elias *et al.*, 2012) and also be used as a reducing agent. These properties provided the potential utilization of Tannic acid in developing active packaging materials.

In the food sector, the incursion of nanotechnological advances is still discreet but it is gaining more and more interest by both scientific and industrial community (Rashidi and Darani 2011). Recently, emulsions with small droplet size, typically from 10 to 100 nm, also called nanoemulsions, are being investigated as lipophilic drug delivery systems in food, cosmetic and pharmaceutical products (Bernardi *et al*., 2011; He *et al*., 2011). Due to their intrinsic properties, they may present several advantages for encapsulating functional lipophilic compounds over conventional emulsions. Reduced droplet size of nanosolutions not only enhances the transport of active molecules through biological membranes but also increase the surface area/volume ratio, thus leading to improved functionality. On the other hand, nanoemulsions are kinetically stable and transparent colloidal dispersions, being suitable for a wide range of practical applications (Solans *et al*., 2005). Therefore, the present work has been designed to develop and standardize edible alginate based active edible packaging films incorporated with natural polyphenol nanosolutions i.e., quercetin and tannic acid and to study the physico-chemical, microbiological quality of pork patties packaged in the above films and to arrive at the efficacy of these films with nansolutions of natural polyphenols and the ability of the above nano polyphenol containing edible coatings to extend the shelf-life of pork patties.

## Materials and Methods

### Quercetin and Tannic Acid Nanosolutions Preparation

Quercetin and Tannic acid polyphenols each at 5 per cent w/v were selected for use in sodium alginate-based films to produce active packaging films. Coarse solutions of above polyphenols were prepared by slow and continuous addition of Quercetin and Tannic acid each at 5 per cent level with distilled water separately. Non-ionic surfactant i.e., tween 80 was added into the above formulations at 1.5 per cent level. The solution was subjected to stirring continuously on a magnetic stirrer (SPINOT 6030) at 3000 rpm during slow addition of polyphenols and tween 80. The formed coarse solution was then subjected to ultrasonication (Qsonica, Q500, USA) at 20 kHz, 200 watts using 20 mm diameter probe for 5 minutes. The temperature of the process was controlled below 10 $^{0}$C until formation of nanosolutions of Quercetin and Tannic acid.

### Alginate Based Film Preparation

Film forming solutions were prepared by addition of sodium alginate at 2 per cent w/v level into distilled water. The solution was subjected to a temperature of 90 $^{0}$C for allowing gelatinization. Glycerol at 4 per cent level was added to the solution at 70 $^{0}$C as plasticizer. After bring down the temperature of the solution to room temperature, Quercetin (QUEN) and Tannic acid (TAN) nanosolutions at a concentration of 50 µl, 75 µl, 150 µl (T1, T2, T3) and 5 µl, 10 µl, 15 µl (T4, T5, T6) respectively were added to the alginate solution to produce different film forming solutions and the film forming solution without nanosolution was taken as control. Two per cent aqueous calcium chloride solution (w/v) at a concentration of 15 ml per 100 ml was added to all film forming solutions separately with continuous stirring to improve the physical properties of films. The solutions were casted onto petri plates having a diameter of 9 centimetres and were kept at 54 $^0$C for 24 hrs approximately in hot air oven to allow drying. The dried films were then removed carefully from the petri plates and stored in desiccators until being evaluated.

The developed edible films were evaluated for different parameters i.e., physico-chemical, mechanical, antioxidant, antimicrobial activity and total phenol content which were presented in Table 1. Based on the analysis of results of the above parameters of the films, best film was selected. Film was tested for its efficacy on pork patties as an active packaging film. The pork patties were prepared and wrapped in the above films and stored at refrigerated temperature (4±1 $^0$C). They were evaluated at periodic intervals for the following parameters.

**Figure 1:** Unpacked pork patties

**Figure 2:** Packaged pork patties with film

**Physico-chemical Characteristics**

***pH***

pH of the preparation was determined by following the method of Trout *et al.* (1992) using deluxe digital pH meter (model 101E). Five grams of representative sample was homogenized with 45 ml of distilled water in a laboratory blender for about one minute. The pH was recorded by immersing the combination glass electrode of digital pH meter in the homogenate. Before the measurement of pH, the pH meter was calibrated with buffer solutions of pH 4 and pH 7 as per user manual instructions to avoid errors.

***Thiobarbituric Acid Reactive Substances (2-TBARS) Value***

TBARS values were determined by the method of Tarladgis *et al.* (1960). Ten grams of sample was blended with 50ml of distilled water in a warring blender for 2 minutes. This mixture was transferred to a 500ml Kjeldahl flask quantitatively. Then the blender was rinsed with 45 ml of distilled water and was transferred quantitatively to the flask to which 5 ml of HCl was added previously. Few glass beads were added to the contents to avoid frothing and bumping. The contents of the flask were heated to 80-100 $^0$C with the help of a heating mantle and 50ml of distillate was collected in a stoppered measuring cylinder. Five ml of thoroughly mixed distillate was pipetted out in duplicates into 20 ml glass stoppered test tubes to which 5 ml of 0.02 M TBA reagent was added. The contents were mixed well and heated in a boiling water bath for 35 minutes. A blank consisting of 5 ml of distilled water and 5 ml TBA reagent was run simultaneously. After cooling the tubes under running water, optical density (OD) was measured in a spectrophotometer at 532 nm.

$$\textbf{TBARS value}(\textbf{mg of malonaldehyde per kg of sample}) = \textbf{OD} \times \textbf{7.8}$$

***Cooking Loss (Per Cent)***

Cooking loss per cent was determined by calculating the difference in weight of pork patties before and after cooking in a water bath for 20 min.

$$\textbf{Cooking yield}(\%) = \frac{\textbf{weight after cooking}}{\textbf{weight of raw emulsion}} \times \textbf{100}$$

$$\textbf{Cooking loss}(\%) = \textbf{100} - \textbf{cooking yield}(\%)$$

***Microbial Analysis***

The microbial quality of preparation was evaluated by estimating the Total plate count (TPC), Psychrophilic Bacterial Count (PBC) and Yeast and Mould counts (YMC) following pour plating technique as per standard procedure of ICMSF (1980).

***i. Preparation of Serial Dilutions***

For microbiological analysis, 5g of representative sample was homogenized with 45ml of 0.1 per cent sterile peptone water in laboratory blender and tenfold serial dilutions were made from each sample by using 0.1 per cent peptone water as diluent.

***ii. Total Plate Count***

Sterile petri plates were inoculated aseptically with 1ml sample from appropriate dilution in duplicates, to which 15-20ml of sterilized plate count agar (Himedia, Mumbai) at 44-46$^0$C was poured gently and the contents were mixed well for even distribution of the sample without air bubbles and plates were allowed to solidify. The plates were then incubated in inverted positions at 37 $^0$C for 24 – 48 hrs. Plates having 25-250 colonies were selected and the colonies were counted. The results were expressed as log units per gram of the sample.

***iii. Yeast and Mould Count***

The yeast and mould count were determined by pour plate method using Potato dextrose agar (PDA). Plates inoculated with sample were incubated at 23-25 $^0$C for 5-7 days. Colonies were counted and expressed as log units per gram of the sample.

***iv. Psychrophilic Count***

Psychrophilic bacterial count (PBC) was recorded on pour plates of plate count agar which were incubated at 4 $^0$C for 11 days period.

***v. Sensory Evaluation***

The pork patties which were wrapped in active edible sodium alginate films along with controls were fried and subjected to a six membered taste panel for sensory evaluation. Colour, appearance, flavour, juiciness, tenderness and overall acceptability were scored on a 9-point hedonic scale.

***vi. Statistical Analysis***

The results were analysed through SPSS (17.0) with n=6. The significance was defined at $P<0.05$ and Tukey’s multiple range tests were used to determine significant difference between the mean values.

## Results and Discussion

In the early phase of study, Sodium alginate films incorporated with different concentrations of nanosolutions were evaluated for quality based on their mechanical, physico-chemical, antioxidant and antimicrobial properties and were presented in Table 1 and Table 2.

**Mechanical Properties of Films**

The results for mechanical properties of Sodium Alginate films incorporated with different concentrations nanosolutions were presented in Table 1.

**Table 1:** Mean (± SE) values of mechanical properties of Sodium Alginate films incorporated with different concentrations of Quercetin ($T_1$, $T_2$ and $T_3$) and Tannic Acid ($T_4$, $T_5$ and $T_6$) nanosolutions

| Film characteristics | Treatment groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | **C** | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | **$T_5$** | **$T_6$** |
| Film thickness (*microns*) | 167.46 ± 2.75$^{a}$ | 166.80 ± 2.84$^{a}$ | 199.45 ± 2.82$^{c}$ | 194.53 ± 2.74$^{c}$ | 166.11 ± 2.36$^{a}$ | 185.13 ± 2.42$^{b}$ | 185.78 ± 2.63$^{b}$ |
| Overall mean | **167.46 ± 2.75** | **186.92 ± 3.80** | | | **179.00 ± 2.58** | | |
| Grammature (*gm/m$^2$*) | 226.75 ± 3.14$^{a}$ | 227.40 ± 3.42$^{a}$ | 255.15 ± 3.97$^{c}$ | 255.23 ± 3.51$^{c}$ | 227.96 ± 3.37$^{a}$ | 243.11 ± 3.33$^{b}$ | 237.20 ± 3.34$^{ab}$ |
| Overall mean | **226.75 ± 3.14** | **245.92 ± 3.74** | | | **236.09 ± 2.36** | | |
| Tensile strength (*N/cm$^2$*) | 0.29 ± 0.004$^{c}$ | 0.28 ± 0.003$^{bc}$ | 0.27 ± 0.003$^{b}$ | 0.24 ± 0.005$^{a}$ | 0.32 ± 0.014$^{d}$ | 0.32 ± 0.003$^{d}$ | 0.37 ± 0.003$^{e}$ |
| Overall mean | **0.29 ± 0.004** | **0.26 ± 0.004** | | | **0.32 ± 0.009** | | |
| Elongation at break (*%*) | 17.45 ± 0.15$^{c}$ | 17.62 ± 0.19$^{cd}$ | 16.12 ± 0.19$^{b}$ | 15.12 ± 0.19$^{a}$ | 18.29 ± 0.15$^{e}$ | 18.12 ± 0.19$^{de}$ | 17.62 ± 0.19$^{cd}$ |
| Overall mean | **17.45 ± 0.15** | **16.29 ± 0.26** | | | **18.01 ± 0.11** | | |

*Means bearing common superscript in each row do not differ significantly ($P \leq 0.05$)*

## Physico-chemical Properties of Films

The results for Physico-chemical properties of Sodium Alginate films incorporated with different concentrations nanosolutions were presented in Table 2.

**Table 2:** Mean (Mean ± SE) values of various Physico-chemical properties of Sodium Alginate films as affected by incorporation of different concentrations of Quercetin ($T_1$, $T_2$ and $T_3$) and Tannic Acid ($T_4$, $T_5$ and $T_6$) nanosolutions

| Film Characteristics | Treatment groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Control** | **Quercetin groups** | | | **Tannic Acid groups** | | |
| | **C** | **$T_1$** | **$T_2$** | **$T_3$** | **$T_4$** | **$T_5$** | **$T_6$** |
| Water vapour permeability (*×$10^{-10}$g m$^{-1}$ s$^{-1}$ Pa$^{-1}$*) | 15.44 ± 0.51$^{b}$ | 14.95 ± 0.43$^{b}$ | 13.15 ± 0.48$^{a}$ | 12.69 ± 0.30$^{a}$ | 13.05 ± 0.39$^{a}$ | 13.16 ± 0.3$^{a}$ | 12.60 ± 0.36$^{a}$ |
| Overall mean | **15.44 ± 0.51** | **13.60 ± 0.32** | | | **12.94 ± 0.20** | | |
| Water sorption (%) | 76.15 ± 1.53$^{d}$ | 72.02 ± 1.98$^{cd}$ | 70.78 ± 1.59$^{bc}$ | 66.80 ± 1.74$^{b}$ | 61.90 ± 1.56$^{a}$ | 59.56 ± 1.73$^{a}$ | 57.31 ± 1.52$^{a}$ |
| Overall mean | **76.15 ± 1.53** | **69.86 ± 1.10** | | | **59.59 ± 0.98** | | |
| Film opacity | 0.04 ± 0.004$^{a}$ | 0.05 ± 0.002$^{ab}$ | 0.07 ± 0.002$^{b}$ | 0.11 ± 0.005$^{c}$ | 0.05 ± 0.011$^{ab}$ | 0.07 ± 0.002$^{b}$ | 0.13 ± 0.013$^{c}$ |
| Overall mean | **0.04 ± 0.004** | **0.08 ± 0.006** | | | **0.08 ± 0.009** | | |
| Anti-oxidant activity (*µg/ml of trolox equivalent*) | 57.63 ± 1.93$^{a}$ | 68.85 ± 1.42$^{b}$ | 69.24 ± 1.80$^{b}$ | 70.57 ± 1.53$^{b}$ | 71.22 ± 1.44$^{c}$ | 77.70 ± 1.57$^{c}$ | 79.40 ± 1.59$^{c}$ |
| Overall mean | **57.63 ± 1.93$^{a}$** | **69.55 ± 0.88** | | | **76.10 ± 1.19** | | |
| Total phenol content (*mg of GA/gm of sample*) | 0.00$^{a}$ | 1.67 ± 0.002$^{b}$ | 1.68 ± 0.001$^{b}$ | 1.68 ± 0.001$^{b}$ | 1.74 ±0.014$^{c}$ | 1.75 ± 0.006$^{c}$ | 1.76 ± 0.002$^{c}$ |
| Overall mean | **0** | **1.68 ± 0.003** | | | **1.75 ± 0.005** | | |
| Antimicrobial activity against *S. aureus* (*diameter in mm*) | 0.00$^{a}$ | 7.83 ± 2.71$^{b}$ | 13.66 ± 1.87$^{bc}$ | 20.33 ± 1.45$^{de}$ | 16.83 ± 1.85$^{cd}$ | 23.50 ± 2.56$^{ef}$ | 26.16 ± 1.40$^{f}$ |
| Overall mean | **0** | **13.94 ± 1.67** | | | **22.16 ± 1.44** | | |
| Antimicrobial activity against *E. coli* (*diameter in mm*) | 0.00$^{a}$ | 8.66 ± 2.84$^{b}$ | 13.33 ± 1.54$^{bc}$ | 16.00 ± 1.65$^{c}$ | 14.50 ± 1.58$^{c}$ | 17.83 ± 1.64$^{cd}$ | 22.33 ± 1.22$^{d}$ |
| Overall mean | **0** | **12.66 ± 1.33** | | | **18.22 ± 1.12** | | |

*Means bearing common superscript in each row do not differ significantly ($P \leq 0.05$)*

In the late phase of the experiment, the edible film with Tannic acid nanosolution incorporated at 15µl level was selected to evaluate its effect on the quality of pork patties. Tannic acid, a gallic ester of D-glucose is recognised by its antioxidant capacity due to its multiple phenolic groups that can interact with biological macromolecules (Aelenei *et al.*, 2009). Development of plasticized film is easy with Tannic acid because of the ability of Tannic acid as a cross-linking agent. The initial phase of study revealed that the film with Tannic acid i.e., formulation $T_6$ had significantly good battier properties and antioxidant activity. Therefore, $T_6$ formulation was used to wrap pork patties and patties were evaluated at 0, 3, 5, 7, 9 and 11 days of refrigerated (4 ± 1 $^0$C) storage.

**Physico-Chemical Characteristics of Pork Patties**

The results for physico-chemical characteristics of pork patties were presented in Table 3.

**Table 3:** Mean (±SE) values of various Physico-chemical characteristics of pork patties wrapped in polyphenolic films

| Treatments | Days of Storage Period | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Day 0$^{NS}$** | **Day 3$^*$** | **Day 5$^*$** | **Day 7$^*$** | **Day 9$^*$** | **Day 11$^*$** | **Overall mean** |
| | | | | **pH** | | | |
| **C** | 5.90 ± 0.01 | 6.13 ± 0.02 | 6.32 ± 0.03 | 6.39 ± 0.03 | 6.42 ± 0.02 | 6.59 ± 0.03 | **6.29 ± 0.04$^y$** |
| **$T_6$** | 5.90 ± 0.02 | 6.06 ± 0.01 | 6.13 ± 0.02 | 6.22 ± 0.01 | 6.31 ± 0.01 | 6.41 ± 0.02 | **6.17 ± 0.03$^x$** |
| **Overall mean** | **5.90 ± 0.02$^a$** | **6.09 ± 0.01$^b$** | **6.23 ± 0.03$^c$** | **6.31 ± 0.03$^d$** | **6.37 ± 0.02$^e$** | **6.50 ± 0.03$^f$** | |
| | | | | **2-TBARS** | | | |
| **C** | 0.19 ± 0.01 | 0.50 ± 0.02 | 1.04 ± 0.02 | 1.86 ± 0.02 | 3.24 ± 0.01 | 4.15 ± 0.01 | **1.83 ± 0.24$^y$** |
| **$T_6$** | 0.15 ± 0.01 | 0.20 ± 0.01 | 0.38 ± 0.01 | 0.70 ± 0.01 | 1.09 ± 0.01 | 1.34 ± 0.01 | **0.64 ± 0.07$^x$** |
| **Overall mean** | **0.17 ± 0.01$^a$** | **0.35 ± 0.04$^b$** | **0.71 ± 0.09$^c$** | **1.28 ± 0.17$^d$** | **2.16 ± 0.32$^e$** | **2.75 ± 0.42$^f$** | |
| | | | | **Cooking Loss** | | | |
| **C** | 6.24 ± 0.01 | 8.71 ± 0.02 | 12.01 ± 0.02 | 13.85 ± 0.01 | 18.37 ± 0.02 | 20.28 ± 0.02 | **13.24 ± 0.83$^y$** |
| **$T_6$** | 6.09 ± 0.02 | 8.30 ± 0.02 | 11.84 ± 0.01 | 13.65 ± 0.02 | 18.01 ± 0.02 | 19.98 ± 0.01 | **12.98 ± 0.83$^x$** |
| **Overall mean** | **6.16 ± 0.02$^a$** | **8.50 ± 0.06$^b$** | **11.93 ± 0.03$^c$** | **13.75 ± 0.03$^d$** | **18.19 ± 0.05$^e$** | **20.13 ± 0.04$^f$** | |

*(P≤0.05); Means bearing common superscript along the row within storage periods and along the column within treatments does not differ significantly. (*) means significant difference between treatments, NS – non significant.*

***pH***

The mean pH values of pork patties wrapped in Tannic acid incorporated films were significantly (P≤0.05) lower than those of patties wrapped in control film. This could be due to lowered production of volatile base alkaline compounds by endogenous enzymes due to the presence of Tannic acid in the treatment formulation (Mohan *et al.*, 2008). During refrigerated storage, irrespective of formulations, the overall pH values of pork patties were increased significantly (P≤0.05) with increasing storage period. This might be due the liberation of ammonia compounds as a result of endoprotease activity or the proteolytic microbial flora present in the raw meat (Mokhtar *et al*., 2012). However, the rate of increase in $T_6$ film wrapped patty was at a slower pace, which can be attributed to the presence of active Tannic acid nanosolution in alginate film which helps in reduction of bacterial activity. The results were well in agreement with Moawad *et al.* (2017) who worked on the effect of combination of Olive oil extract and Tannic acid on quality of refrigerated beef patties, Annapareddy *et al.* (2015) in mutton balls applied with polymer coatings enriched with natural spice oils and Prathyusha *et al*. (2014) in chicken nuggets coated with edible polymer coatings enriched with natural antioxidants.

***Thibarbituric Acid Reactive Substances (2-TBARS)***

The TBARS value of 2 mg MDA /kg was regarded as the limit beyond which the product could normally develop objectionable odour and taste. In this study the initial TBA values of the pork patties wrapped in control film was in the range of 0.15 to 0.19 mg MDA / kg and the value exceed 2 mg/ kg on day 9 for control samples. However, the TBA values of $T_6$ film wrapped patty were well within the limits till day 11. Decreased TBARS values were due to highest antioxidant activity exhibited by Tannic acid present in the film. The high antioxidant effect of Tannic acid might be due to its chelating ability towards non-haeme iron in the minced meat. Iron is considering a strong pro-oxidant transitional metal that can increase lipid oxidation in meat. Because of their chemical structure Tannic acid could inhibit hydroxyl radical formation through Fenton reaction (Lopes *et al.*, 1999). The results were well in correlation with those of Mallika et al. (2017) in the study on the quality of chicken balls on application of edible film wraps enriched with natural spice oils, Moawad *et al.* (2017) who worked on the effect of combination of Olive oil extract and Tannic acid on quality of refrigerated beef patties, Maqsood and Benjakul (2010) in Tannic acid combined Modified Atmospheric Packaged refrigerated ground beef and Al-hijajin *et al*. (2016) who worked on the effect of Tannic acid on lipid peroxidation of raw and cooked chicken breast meat.

### *Cooking Loss*

The mean cooking loss values of pork patties wrapped in Tannic acid incorporated films were significantly ($P<0.05$) lower than those of patties wrapped in control film. Lowered cooking loss and thawing loss might be associated with lower evaporation rate respiration rate due to wrapping in films. (Mallika *et al.* 2018). Irrespective of type of formulation, cooking loss of pork patties was increased with increasing storage period. The results were well in agreement with those of Mallika *et al.* (2018) in pork patties coated with calcium alginate, Annapareddy *et al.* (2015) in mutton balls applied with polymer coatings enriched with natural spice oils, Prathyusha *et al*. (2014) in chicken nuggets coated with natural antioxidants enriched edible polymer coatings.

### *Microbial Analysis*

The results for microbiological quality parameters of pork patties were presented in Table 4.

**Table 4:** Mean (±SE) values of microbiological quality parameters of pork patties wrapped in edible polyphenolic films

| Treatments | Days of Storage Period | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Day 0$^{NS}$** | **Day 3$^{*}$** | **Day 5$^{*}$** | **Day 7$^{*}$** | **Day 9$^{*}$** | **Day 11$^{*}$** | **Overall mean** |
| **Total Plate Count** | | | | | | | |
| **C** | 4.61 ± 0.01 | 5.11 ± 0.01 | 5.45 ± 0.01 | 5.73 ± 0.01 | 6.65 ± 0.01 | 6.95 ± 0.01 | **5.75 ± 0.13$^{y}$** |
| **$T_6$** | 4.35 ± 0.01 | 4.72 ± 0.01 | 4.93 ± 0.01 | 5.10 ± 0.01 | 5.64 ± 0.03 | 5.80 ± 0.01 | **5.09 ± 0.08$^{x}$** |
| **Overall mean** | **4.48 ± 0.03$^{a}$** | **4.91 ± 0.05$^{b}$** | **5.19 ± 0.07$^{c}$** | **5.42 ± 0.09$^{d}$** | **6.14 ± 0.15$^{e}$** | **6.37 ± 0.17$^{f}$** | |
| **Yeast and Mould Count** | | | | | | | |
| **C** | 2.50 ± 0.01 | 2.70 ± 0.01 | 3.08 ± 0.02 | 3.23 ± 0.02 | 4.04 ± 0.02 | 5.34 ± 0.01 | **3.48 ± 0.16$^{y}$** |
| **$T_6$** | 2.46 ± 0.02 | 2.58 ± 0.01 | 2.93 ± 0.01 | 3.09 ± 0.01 | 3.81 ± 0.02 | 4.99 ± 0.02 | **3.31 ± 0.14$^{x}$** |
| **Overall mean** | **2.48 ± 0.01$^{a}$** | **2.64 ± 0.02$^{b}$** | **3.00 ± 0.02$^{c}$** | **3.16 ± 0.02$^{d}$** | **3.93 ± 0.03$^{e}$** | **5.16 ± 0.05$^{f}$** | |

*($P\leq0.05$) means bearing common superscript along the row within storage periods and along the column within treatments do not differ significantly. (*) means significant difference between treatments, NS – non significant.*

#### **i.** *Total Plate Count*

The mean total plate count values of pork patties wrapped in Tannic acid incorporated films were significantly ($P\leq0.05$) lower than those of patties wrapped in control film. During storage period the total plate counts increased with increasing storage period. However lower counts were noticed in patties wrapped in $T_6$ film than patties wrapped in control film. Unacceptable TPC values were found in control treated patties on day 7 under refrigerated storage temperature whereas the same was observed on 11$^{th}$ day for Tannic acid treated patties. The decreased values indicated strong antimicrobial activity of Tannic acid which was due to combined effects of adsorption of polyphenols to bacterial membranes and subsequent leakage of cellular contents and the generation of hydro peroxides from polyphenols (Negi *et al.*, 2012). The inhibitory effect of phenolic compounds also could be explained by interaction with enzymes, substrate and metal ion deprivation (Duman *et al.*, 2009). The results were well in agreement with those of Moawad *et al.* (2017) who worked on the effect of combination of Olive oil extract and Tannic acid on quality of refrigerated beef patties, Annapareddy *et al*. (2015) in mutton balls applied with polymer coatings enriched with natural spice oils and Prathyusha *et al*. (2014) in chicken nuggets coated with natural antioxidants enriched edible polymer coatings.

### *ii. Yeast and Mold Count*

The mean total yeast and mould count values of pork patties wrapped in Tannic acid incorporated films were significantly (P≤0.05) lower than those of patties wrapped in control film. The fungal activity of Tannic acid might be due to the astringent effect of Tannic acid to many microbial enzymes such as cellulose, pectinase, and xylanase and its toxicity on the cell wall membranes of microorganisms and their ability to chelate metals which reduce the availability of essential metal ions such as iron which is important for metabolic activities of microorganisms (Okuda *et al.*, 2011). The results were well in correlation with Annapareddy *et al*. (2015) in mutton balls applied with polymer coatings enriched with natural spice oils and Prathyusha *et al*. (2014) in chicken nuggets coated with natural antioxidants enriched edible polymer coatings.

### *iii. Psychrophilic Count*

There were no psychrophilic bacteria counts observed in both control and $T_6$ film wrapped patties. This might be due to the temperature variance for growth of psychrophilic bacteria. The results were in agreement with Biswas *et al*. (2004) in enrobed precooked pork patties under chilled and frozen storage conditions, Cholan (2008) in low-fat chicken nuggets and Chandralekha *et al.* (2010) in refrigerated chicken meat balls added with natural and synthetic antioxidants.

### *iv. Sensory Evaluation*

The results for Sensory parameters of pork patties were presented in Table 5.

**Table 5:** Mean (Mean ± SE) values of various Sensory parameters of pork patties as affected by wrapping in control and $T_6$ films

| Treatments | Days of Storage Period | | | | | | |
|---|---|---|---|---|---|---|---|
| | Day 0$^{NS}$ | Day 3$^*$ | Day 5$^*$ | Day 7$^*$ | Day 9$^*$ | Day 11$^*$ | Overall mean |
| **Colour** | | | | | | | |
| **C** | 8.0 5± 0.07 | 7.43 ± 0.08 | 6.51 ± 0.09 | 5.21 ± 0.09 | 4.1 ± 0.08 | 3.71 ± 0.09 | **5.85 ± 0.27$^x$** |
| **$T_6$** | 8.05 ± 0.07 | 7.51 ± 0.09 | 7.05 ± 0.07 | 6.68 ± 0.09 | 6.05 ± 0.09 | 5.95 ± 0.08 | **6.88 ± 0.13$^y$** |
| **Overall mean** | **8.05 ± 0.05$^f$** | **7.47 ± 0.06$^e$** | **6.78 ± 0.09$^d$** | **5.95 ± 0.23$^c$** | **5.108 ± 0.29$^b$** | **4.83 ± 0.34$^a$** | |
| **Flavour** | | | | | | | |
| **C** | 8.13 ± 0.09 | 7.01 ± 0.08 | 6.60 ± 0.09 | 5.80 ± 0.09 | 5.00 ± 0.09 | 4.00 ± 0.08 | **6.09 ± 0.23$^x$** |
| **$T_6$** | 8.13 ± 0.09 | 7.86 ± 0.08 | 7.31 ± 0.10 | 6.83 ± 0.10 | 5.98 ± 0.08 | 5.60 ± 0.14 | **6.95 ± 0.16$^y$** |
| **Overall mean** | **8.13 ± 0.06$^f$** | **7.44 ± 0.14$^e$** | **6.95 ± 0.12$^d$** | **6.31 ± 0.16$^c$** | **5.40 ± 0.16$^b$** | **4.80 ± 0.25$^a$** | |
| **Tenderness** | | | | | | | |
| **C** | 8.45 ± 0.08 | 7.30 ± 0.09 | 6.95 ± 0.08 | 5.85 ± 0.08 | 4.73 ± 0.10 | 3.91 ± 0.08 | **6.20 ± 0.26$^x$** |
| **$T_6$** | 8.45 ± 0.08 | 7.96 ± 0.08 | 7.50 ± 0.08 | 7.00 ± 0.08 | 6.13 ± 0.10 | 5.81 ± 0.10 | **7.14 ± 0.16$^y$** |
| **Overall mean** | **8.45 ± 0.05$^f$** | **7.63 ± 0.11$^e$** | **7.22 ± 0.10$^d$** | **6.42 ± 0.18$^c$** | **5.43 ± 0.22$^b$** | **4.86 ± 0.29$^a$** | |
| **Juiciness** | | | | | | | |
| **C** | 8.60 ± 0.09 | 7.31 ± 0.09 | 6.80 ± 0.10 | 5.88 ± 0.08 | 4.88 ± 0.08 | 4.00 ± 0.09 | **6.24 ± 0.26$^x$** |
| **$T_6$** | 8.60 ± 0.09 | 7.81 ± 0.10 | 7.16 ± 0.10 | 6.28 ± 0.09 | 5.73 ± 0.10 | 5.35 ± 0.06 | **6.82 ± 0.19$^y$** |
| **Overall mean** | **8.60 ± 0.06$^f$** | **7.56 ± 0.10$^e$** | **6.98 ± 0.09$^d$** | **6.08 ± 0.08$^c$** | **5.30 ± 0.14$^b$** | **4.67 ± 0.21$^a$** | |
| **Overall Acceptability** | | | | | | | |
| **C** | 8.30 ± 0.05 | 7.26 ± 0.05 | 6.70 ± 0.05 | 5.64 ± 0.04 | 4.69 ± 0.05 | 3.90 ± 0.03 | **6.08 ± 0.25$^x$** |
| **$T_6$** | 8.30 ± 0.02 | 7.79 ± 0.07 | 7.25 ± 0.05 | 6.59 ± 0.12 | 5.97 ± 0.02 | 5.67 ± 0.04 | **6.93 ± 0.16$^y$** |
| **Overall mean** | **8.30 ± 0.01$^f$** | **7.52 ± 0.09$^e$** | **6.98 ± 0.09$^d$** | **6.12 ± 0.15$^c$** | **5.33 ± 0.19$^b$** | **4.79 ± 0.26$^a$** | |

*(P≤0.05) means bearing common superscript along the row within storage periods and along the column within treatments do not differ significantly. (*) means significant difference between treatments, NS – non significant.*

There was significant difference (P≤0.05) between all the sensory attributes (colour, flavour, tenderness, juiciness and overall acceptability) of pork patties wrapped in $T_6$ film (sodium alginate + 15µl of Tannic acid nanosolution) and patties wrapped in control film. The mean values of sensory attributes (colour, flavour, tenderness, juiciness and overall acceptability) of pork patties wrapped in Tannic acid incorporated films were significantly ($P \leq 0.05$) higher than those of patties wrapped in control film. It is obvious that the sensory scores decreased significantly ($P \leq 0.0.5$) in both treatment and control groups with increasing refrigerated storage time. This might be due to microbial effect, oxidation of lipid and degradation of protein in the patties. But Tannic acid treated patties exhibited high scores in all sensory attributes compared to control which confirms the sensory stability characteristics of Tannic acid. The results were well in correlation with those of Moawad *et al*. (2017) who worked on the effect of combination of Olive oil extract and Tannic acid on quality of refrigerated beef patties, Annapareddy *et al.* (2015) in mutton balls applied with polymer coatings enriched with natural spice oils and Prathyusha *et al.* (2014) in chicken nuggets coated with natural antioxidants enriched edible polymer coatings.

## Conclusion

The results indicated that the natural polyphenols can be incorporated into edible films without having a detrimental effect on product quality producing a healthy meat product. Moreover, during refrigerated storage panel members preferred patties wrapped in Tannic acid incorporated films over control patties. This could be due to the appealing nature of films wrapped over patties. Tannic acid was a potent preservative which enhanced odour, lipid and microbial safety to maintain the sensory attributes of pork patties.

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## Conflict of Interests

There is no conflict of interest.

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