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Original Research

Development of Edible Active Packaging Films from Natural Polyphenol Loaded Nanosolutions

V. Giridhara Rao*[1], E. Naga Mallika[2], B. Eswara Rao[3] and T. Srinivasa Rao[4]

[1]Assistant Professor, Department of Livestock Products Technology, NTR College of Veterinary Science, Gannavram, Andhra Pradesh, INDIA
[2]Associate Professor, Department of Livestock Products Technology, NTR College of Veterinary Science, Gannavram, Andhra Pradesh, INDIA
[3]Professor, Department of Livestock Products Technology, NTR College of Veterinary Science, Gannavram, Andhra Pradesh, INDIA
[4]Associate Professor, Department of Veterinary Public Health and Epidemiology, NTR College of Veterinary Science, Gannavram, Andhra Pradesh, INDIA

*Corresponding Author: vanagiri1993@gmail.com

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Abstract

The study was conducted to develop and evaluate alginate based active edible films with nanoemulsions of polyphenols. Films were developed using sodium alginate incorporating polyphenol nanosolutions of Quercetin and Tannic acid and their mechanical and physico-chemical parameters were compared. The film thickness and grammature ranged from 166.80 ± 2.84 to 199.45 ± 2.82 microns and 227.40 ± 3.42 to 255.23 ± 3.51 gm/m2 respectively which were significantly higher in Quercetin nanoemulsion incorporated films than Tannic acid nanosolution incorporated films and control (without nanoemulsion). The per cent elongation at break, tensile strength, opacity mean values were significantly higher in Tannic acid incorporated films than Quercetin incorporated films and control films. The water vapour permeability and water sorption values were found to be lower in Tannic acid nanosolution incorporated films whereas antioxidant activity, phenol content and antimicrobial activity of Tannic acid incorporated films were significantly higher than Quercetin incorporated films.

Keywords: Active Packaging, Edible Films, Nano Solutions, Quercetin, Tannic Acid

Introduction

Application of edible films as active packaging materials moves forward the quality and security of foods besides reducing the menace of using too much of plastics. Edible films have long been known to guard perishable meals merchandise from deterioration and loss of quality. Besides acting as a barrier against moisture, gases, and volatiles mass diffusion, edible films can also act as carriers for a wide range of food additives, including flavouring agents, antioxidants and vitamins and can be considered in food preservation due to their ability to extend shelf life (Sallam *et al.,* 2007). Various bio-preservatives like Polyphenols could be incorporated into edible films to broaden their antimicrobial and antioxidant properties. These are a prime group of noticeably powerful antioxidants, because they showcase effective free radical scavenging pastime and safety towards oxidation of transition metals and lipid peroxidation (Zhou & Elias, 2013). Tannins have extensive spectrum and antimicrobial activity against bacteria like Staphylococcus aureus, Escherichia coli and most of them are able to suppress a number of microbial virulence and display synergism with antibiotics (Rodriguez V M J *et al.*, 2010). Quercetin is a natural polyphenol that with highest anti-radical action along with the potential to behave as a scavenger of loose radicals and an inhibitor of lipid peroxidation. It has antioxidant activity, highlighted by antimutagenic (Gupta *et al.,* 2010) and antitumorigenic (Wang *et al.,* 2012) properties. Quercetin also proved to be powerful against wide range of microbes including both Gram positive and Gram-negative bacteria. Tannic acid (TA) is a glucoside of gallic acid polymer with a couple of phenolic hydroxyl groups that are found in much vegetation (Gao Z *et al.,* 2014). This is an appealing molecule regarded to have antioxidant, antitumor and antibacterial hobby (Zhou L *et al.,* 2013) and also be used as a reducing agent.

In the food sector, the invasion of nanotechnological advances continues to be discreet but it is gaining an increasing interest (Rashidi & Khosravi-Darani, 2011). Of late, emulsions with nano droplet size, are being investigated as lipophilic drug delivery systems in food, cosmetic and pharmaceutical commodities (Bernardi *et al.,* 2011). Reduced droplet size of nano solutions not only enhances the carry of active molecules via biological membranes but also augment the surface area/volume ratio, consequently leading to better functionality. Conversely nanoemulsions are kinetically stable and see-through colloidal dispersions, and are apposite for a wide scope 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 physical, mechanical, anti-microbial and anti-oxidant properties of the edible films incorporated with nanopolyphenols.

Materials and Methods

Quercetin and Tannic Acid Nanosolutions Preparation

Quercetin and Tannic acid polyphenols each at 5 per cent w/v were selected for using 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 20 mm diameter probe for 5 minutes. The temperature of the process was controlled below 10°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°C for allowing gelatinization. Glycerol at 4 per cent level was added to the solution at 70°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 and 5 µl, 10 µl, 15 µl respectively were added to the alginate solution to produce different film forming solutions and film forming solution without nanosolution was taken as control. 2 per cent w/v aqueous calcium chloride solution 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°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.

Characterization of Films

Mechanical properties of films

The thickness of each film was measured in microns by electronic micrometer (0-25 mm) with an accuracy of 0.001 mm. The average of at least 10 random measurements was represented as film thickness. The grammature was after estimated as per the procedure demonstrated by Geraldine *et al.* (2008). Grammature was determined by dividing film weight in grams with its area in square metres.

$$\text{Grammature} \frac{\text{gm}}{\text{m2}} = \frac{\text{Weight of the film(gms)}}{\text{Area(m2)}}$$

The Tensile Strength value of the edible film was recorded as per the method of Berry and Stiffler (1981) with texture profile analyzer. Six observations were recorded for each sample to obtain the average value of shear force in Newton/cm2. Per cent elongation at break was measured according to method described by Soni *et al.* (2015).

Physico-chemical Properties of Films

Water Vapour Permeability

The water vapour permeability (WVP) of the films was measured gravimetrically based on ASTM E96-92 method described by Casariego *et al.* (2009). The film was sealed on the top of a glass permeation cup containing distilled water (RH 100%) at 20 ºC and placed in a desiccator which was maintained at 20°C and 1.5% RH and a vapour pressure of 28.044 Pa containing silica gel. The cups were weighed every hour for a period of 8 h. The water transferred through the film and adsorbed by the desiccant was determined from weight loss of the permeation cups. The slope of weight loss versus time was obtained by linear regression curve. WVP of the films was calculated as follows:

$$\text{water vapour permeability } (\times 10\text{-}10\text{g m-1 s-1 Pa-1}) = \frac{\Delta g}{\Delta t} \times \frac{X}{A.\ \Delta P}$$

Where,

$\Delta g/\Delta t$ - Rate of weight change (g/h),

x - Film thickness (m),

A - Permeation area (m2),

ΔP - Partial pressure difference across the film (4244.9 Pa at 30◦C).

Water Sorption Kinetics

The water sorption of edible sodium alginate films was evaluated by following the method of Lavorgna *et al.* (2010). The film samples were cut into small pieces of 2 cm × 2 cm size and placed in desiccator overnight and weighed to obtain their dry mass. Weighed samples were placed in closed beakers containing 30 ml of water (pH 7) and stored at 25°C. The swelling was evaluated by periodically measuring the weight increment of the samples. The film's wet surface was gently blotted with a tissue paper before weighing each time. The water gain of each sample was calculated by using following formula:

$$\text{Water gain\%} = \frac{\text{weight of wet film-weight of dry film}}{\text{weight of dry film}} \times 100$$

Light Transmission and Film Opacity

Transmission and opacity of the films were evaluated according to the method of Tunc and Duman (2010). The films were cut into rectangular pieces and were placed in the spectrophotometer cell. An empty compartment was used as a reference in the measurements. The light barrier properties of the film samples were measured by scanning

the samples at wavelengths between 200 and 800 nm using a UV spectrophotometer. Procedure was repeated for six replicates of each film. The transparency was calculated using the following equation:

Opacity=ABS600X

Where,

ABS600 - value of absorbance at 600 nm,

X - Film thickness in mm.

Antioxidant Activity

The antioxidant activity was determined by DMPD free radical scavenging assay as described by Fogliano *et al.* (1999). The compound N, N-dimethyl-1, 4-diaminobenzene (DMPD) is converted in solution to a relatively stable and coloured radical form by the action of ferric salt. After addition of a sample containing free radicals, these are scavenged and as a result of this scavenging, the coloured solution was decolourized.

Preparation of DMPD Solution

DMPD, 100 mM, was prepared by dissolving 209 mg of DMPD in 10 ml of deionised water. 1 ml of this solution was added to 100 ml of 0.1 M acetate buffer (pH 5.25) and the coloured radical cation (DMPD+) was obtained by adding 0.2 ml of a solution of 0.05 M ferric chloride (final concentration 0.1 mM). One millilitre of this solution was directly placed in a 1 ml plastic cuvette and its absorbance at 505 nm was measured. An optical density of 0.900 ± 0.100 unit of absorbance was obtained and it represents the uninhibited signal. The optical density of this solution, which is freshly prepared daily, is constant up to 12 h at room temperature.

DMPD Reagent Preparation

Solution 1: acetate buffer (0.2 mol·L-1, pH 5.25)

1a) 2.17 g of sodium acetate trihydrate was dissolved in 80 ml of ACS water.
1b) 300 µl of concentrated acetic acid (>99.5% v/v) was diluted to a volume of 20 ml with ACS water.

These two solutions were mixed to reach the pH 5.5

Solution 2: 0.74 mmol·L-1 ferric chloride: 1 mg of FeCl3.6H2O was dissolved with ACS water to a volume of 5 ml.

Solution 3: (36.7 mmol·L-1 DMPD) 25 mg of DMPD was dissolved in 5 ml of ACS water. This solution must be prepared at the time of use due to its low stability.

These three solutions (solutions No. 1, 2 and 3) were mixed in a 20:1:1 (v/v/v) ratio.

A 2.95 ml volume of above reagent was pipetted into a plastic cuvette. Then 50µl of film forming solution was added and absorbance was measured at 505 nm wavelength after 10 minutes at 25°C. Standard was prepared by adding 2.95 ml of DMPD reagent and 50 µl of trolox solution and Antioxidant activity was expressed as µg/ml of trolox equivalent.

$$\text{DMPD scavenging effect(D.SE\%)} = 1 - \frac{As}{Ac} \times 100$$

Where,

As – absorbance of sample,
Ac – absorbance of the standard.

$$\text{Trolox equivalent}\mu g/ml = D.SE\ \%0.7293 - 1.3857$$

Anti-microbial Activity

The anti-microbial activity was determined for the film forming solutions (S1 to S6) against Staphylococcus aureus and E. coli bacteria by using the agar well diffusion method of Pellissari *et al.* (2009) with slight modifications. 6 to 8 mm diameter hole was punched aseptically on Muller Hinton agar plates spreaded with 0.1 ml of inoculum with 105-106 CFU/ml of bacterial culture, standardised using McFarland scale. The floor of the well was sealed with agarose to avoid diffusion of solution beneath the agar. Using a sterile tip, a volume of 20 µl from each formulation of the film forming solution was introduced into the wells. The plates were incubated at 37±1°C for 24 h. The diameter of the zone of inhibition around wells was measured and equated against an ABST zone of inhibition scale and compared with standard antibiotic zones.

Total Phenol Content

The total phenol content of film forming was determined by using the method of Gulcin *et al.* (2005). 0.5 ml of sample from each filming solution was oxidized with 0.5 ml of Folin Ciocalteu's reagent in a volumetric flask. 1.5 ml of the 7.5% saturated sodium carbonate solution was added to each flask to neutralize the reaction followed by adjusting the volume to 23 ml with distilled water. The contents in the tubes were thoroughly mixed and allowed to stand at ambient temperature for 2 h until the characteristic blue colour developed. Absorbance of the clear supernatant was measured at 725 nm using a spectrophotometer. The total phenol content in film forming solution was calculated based on a standard curve prepared using gallic acid and expressed as milligrams of gallic acid equivalent (GAE) per gram of sample. Standard calibration was made from 15 to 500 µg/ml of gallic acid (R2 = 0.829). The overall mean values for the absorbance of different concentrations of gallic acid at 725 nm were presented in Table 1 and standard calibration curve was presented in Fig. 1.

Standard calibration curve was plotted using absorbance gallic acid at different concentrations i.e., 15.625, 31.25, 62.50, 250 and 500 in µg/ ml and the standard equation was depicted from the standard calibration curve.

Standard equation

$$\text{Absorbance } (\lambda 725) = 0.358x - 0.579$$

The total phenol content was calculated using the above standard equation and expressed as milligrams of gallic acid equivalent per gram of sample.

Table 1: Mean ± SE Absorbance values of gallic acid at different concentrations

Concentration of GA (*µg/ ml*)	**Absorbance (Mean ± SE)**
15.625	0.084 ± 0.02
31.25	0.156 ± 0.04
62.5	0.266 ± 0.03
1250	0.520 ± 0.02
250	1.070 ± 0.01
500	1.98 ± 0.013

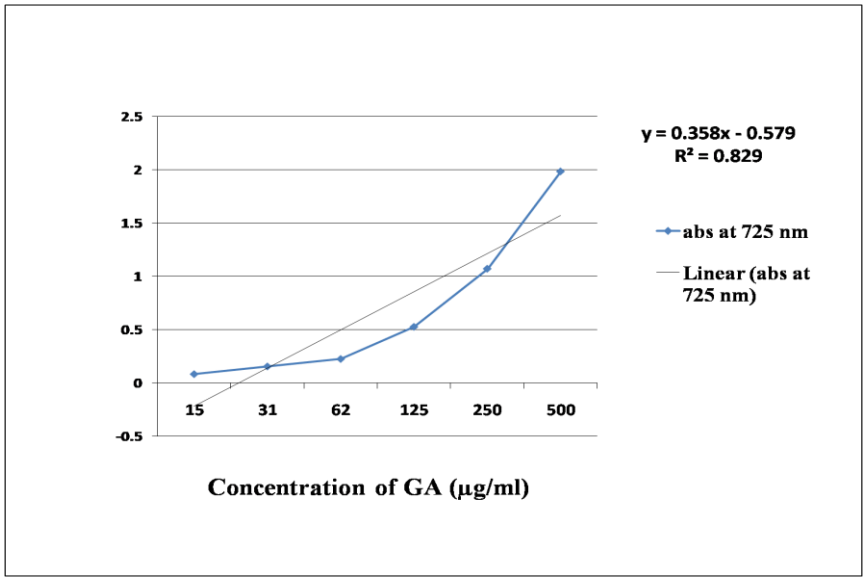

Figure 1: Standard calibration curve of gallic acid equivalent

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 whether there is significant difference between the mean values.

Results and Discussion

The quality of the Sodium alginate films incorporated with polyphenol nanosolutions were presented in Table 2 and 3.

Mechanical Properties of Films

The films with Quercetin nanosolution exhibited significantly (P<0.05) higher thickness and grammature values compared to control and Tannic Acid nanosolution incorporated films. Increase in film thickness & grammature with the incorporation of Quercetin was probably due to increased viscosity of the film forming solutions with more viscous biopolymer and polyphenol molecules resulting in thicker films (Taylor *et al.*, 2005 and Naga Mallika *et al.*, 2017). Irrespective of type of polyphenol incorporated the thickness and grammature were increased with increase in the concentration of active ingredient incorporated. This was probably due to the incorporation of air into matrix during cross linking of base matrix with polyphenol while mixing and the strong network formed might prevent subsequent release of the air (Hager *et al.*, 2012). The results were well in agreement with those of Giteru *et al.* (2015) and Hager *et al.* (2012). The films incorporated with Tannic acid had significantly (P<0.05) higher tensile strength than the other films. This could be due formation of cross linkage within the film structure which resulted in an increased mechanical strength due to addition of tannic acid (Hager *et al.*, 2012). The increase in the level of incorporation of Tannic acid into the films had lead to an increased trend in tensile strength of the films. This could possibly be due to the reinforcement effect tannic acid throughout the biopolymer matrix and formation of stable network at high concentration (Rivero *et al.*, 2010). But the addition of quercetin had recorded a decreasing trend with increase in the concentration of incorporation. The change in the intramolecular binding with the addition of Quercetin might be the reason for this trend (Souza *et al.*, 2015). The addition of hydrophobic agent like Quercetin could induce the development of structural discontinuities thus reducing the strength of the film (Rubilar et al., 2013). The results were well in accordance with those of Rivero *et al.* (2010), Cao *et al.* (2007), Mathew and Abraham (2008) and Souza *et al.* (2015). The films incorporated with Tannic acid nanosolution had significantly (P<0.05) higher percent elongation at break values than Quercetin incorporated film. Irrespective of the type of polyphenol incorporated the mean percent elongation at break were decreased with increase in the concentration of active ingredient incorporated. This was the result increased of intermolecular cross links coupled with decreased in intermolecular distance (Mathew and Abraham, 2008). The results were on par with those of Rivero *et al.* (2010), Hager *et al.* (2012) and Giteru *et al.* (2015).

Table 2: Mean (Mean ± SE) values of different mechanical properties of Sodium Alginate films as affected by incorporation of different concentrations of Quercetin (T1, T2 and T3) and Tannic Acid (T4, T5 and T6) 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<0.05)

Table 3: Mean (Mean ± SE) values of various Physico-chemical properties of Sodium Alginate films as affected by incorporation of different concentrations of Quercetin (T1, T2 and T3) and Tannic Acid (T4, T5 and T6) nanosolutions

Film characteristics	Treatment groups						
	CONTROL	**QUEN groups**			**TAN groups**		
	C	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
Water vapour permeability ($\times 10^{-10} g\ m^{-1}\ s^{-1}\ Pa^{-1}$)	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<0.05)

Physico-Chemical Properties of Films

The mean water vapour permeability values of polyphenol incorporated films were significantly (P<0.05) lower than the control films. The lower water vapour permeability of all other treated films could be due to tight network formed by cross linking capacity of Tannic acid with base matrix and its ability to occupy previously hydrophilic hydroxyl groups in the film and trapping of small air bubbles inside the matrix, resulting in reduced water molecule sorption in the cross-linked film (Hager *et al.,* 2012). The results were in conformity with those of Hager *et al.* (2012), Wang *et al.* (2017) and Souza *et al.* (2015). The films water solubility can influence its use for protection of the packaged product from the external environment. The film might be insoluble especially when applied to high moisture foods like meat (Giteru *et al.,* 2015). The films incorporated with Tannic acid nanosolution had significantly (P<0.05) lower water sorption values than their counterparts. The decreased water sorption of Tannic acid incorporated films might be due to the increased degree of cross linking with higher number of cross links resulting in low polymer mobidity thus reducing three dimensional rearrangements of the network during moisture sorption (Coussy *et al.,* 2011). The results were well in accordance with those of Zhang *et al.* (2010). Films with lower light transmission and higher opacity values could prevent lipid oxidation induced by UV light in a food system. The mean opacity values of active edible films incorporated with different concentrations of nanoemulsions of Quercetin and Tannic acid differed significantly (P<0.05) with control. The mean opacity values of polyphenols incorporated films were significantly (P<0.05) higher than control film values. In both Quercetin nanoemulsion and Tannic acid nanosolution incorporated films, the opacity values were significantly (P<0.05) increased with increasing the level of incorporation of polyphenol. However, T3 and T6 formulations had significantly (P<0.05) higher film opacity than all the other formulations. This might be due to the fact that alginate film containing polyphenols could act as good barriers for UV and visible light there by reducing the light induced lipid oxidation (Wang *et al.,* 2016). The results were in harmony with those of Wang *et al*. (2016) in chitin films incorporated with Tannic acid and Souza *et al.* (2015) in chitosan films incorporated with Quercetin.

Antioxidant activity indicates the ability of the films to scavenge free radicals. Determination of radical scavenging of the films is important because of harmful effects of free radicals in foods and biological systems. The mean antioxidant activity values of polyphenol incorporated films were significantly (P<0.05) higher than that of control. The films incorporated with Tannic acid had significantly (P<0.05) higher antioxidant activity value in terms of µg of trolox equivalent than Quercetin incorporated films. The antioxidant activity of Tannic acid incorporated films had increased significantly (P<0.05) with increasing concentration of polyphenol incorporation. This might be due to easy assembly of large amounts of Tannic acid onto the alginate films due strong hydrogen bonds and hydrophobic interaction between polymer and Tannic acid (Wang *et al.,* 2017). The antioxidant activity of Quercetin nanoemulsion incorporated films was also significantly (P<0.05) higher than control films. The results were well in agreement with those of Wang *et al.* (2017) in chitin film incorporated with Tannic acid, Zhang *et al.* (2007) in chitosan nanoparticles loaded with Quercetin. The mean antimicrobial activity values of polyphenol incorporated films were significantly (P<0.05) higher than that of control. The films incorporated with Tannic acid had significantly (P<0.05) higher antimicrobial activity values than Quercetin incorporated films. Irrespective of type of film, the antimicrobial activity increased with increased concentration of polyphenol incorporation. Tannic acid was found more effective on *S. aureus* than *E. coli* as indicated by the increase in the zone of inhibition with increase in the concentration, which was higher with Tannic acid. The antimicrobial activity of Tannic acid could be attributed to its strong chelating property towards divalent cations present on the surface of cell (Bakar *et al.,* 2017). The mechanism behind the antibacterial activity of Tannic acid could be related to its ability to inactivate enzymes and the surface proteins on the bacterial cells because of strong interaction with proteins through hydrogen binding and hydrophobic interactions (Kim *et al.,* 2010). The difference in the cell wall structures of G +ve and G –ve bacteria made *S. aureus* more vulnerable to Tannic acid attack (Fu *et al.,* 2015). Quercetin incorporated films also had significant (P<0.05) antimicrobial activity against *S. aureus* and *E. coli* when compared to control films. This could be due to a number of factors including the inhibition of nucleic acid synthesis i.e., the action of Quercetin on inhibition of DNA gyrase, permeability increases of bacterial internal membrane and dissipation of membrane potential of bacteria (Cushnie *et al.,* 2005). The results were well in accordance with Wang *et al.* (2017) who observed increased diameter of inhibition zones for Tannic acid incorporated films compared to control film and Widsten *et al.* (2017) who noticed antimicrobial activity of Tannic acid when applied on to the commercial films. The films incorporated with Tannic acid had significantly (P<0.05) higher phenolic values than Quercetin incorporated films. 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).

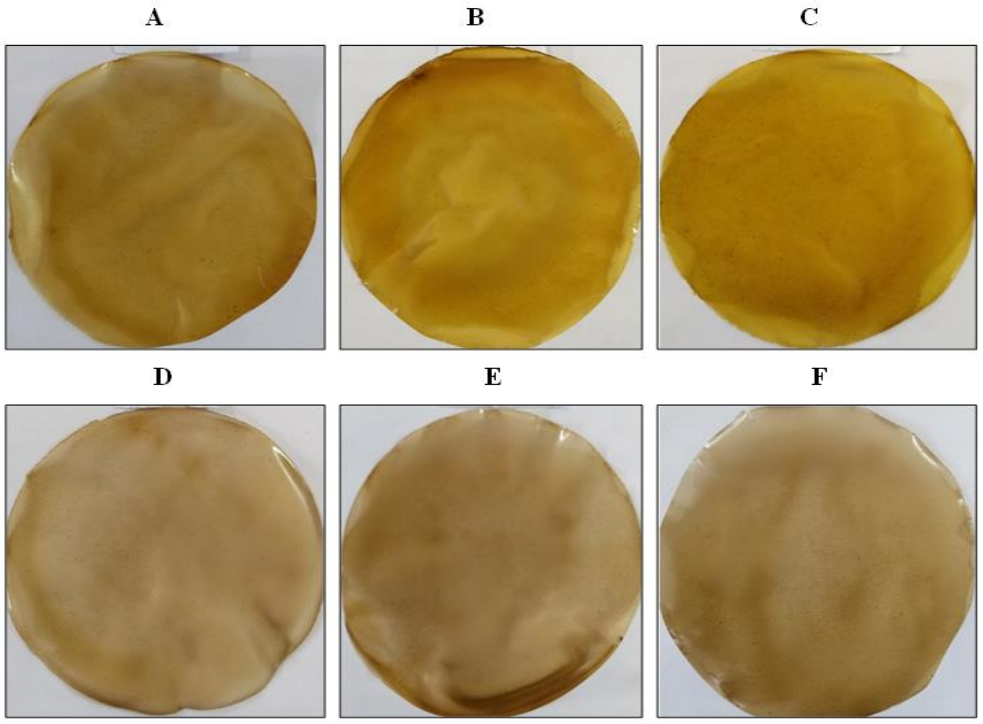

Figure 2: Sodium Alginate film with different concentrations of nanosolution
Fig. 2A: SA film + 50 µl 0f QUEN, **Fig.2B:** SA film + 75 µl 0f QUEN, **Fig. 2C**: SA film + 150 µl 0f QUEN, **Fig.2D:** SA film + 5 µl 0f TAN, **Fig.2E:** SA film + 10 µl 0f TAN, **Fig.2F:** SA film + 10 µl 0f TAN, *(SA-Sodium Alginate, QUEN–Quercetin Nanosolution, TAN-Tannic Acid Nanosolution)

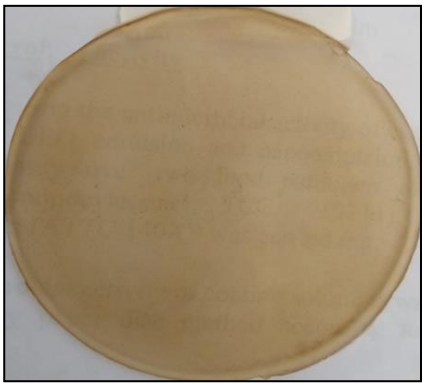

Figure 3: SA film without Nanosolution (control)

Conclusion

Edible films are alternative food packaging to improve the quality and safety of food products. In this study, sodium alginate-based films were developed with incorporation of nanosolution of Quercetin and Tannic acid in different concentration. A concentration of 150 µl of Quercetin and 15 µl of Tannic acid yielded better films. The films with Tannic acid at a concentration of 15 µl (T6) has good film forming properties along with better antioxidant and antimicrobial activity to form a part of active edible packaging. This study reveals the potential application of edible films as carriers of antioxidant and antimicrobial agents in packaging to extend the shelf-life of the packaged product.

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Conflict of Interests

There is no conflict of interest.

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