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Naga Mallika E [α], Haritha M [σ], Eswara Rao B [ρ], Srinivasa Rao T [ω] & Reddy GVB [¥]

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The mean thickness, grammature, opacity values, tensile strength and percent elongation at break were altogether ($P<0.05$) higher in cardamom oil nanoemulsion incorporated films contrasted with films with gingeroil nanoemulsion and control. The water Vapour permeability estimates were higher in films with ginger essential oil nanoemulsion contrasted with films of cardamom essential oil nanoemulsion. The water sorption kinetics estimations of control films were essentially ($P<0.05$) higher than the remainder of details.

The mean Antioxidant value of ginger essential oil nanoemulsion and cardamom basic oil nanoemulsion films were 80.37 and 98.06 and cardamom basic oil nanoemulsion films were essentially ($P<0.05$) higher in their antioxidant competence than ginger essential oil nanoemulsion and control films. The mean log decrease of both E.coli and S.aureus were 1.66, 2.87 and 1.54, 2.91 for Ginger and cardamom oil added films correspondingly. The log decrease was critical for both E.coli and S.aureus yet high with S.aureus. Contingent upon the investigation of above quality parameters, film with 100μl of CEON (Cardamom Essential Oil Nano Emulsion) was chosen as best film and used to assess it's capability to expand the shelf life of chicken patties. The outcomes showed that cardamom essential oil nanoemulsion can be effectively incorporated into sodium alginate films and can successfully be utilized as a food wrap and as a palatable packaging for meat and meat items.

I. Introduction

The quality and safety of meat is profoundly subjected to the applied packaging materials. Essentially fresh and handled meat products are being packaged for averting ruining and to postpone their deterioration. Packaging additionally assists in diminishing the weight loss also which is imperative in terms of economics. (Fani et al. 2018). The current methods of packaging range from an over-wrap packaging and vacuum packaging to Modified Atmospheric packaging. As of late, a progression of new packaging advancements and materials has been created including Active packaging, intelligent packaging, edible coatings or films.

Expanding enthusiasm towards utilization of edible coatings and films was escalated in the ongoing past. Taking into account of their ecological agreeable nature, a few biopolymers, for example, starch, cellulose, chitosan, gums, alginate and protein (kheziran 2018, Jridi 2018, Romanvi 2017, Mallika 2018) can be utilized as base materials for producing edible films, as these materials offer chance of acquiring films with added advantage of viable addition of essential oils, so as to create self-motivated active packaging films. These films can perhaps assure food quality and safety, stretch the shelf life of food, decrease environmental effect on food and can expand appeal of the packaged item. But, just a set number of innovations are appropriate to meat and meat items.

Alginate is a characteristic polysaccharide extracted from the cell walls of brown sea weed and could be utilized in food industry, and is known for its biodegradability, non-harmfulness, biocompatibility, low cost and extraordinary colloidal properties which incorporate thickening, stabilizing, suspending film forming, gel creating and emulsion settling properties. Alginate films forces an incredible barrier property to oxygen and carbon dioxide, great mechanical properties and it could likewise be a decent transporter of various added substances (Kafrani et al 2016).

Essential Oils are the common enemies of oxidants and when joined straightforwardly in to the food item may adjust the taste. To stretch out the time span of usability of the food product and to shield the item from oxidation and deterioration, Essential oils can be effectively included into the edible films. They could be gradually delivered on to the food surface from the film and can stay in adequate fixation, for lengthening the shelf life of the food.

The essential oils of ginger have been accounted for their solid antimicrobial, antifungal and cancer prevention performance. (Singh et al., 2008; Noori et al. 2018). The green cardamom (Elettaria cardamomum) is local to South Asia and its basic oil,

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have curative advantages like antibacterial, antifungal, anticancerous, antispasmodic, gastroprotective and anti inflammatory activity (Mejdi et al.,2016). Essential Oils when combined directly into the food may change the organoleptic properties of the food besides causing toxicity at high concentrations or they may lose their activity while responding with environmental variables such as cooking and addition of other food ingredients. To overcome these issues, a new approach is to encapsulate active compounds and to enhance their transport by developing of nanoemulsions. Nano emulsions are colloidal scatterings framed by the blend of two immiscible stages and balanced out by a surfactant, with oil droplets of size in the scope of 20-200nms. They are optically transparent in contrast with customary emulsions and this feature is a bit of advantage for food application.

II. Materials and Methods

With this in the present investigation, an attempt was made to develop sodium alginate based edible films incorporated with natural essential oil nanoemulsions i.e., ginger and cardamom and to evaluate their effect on the quality of chicken patties. Based on the results obtained, best film was selected and the film was applied to the product and the shelf-life of the product was studied under refrigeration (4±1°C) temperature at regular intervals to record the effect of nanoemulsions of essential oil loaded sodium alginate films on quality of chicken patties and to record the efficacy of the film as an active packaging.

The experiment was conducted in two parts. Ginger and cardamom essential oils were made into nanoemulsions and they were incorporated in to sodium alginate based films to prepare active packaging films. Their quality was tested and after analysing their activity during experiment two, the best film from the above experiment was applied on to chicken meat patties and quality of chicken patties was studied in order to evaluate the efficacy of the films as active packaging films.

a) Preparation of Essential Oil Nanoemulsions

Ginger essential oil (GEO) and cardamom essential oil (CEO) each at 5 per cent v/v were selected for using in sodium alginate based films to produce active packaging films. Coarse emulsions of above essential oils were formed by continuous stirring and tween 80 was added at 1.5 percent level as surfactant. The formed coarse emulsion were ultrasonicated (Qsonica, Q500, USA) at 20 KHZ, 200 watts with 20mm diameter probe for 5minutes. The temperature of the process was controlled at less than 10^0C until formation of nanoemulsions of ginger essential oil (GEON) and cardamom essential oil (CEON).

b) Preparation of Film Forming Solutions

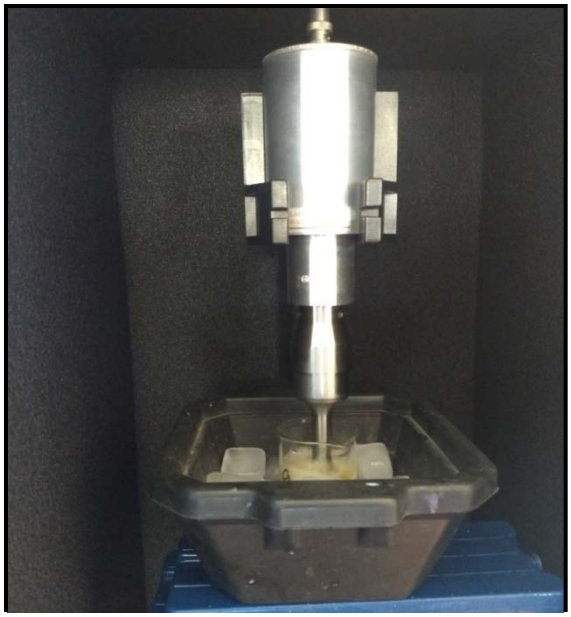
Film forming solutions were prepared with 2% sodium alginate. Glycerol at 4 per cent level was added as plasticizer. After the temperature reached to 37^0C GEON at 10, 20 and 50 μl and CEON at 10,50 and 100 μl were added to the alginate solution to produce six different film forming solutions i.e., Sodium alginate film forming solution with 10 μl of GEON (S_1), with 20 μl of GEON (S_2), with 50 μl of GEON (S_3), with 10 μl of CEON (S_4), with 50 μl of CEON (S_5), with 100μl of CEON (S_6).

c) Preparation of Active Edible Films

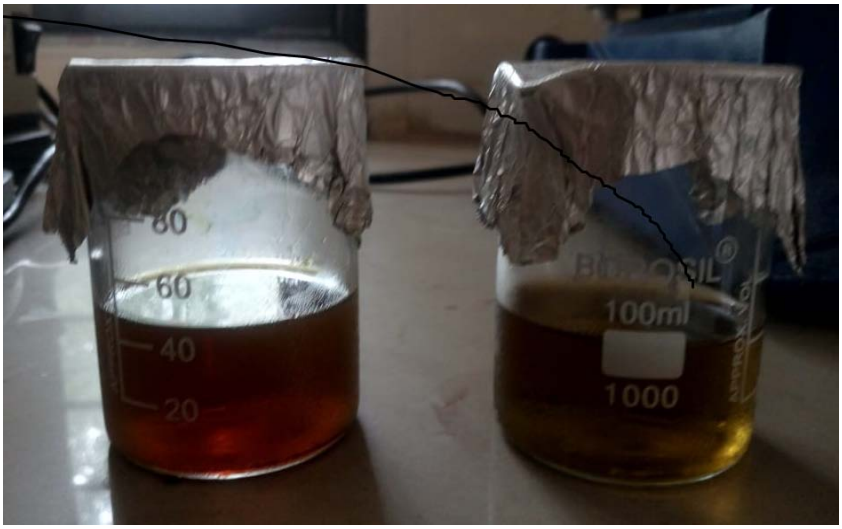
2%v/v aqueous calcium chloride solution at a concentration of 15 ml per 100ml of solution was added to all film forming solutions separately with continuous stirring to improve the physical properties of films. The solutions were casted onto petri plates and were allowed to dry to form six different types of films viz T_1, T_2, T_3, T_4, T_5, T_6 from S_1, S_2, S_3, S_4, S_5, S_6 respectively. The dried films were then removed carefully from the petri plates and stored in desiccators until being used for further studies.

The films were evaluated for different parameters and the results were analysed through SPSS (20.0) with n = 6.

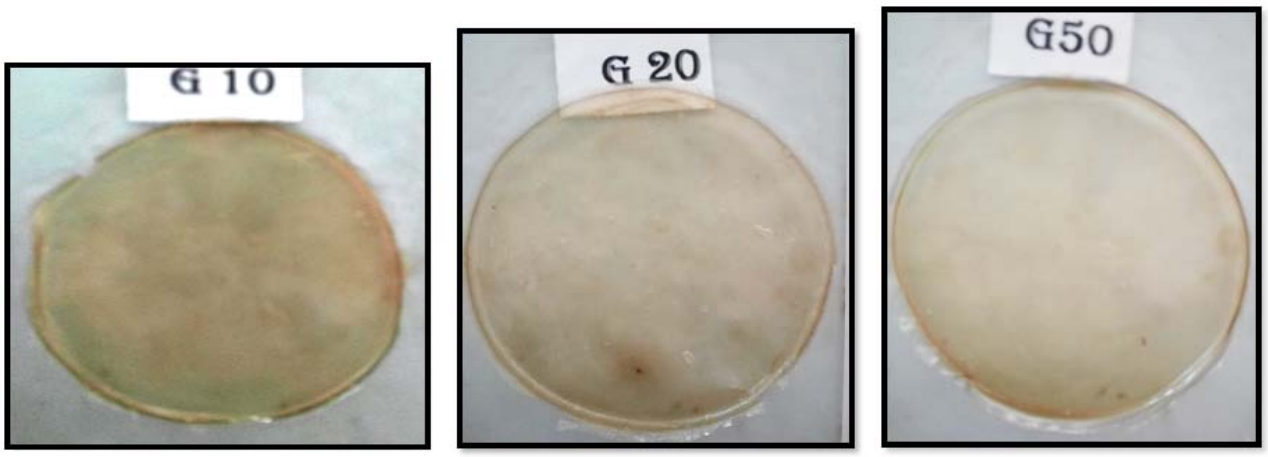
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Preparation of nanoemulsion by ultrasonication

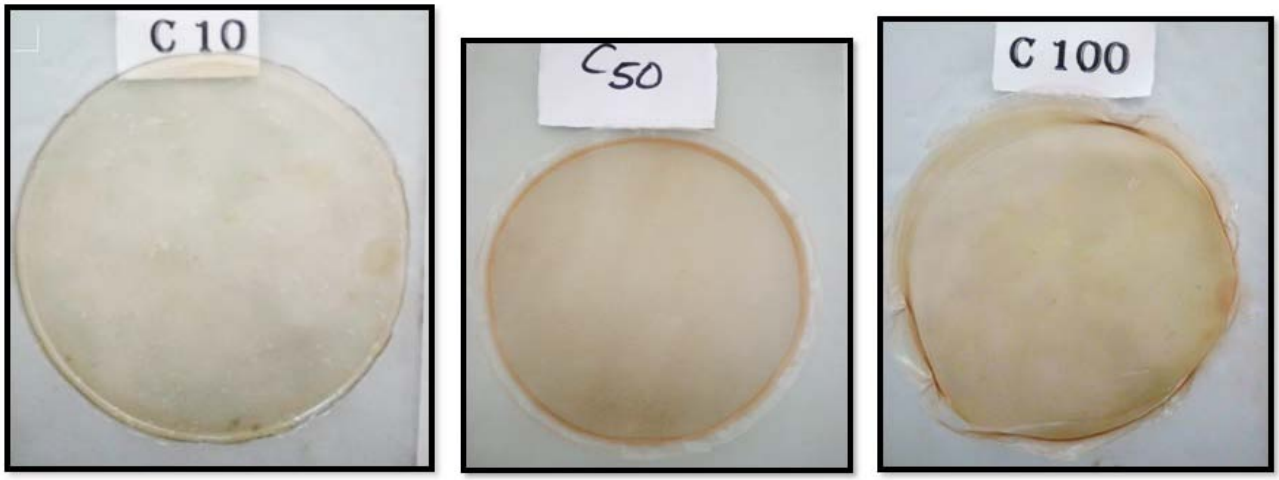


Nanoemulsions of Ginger essential oil (left) and Cardamom essential oil (right)



Sodium alginate films incorporated with different concentrations of ginger essential oil nanoemulsions

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Sodium alginate films incorporated with different concentrations of cardamom essential oil nanoemulsions

The thickness of each film was measured in *microns* by electronic micrometer (0-25 mm) with an accuracy of 0.001 mm..The average thickness for at least ten random locations has represented as film thickness. The grammature was estimated as per the procedure demonstrated by Geraldine *et.al* (2008).

The water vapour permeability (WVP) of the films was measured gravimetrically based on ASTM E96-92 method as described by Casariego *et al.* (2009). The film was sealed on the top of a glass permeation cup containing distilled water of 20 ºC ,100% RH; 2.337 & 103 Pa vapour pressure 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:

$$\boldsymbol{water\ vapour\ permeability(WAP)} = \frac{\Delta \boldsymbol{g}}{\Delta \boldsymbol{t}} \times \frac{\boldsymbol{X}}{\boldsymbol{A}.\Delta \boldsymbol{P}}$$

Where,

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

x - Film thickness (m),

A - Permeation area (m),

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

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 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:

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

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 three replicates of each film. The opacity was calculated using the following equation:

$$\boldsymbol{Opacity} = \frac{\boldsymbol{ABS600}}{\boldsymbol{X}}$$

Where,

ABS600 - value of absorbance at 600 nm,

X - film thickness in mm.

In vitro assessment of the antibacterial activity of essential oil nano emulsions was carried out in accordance with the method of Wang et al 2015 with slight modification. In brief, 6 hrs cultures of S.aureus and E.coli were diluted McFarland standard 1 i.e., 0.5 to adjust a microbial count of approximately 1×10^8 cfu/mL. 0.1% Peptone water was sterilized and serial dilutions were prepared and kept for overnight for sterility checking.1 ml of test microbial solution that is adjusted to McFarland standard 1 was taken in to test tube

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containing 9ml peptone water and serial dilutions were made. For testing of antibacterial activity the nano emulsions of ginger and cardamom essential oils were aseptically prepared and the 10, 20, 50 µl of giner essential oil nanoemulsion and 10, 50, 100 µl of cardamom essential nanaemulsion were transferred to test tubes containing serially diluted test microbe solution and incubated for 24 h at 37 ^{0}C. Distilled water was used as control. For determination of the colonies, the incubated samples with different serial dilutions were plated onto agar plates (plate count agar) and incubated for 24 h at 37 ^{0}C. Subsequently, colonies were counted for E.coli and S. aureus for each concentration of essential oils and total cfu (colony forming units) was determined, and growth reduction calculated according to Eq. (1). A logarithmic microbial growth reduction of less than 0.5 represents no antibacterial activity. Values between 0.5 and 1 are rated as a slight, values greater than 1 and less or equal to 3 as a significant, and a log reduction greater than 3 as a strong antibacterial activity.

Log growth reduction $_{(24h)}$ = log cfu (control) $_{(24h)}$ - log cfu (sample) $_{(24h)}$ ..... (1)

The anti-microbial activity was determined for the films (T_1 to T_6) by the agar diffusion method, of Pelissari *et al.* (2009).The edible films were aseptically cut in to 2- mm discs and placed on muller hinton agar plates spreaded with 0.1 ml of inocula with $10^5 - 10^6$ CFU/ml of bacterial culture, standardised against McFarland scale. The plates were incubated at 37±1°C for 24 h. The diameter of the zone of inhibition around the discs was measured and equated against an ABST zone of inhibition scale and compared with standard antibiotic zones.

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 radicles, these are scavenged and as a result of this scavenging, the coloured solution is decolourized.

d) 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.

e) 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 $FeCl_3.6H_2O$ 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 sample 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.

$$\boldsymbol{DMPD\ scavenging\ effect(D.SE\%) = \left(1 - \frac{A_s}{A_c}\right) \times 100}$$

Where,

A_s – absorbance of sample,

A_c – absorbance of the standard.

$$\boldsymbol{Trolox\ equivalent(\mu g/ml) = \frac{D.SE\ \%}{0.7293} - 1.3857}$$

f) Tensile Strength and % Elongation at Break

The tensile strength and % elongation at break was measured following the procedure demonstrated by Soni A *et.al* (2016). The mechanical properties of the films were measured by means of its tensile strength (TS) and % elongation at break (EAB).

The TS value of the edible film was recorded as per the method of Berry and Stiffler 1981.The TS of the film was measured with texturometer. Each film was cut into 8 × 2 cm strips and then mounted between grips onto the texturometer and stretched until they broke. Six observations were recorded for each sample to obtain the average value of shear force in $\boldsymbol{newton/cm^2 (N/cm^2)}$

Elongation at break was measured according to the method of Soni A *et.al* (2016). for calculation of per cent elongation at break, films were cut into 8 × 2 cm strips, and fixed on a manually formed scale. One end of the film was fixed and other end is stretched manually until it was broken. The EAB of the films was calculated as follows:

$$\boldsymbol{elongation\ at\ break(\%) = \frac{B - A}{A} \times 100}$$

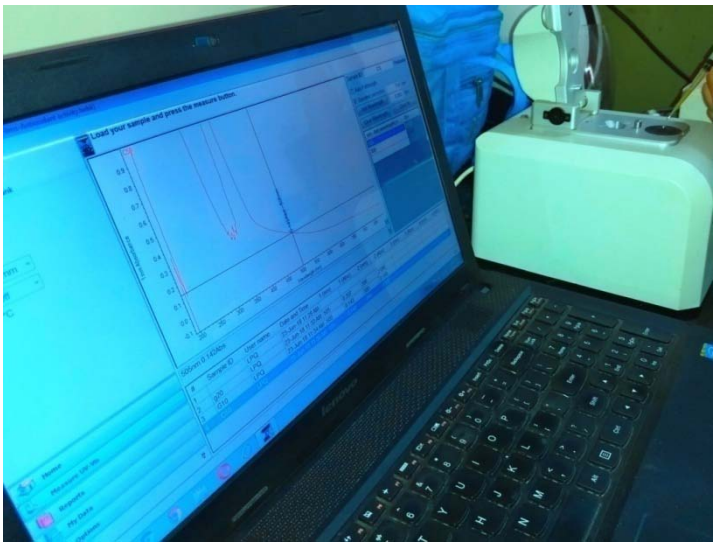
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where,

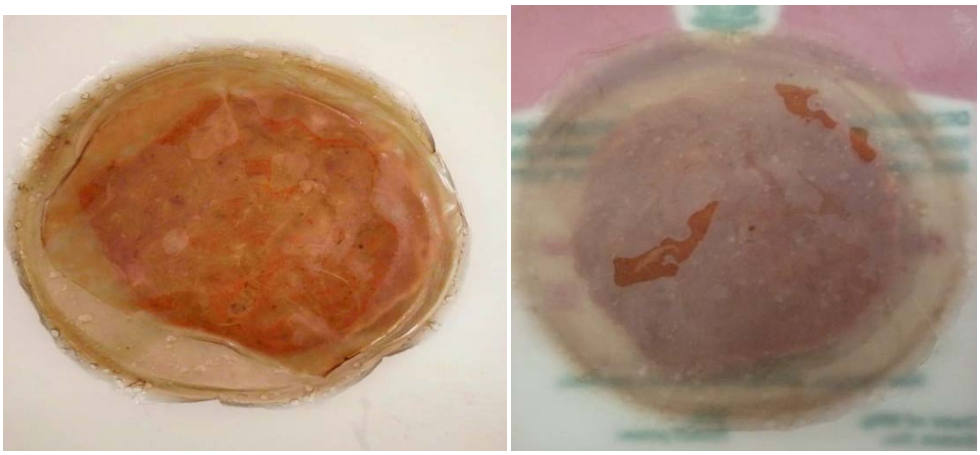
A – intial length of the film and

B – Final length of the film after stretching/at the time of break.

The results of the above parameters were analysed and depending on the activity of the films, the best film was selected and the selected film was proved for its efficacy extend in the shelf life of the product packed in it. The data was subjected to statistical analysis using SPSS IBM, version 20.0.(n=6)



Measuring the absorbancy values for Antioxidant activity



Product Stored in the film

III. Results & Discussion

Thickness of edible film is related to transperancy, water vapour permeability and mechanical properties of the films. (Ejaz et al.2018). The mean thickness and grammature values of both GEON and CEON incorporated films were significantly ($P<0.05$) higher than the control films. The films incorporated with CEON had significantly higher thickness and grammature values than GEON incorporated films. Irrespective of the type of essential oil incorporated the mean thickness and grammature of the films were increased with increasing concentrations of active ingredients incorporated. This might be due to the formation of thin layer over the surface of film due to the hydrophobic interactions between the oils and polysaccharide film (Ejaz et al.2018). The results obtained in the present study were in accordance with Ejaz et al. (2018) in bio nanocomposite gelatin film with clove oil and zinc oxide nano rods and Benavides et al. (2012) in alginate films with oregano oil.

Water vapour permeability measures the diffusion of water molecules through the cross section of the film and can give an estimation of its barrier property. To prevent dehydration of foods, films used as packaging or coating must control the moisture transport from the product to the environment. Hence WVP of edible films should as low as possible (Fabra et al.2008). Generally WVP depends on the diffusivity and solubility of water molecules in the film matrix (Wang et al. 2013).

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The mean water vapour permeability of films incorporated with 100 μl of ginger emulsion oil nano emulsion was significantly ($P<0.05$) higher than the other films. The mean WVP of films incorporated with CEON was significantly ($P<0.05$) lower than GEON incorporated films and control. In GEON films, the increase in the concentration of nano emulsion water vapour permeability values were increased significantly ($P<0.05$). This might be due to an increase in the number and size of the holes appearing in the polymer matrix (Chen et al.2016). The increase in the concentration of CEON incorporation leads to a decrease in the water vapour permeability of the films. This might be due to the hydrophobic nature of essential oil that inhibits the water transmission across the film. The WVP of polymer films depends on the hydrophilic and hydrophobic ratio of the film constituents. This could be the possible reason for variation in WVP trend in different treatments (Soni et al. 2016). These results were well in accordance with those of Soni et al. 2016 in oregano and thyme oil incorporated carrageenan based edible films for packaging of chicken patties and Chen et al. 2016 with chitosan films containing Cinnamaldehyde nanoemulsions.

The films water solubility can influence its use for protection of the packaged product from the external environment. The film might be water insoluble especially when applied to high moisture foods like meat (Giteru et al. 2017).

The mean water sorption of both GEON and CEON incorporated films was significantly ($P<0.05$) lower than the control films. The films incorporated with CEON had significantly ($P<0.05$) lower water sorption values than GEON incorporated films. The reduced film solubility in essential oil nano emulsions incorporated films might be due to its hydrophobicity (Soni et al. 2016). Irrespective of the type of essential oil incorporated the mean water sorption kinetic values of the films was decreased with increasing concentration of active ingredients incorporated. This might be due to the higher degree of substitution of water by oil droplets which could lower hydrophilicity of film, thereby lowering the soluble matter of films (Dammak et al. 2017).The results were well in accordance with those of Soni et al. (2016) in essential oil incorporated carrageenan based edible films and Rostamzad et al. (2016) in fish protein films with nanoclay and transglutaminase for food packaging.

Films with lower light transmission and higher opacity values could prevent oxidation induced by UV light in a food system (Rostamzad et al 2016). The mean opacity values of both GEON and CEON incorporated films was significantly ($P<0.05$) higher than the control films. The films incorporated with CEON had significantly ($P<0.05$) higher opacity than GEON incorporated films. Irrespective of the type of essential oil incorporated the mean opacity of the films was increased with increasing concentrations of active ingredients incorporated. This could be attributed to decrease in light transmission and due to the light scattering or interference of light passage by the lipid droplets in essential oils (Ejaz et al. 2018).

This would indicate that alginate films containing essential oils could act as good barriers for UV and visible light thereby reducing the light induced lipid oxidation. These results were in accordance with those of Ejaz et al. (2018) in gelatin composite films with clove essential oil and zinc oxide nanorods and Soni et al. (2016) in essential oil incorporated carrageenan based edible films.

Anti oxidant activity evaluates the capacity of the films to scavenge free radicals. Determination of radical scavenging activity of films is important because of harmful effects of free radicals in foods and biological systems (Noori et al.2018).

The mean anti oxidant activity in μg/ml trolox equivalent of active edible films incorporated with different concentrations of GEON and CEON was significantly ($P<0.05$) higher than the control films. The films incorporated with CEON had significantly ($P<0.05$) higher anti oxidant values than GEON incorporated films. This could be due to the presence of chemical components such as α-zingiberene and α-terpinyl acetate in ginger and cardamom essential oils which were known to have strong anti oxidant activity (Noori et al. 2018, Kandikattu et al.2017). Irrespective of the type of essential oil incorporated the mean anti oxidant activity in μg/ml of trolox equivalent was increased with increasing concentrations of active ingredients incorporated. The formation of nano emulsion had decreased the degradation of essential components coupled with increasing the surface area of essential oils in the film thereby achieving a fast and efficient free radical absorption with increasing concentrations of essential oil incorporation (Noori et al. 2018).

The higher antioxidant of CEON incorporated films might be due to the assembly of large amounts of essential oil on to the alginate film due to the strong hydrophobic interactions between polymer and essential oil (Wang et al. 2017). The results were well in agreement with Noori et al (2018) in chicken breast fillets added with nanoemulsion of Ginger Essential oil and Kandikattu et al. (2017) on anti inflammatory and anti oxidant effects of Cardamom.

Anti microbial activity was evaluvated against gram- negative *(E.coli*) and gram- positive bacteria (*S.aureus*) to gain a better understanding of the mechanisms of anti bacterial activity and were expressed in log CFU/ml. The mean *E.coli* and *S.aureus* counts of both GEON and CEON incorporated films were significantly ($P<0.05$) lower than the control films. The films incorporated with CEON had significantly ($P<0.05$) lower counts than GEON incorporated films. Irrespective of the type of essential oil incorporated the

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mean *E.coli* and *S.aureus* counts were decreased with increasing concentration of active ingredients incorporated, into the films and especially with the formulations T_3 and T_6 . The mean log reduction of GEON and CEON incorporated films against both *E.coli* and *S.aureus* were 1.66, 2.87 and 1.54, 2.8 respectively. The log reduction was significant for both *E.coli* and *S.aureus* but high with *S.aureus*. The mean log reduction in the bacterial counts might be due to the presence of α – zingiberene, β- sesquiphellandrene and zingiberenol in ginger essential oil (Noori et al. 2018) and α-terpinyl acetate and 1-8 cineole in cardamom essential oil (Mejdi et al 2016). The mode of action of the Essential oils against bacteria is thought to be due to the hydrophobic nature of oils, the active components cross bacterial membrane easily leading to loss of ions which results in reduction of the electric potential of membrane and loss of function of protons, thus decreasing ATP which promotes cell death of bacteria. A reduction in droplet size of essential oils by nano emulsion formation would allow anti microbial compounds to penetrate faster in to the bacterial cells, thus a higher anti microbial behavior was observed with increasing concentration of essential oil nano emulsion incorporation. The results were in accordance with Noori et al. (2018) with nano emulsions of Ginger essential oil on chicken breast fillets, Kandikattu et al.(2017) with Cardamom essential oil and Trujillo et al.(2015) with lemon grass essential oil.

Edible packaging films must with stand the normal stresses encountered during its application and the subsequent shipping, storage and handling of the food. To maintain its integrity and barrier properties, high puncture strength is required. Therefore film tensile strength is of most important in accordance with the intended application of the film. The mean Tensile strength and percent elongation at break values of both GEON and CEON incorporated films were significantly ($P<0.05$) higher than the control films. The films incorporated with CEON had significantly ($P<0.05$) higher Tensile strength and percent elongation at break than GEON incorporated films. Irrespective of the type of essential oil incorporated the mean tensile strength and per cent elongation at break of the films increased with increasing concentration of active ingredients incorporated. This might be due to a kind of cross linking between chemical components of essential oils and alginate base matrix which developed a resistant and elastic film matrix structure (Dammak et al. 2017). The Nano emulsions with droplets of oil loaded into sodium alginate were liquid at 25^0 C and its presence in the film structure at nanoscale size can easily be deformed enhancing the film flexibility, thereby increasing the tensile strength and percent elongation of the films with increasing concentration of essential oil nano emulsion incorporation (Dammak et al. 2017). The results were in accordance with Dammak et al. 2017 in properties of active gelatin films with rutin loaded nano emulsions.

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