Rubia cordifolia based Novel Edible Film for Improved Lipid Oxidative and Microbial Stability of Meat Products

Rubia cordifolia based edible film

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Abstract

Rubia cordifolia-based bioactive edible film was developed for the preservation of meat products. The film was developed using different concentrations of *R. cordifolia* viz. 0.50% (T_1), 0.75% (T_2), 1.0% (T_3) and 0.0% (control) and their efficacy was assessed using chicken nuggets as a model system. The samples were analysed on 0, 15, 30, 45 and 60 days of refrigerated (4±1 °C) storage. Addition of *R. cordifolia* increased ($P<0.05$) total phenolic and total flavonoid content and reduced ($P<0.05$) free fatty acids, thiobarbituric acid reacting substances and microbial counts [total plate, psychrophilic, yeast/mould and anaerobic counts] while improving the sensory quality of the products. Addition of *R. cordifolia* significantly increased the thickness, opacity and moisture content (%) whereas decreased the solubility (%) of the films. The *R. cordifolia* based edible film significantly improved the lipid oxidative and microbial stability of the model meat product during refrigerated storage and can have commercial applications.

Keywords: *Rubia cordifolia;* edible film; meat products, lipid oxidative stability; microbial stability; sensory quality

1. Introduction

Rubia cordifolia, commonly known as Indian Madder, is a highly valuable and well-known medicinal plant that belongs to the coffee family, *Rubiaceae* (Chandrashekar et al., 2018). The root extract of *R. cordifolia* has a potent antioxidant, antimicrobial, antiviral and free radical scavenging activity owing to a range of bioactive metabolites (Chandrashekar et al., 2018; Martelli & Giacomini, 2018). Recently, *R. cordifolia* mediated nanoparticles were developed by Ahn, Jin, and Park (2019) who observed antioxidant and free radical scavenging properties of the developed silver nanoparticles. However, no study has explored the potential of *R. cordifolia* as an active ingredient for edible and biodegradable films for food applications. Being a rich source of polyphenols, flavonoids, and free radical scavengers (Chandrashekar et al., 2018; Martelli & Giacomini, 2018), its use as a bioactive ingredient in food packaging is highly justifiable.

Plant extracts have been widely used in meat and meat products (Kaur et al., 2021; Kalem, Bhat, Kumar, & Jayawardena, 2018; Dua, Bhat, & Kumar, 2016, 2015a, b; Singh et al., 2015a, b, 2014a, b, 2012; Jamwal et al., 2015) with a significant improvement in colour and lipid stability and storage quality (Bhat, Kumar, & Kumar, 2015a, b; Kaur, Kumar, Bhat, & Kumar, 2015; Kumar, Bhat, & Kumar, 2015, 2016). Unfortunately, plant extracts at their effective concentrations can also have a negative impact on sensory quality (Zargar, Kumar, Bhat, & Kumar, 2017, 2016; Kaur, Kumar, & Bhat, 2015a, b, c). Further, the efficacy of these extracts can be reduced at the higher cooking temperatures of meat products (Noor et al., 2018a, b, 2017). This explains the use of edible and biodegradable films as carriers of the bioactive plant ingredients to control the lipid oxidative and microbial changes in meat products. Edible or biodegradable films have recently attracted great attention from meat researchers due to their special properties, such as use of natural food-grade materials, non-toxic, bioactive, and biodegradable properties (Xiong, Chen, Warner, & Fang, 2020). Addition of plant metabolites to the films, such as phenolic compounds with high antioxidant activity and reducing power, can reduce the effect of storage on the quality of meat products (Sharma et al., 2021). The primary and secondary metabolites of these plant extracts can promote radical scavenging activity (Ahn et al., 2019) and can slow lipid and protein oxidation of meat and meat products during refrigerated storage (Kumar, Bhat, & Kumar, 2013). The objective of the present study was to develop an alginate and maltodextrin based edible film containing *R. cordifolia* as a novel bioactive ingredient and to evaluate its impact on lipid oxidative stability and storage quality during refrigerated storage using chicken nuggets as a model system.

2. Materials and methods

2.1. Spice mix, condiments and chemicals

The spice mix formula standardized by Bhat, Pathak, and Fayaz (2013) was developed in the laboratory and contained cloves 2%, nutmeg 2%, bay leaves 2%, mace 2%, black cardamom 5%, degi mirch (*Capsicum annum*) 5%, white pepper 5%, cinnamon 6%, green cardamom 6%, red chilli 8%, black pepper 10%, aniseed 12%, cumin seed 15% and coriander 20%. The onion, garlic and ginger were used in the condiment mixture in a ratio of 3:2:1, respectively, and were ground to the consistency of a fine paste in a mixer-grinder (Bhat & Pathak, 2012). All the chemicals were of analytical or food grade and were purchased from standard firms such as Hi-Media, Qualigens and Sigma-Aldrich.

2.2. *Rubia cordifolia*

The *R. cordifolia* purified extract was purchased from 'The Himalaya Drug Company (India)' available for human consumption as a general health supplement. This root extract contained antimicrobial compounds such as munjistin (xanthopurpurin-2-carboxylic acid), purpurin (trihydroxy anthraquinone), peudopurpurin (purpurin-3-carboxylic acid) and free alizarin and its glucoside, in addition to other metabolites with antioxidant properties (The Himalaya Drug company, 2020). Four concentrations of the extract viz. T_1 (0.50%), T_2 (0.75%), T_3 (1.0%) and control (0.0%) were incorporated in the films.

2.3. Preparation of the film

Maltodextrin and alginate based edible film was developed using the method described by Sharma et al. (2021). To prepare the film, glycerol (20 g) was added to the mix of sodium alginate (5 g) and maltodextrin powder (45 g). In case of the *R. cordifolia* based films, the powdered extract was also added to this mix. This mixture was dissolved in double distilled water (210 ml) and blended for 10 min to prepare a homogenous solution that was stirred for 4 h using a magnetic stirrer and a flea. This solution (50 ml) was laid out on cellophane coated glass tray (30×20 cm) using a glass rod. These uniform thin sheets were removed from the cellophane after ambient drying for 48 h and dipped in a solution of $CaCl_2$ (2.75 g) and carboxymethylcellulose (0.9 g) in water (49 ml) for 30 min. The films were used after another drying period of 24 h.

2.4. Preparation of chicken nuggets

The chicken meat was purchased from the local market, transported to the laboratory in a chilly bin on ice, deboned manually, packed in sterilized polythene bags and stored at -18 °C till used (Kumar, Bhat, Kumar, & Singh, 2012; Pathak, Bhat, Bukhari, & Ahmad, 2009a, b). The meat emulsion for nuggets was prepared according to the method described by Kumar, Kumar, and Bhat (2012) and Bhat and Pathak (2009). The frozen meat was partially thawed at refrigerated temperature and sliced into small chunks which were minced in a meat mincer using 6 mm plate. Meat emulsion was prepared in a Sirman bowl chopper (MOD C 15 2.8G 4.0 HP, Marsango, Italy) by blending minced meat (68.20%) with curing ingredients (salt 2%, sugar 0.5%, sodium hexametaphosphate 0.3% and sodium nitrite 150 ppm) for 1.5 min. This was followed by the serial addition of crushed ice (10%) and refined soyabean oil (10%) with 1 min of blending after addition of each. This was followed by addition of all other ingredients (spice mixture 2%, condiments 3% and refined wheat flour 4%) and blending till emulsion was ready. The emulsion was filled into greased stainless-steel moulds and steam cooked at 121 °C for 30 ± 2 min. The chicken nuggets were cooled and were wrapped in the edible films [T_1 (0.50%), T_2 (0.75%), T_3 (1.0%) and control (0.0%)] and packaged under vacuum (polyethylene/aluminium packs, 220 micron thick) and stored at refrigeration temperature (4±1 °C) for 60 days. The samples were taken on 0, 15, 30, 45 and 60th day and were evaluated for various quality parameters.

2.5. Physicochemical and microbiological parameters

The pH of the samples was determined by the method of Bhat, Morton, Mason, and Bekhit (2018a, b) using Ultra Turrex T10 tissue homogenizer (Janke and Kenkel, IKA labor Technik, Germany) and a digital pH meter (Product code 35613424, Oakton instruments, Singapore). The method described by Bhat et al. (2019a, b, c) was used to measure the moisture content of the products using a hot air oven. The method described by Mahajan, Bhat, and Kumar (2015a, b) was used to measure thiobarbituric acid reacting substances (TBARS). The optical density was recorded at 538 nm and the values was expressed as mg malondialdehyde per kg of sample. The method described by Mahajan, Bhat, and Kumar (2016a, b) was used to measure free fatty acids (FFA, % oleic acid) using the given formula.

$$\text{Free fatty acids (\% oleic acid)} = \frac{(0.1 \times \text{ml of KOH used} \times 0.282)}{\text{wt. of sample}} \times 100$$

The microbial counts viz. total plate, psychrophilic, coliform, anaerobic and yeast/mould counts were determined following the methods described by Bukhari, Pathak, Bhat, and Ahmed (2012, 2013, 2014) and Kumar, Bhat, and Kumar (2011). Stored samples were opened in a laminar flow chamber sterilized by ultraviolet irradiation. Ten grams of the sample was taken aseptically and blended with 90 ml of 0.1 percent sterile peptone water with a pre-sterilized blend. Serial ten-fold dilution was made in pre-sterilized tubes containing 9 ml volume of 0.1 percent peptone water. The sample preparation was done near flame under laminar flow (Thermo Electron Corporation D-63505 Langenselbold, Robert Boschstr. 1, Germany). Duplicate sets of sterilized Petri plates containing media were inoculated aseptically with aliquots from appropriate dilution. Following incubation, plates showing 30-300 colonies were counted and expressed as $\log_{10}$ cfu/g of the sample (Ahmed, Pathak, Bhat, & Bukhari, 2014).

2.6. Total phenolics and total flavonoids

Total phenolic and flavonoid contents of the extracts were determined using the methods described by Sharma et al. (2021). A final concentration of 0.1 mg/ml was used to evaluate the samples and the results were expressed as mg/g Gallic acid equivalents and mg/g Quercetin equivalents for total phenolic and flavonoid content, respectively.

2.7. Characterization of the film

Thickness of the developed film was measured at eight different locations using a micrometre (0-10 mm, Swastik scientific company, Mumbai, India). The method of Sharma et al. (2021) was used to determine the opacity by measuring the absorbance of a rectangular piece of a film at 550 nm. The transparency of a film was calculated by the ratio of absorbance to that of the thickness and six measurements were carried out for each film. The lower absorbance values suggested higher transparency. The method of Sharma et al. (2021) was followed to measure the moisture content by drying the small pieces (2-3 cm) of a film in a hot air oven at 100 °C for 12 h and the weight loss was expressed on percentage basis. The solubility of the films was measured in water by the method of Sharma et al. (2021). Pre-weighted small-dried pieces (2-3 cm) of a film (dried in a hot air oven at 100 °C for 12 h) were dissolved in distilled water and stirred gently for 2 min at room temperature. The pieces were dried at 105 °C in an oven till the weight became stable and the weight difference before and after dissolution was expressed in percentage.

2.8. Sensory evaluation

Sensory evaluation of the product samples was carried out for colour and appearance, flavour, texture, juiciness and overall acceptability by a mixed gender panel of trained members composed of scientists and research scholars based on a 9-point hedonic scale, wherein 9 denoted “extremely liked” and 1 denoted “extremely disliked” (Bhat, Pathak, Bukhari, Ahmad, & Bhat, 2010). Ten members of the panel replicated the experiment thrice. Samples were presented to the panellists and the serving order of the samples was randomized. Three-digit coded samples were served at room temperature (25 ^{o}C) and water was given for oral rinsing between the samples to avoid carry-over effect.

2.9. Statistical analysis

The experiments were repeated six times (n=6) and the data compiled were analysed by one-way ANOVA (film characteristics) or two-way ANOVA (other parameters) except for sensory evaluation that was analysed using a repeated measurements ANOVA to investigate the effect of treatments and storage time by General Linear Model using Statistical Package for Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA). The measured variables were set as dependent variables and the results were presented as means ± standard errors. The fixed effects included in the model were treatments, storage time and their interactions. The random effects in the model were effects for batches and their interactions with fixed effects. For sensory analysis, treatment was considered as the main effect and panellists as random variable. The effect of storage time and treatments were analysed using Duncan's multiple range tests at 0.05 level of significance.

3. Results and Discussion

3.1. Oxidative stability

The mean values of total phenolic content (mg Gallic acid equivalents/g) and total flavonoid content (mg Quercetin equivalent/g) of the edible films containing different concentrations of *R. cordifolia* viz. T_1 (0.50%) T_2 (0.75%) and T_3 (1.0%) are presented in Figures 1 and 2. The total phenolic content (mg Gallic acid equivalents/g) and total flavonoid content (mg Quercetin equivalent/g) of the edible films showed a significant increase on addition of *R. cordifolia*. The mean values for both total phenolic and total flavonoid contents were higher on 0th day and showed a decreasing trend with increasing storage time. This increase in the oxidative capacity of the bioactive films might be attributed to the phenolic compounds, flavonoids and other bioactive phytochemicals present in *R. cordifolia* (Chandrashekar et al., 2018; Martelli & Giacomini, 2018). Several studies have reported an increase in the total phenolic and total flavonoid contents of the edible films incorporated with bioactive plant extracts (Sharma et al., 2021; Akcan, Estevez, & Serdaroglu, 2017).

3.2. Physicochemical properties of the film

The mean values of various physicochemical properties of the edible film containing different concentrations of *R. cordifolia* viz. control (0.0%), T_1 (0.50%) T_2 (0.75%) and T_3 (1.0%) are presented in Figures 3, 4 and 5. Addition of *R. cordifolia* affected the physicochemical properties of the film and a significant decrease was recorded in the solubility (%) whereas a significant increase was observed in the thickness (mm), opacity and the moisture content (%) of the bioactive films compared to the control film. The thickness is a significant factor that can affect the permeability, transparency and mechanical properties of an edible film (Zhang et al., 2020). The mean values of the thickness of the films were 0.05, 0.07, 0.08 and 0.09 mm for control, T_1, T_2 and T_3 films, respectively. While previous studies have reported similar values for the thickness of the films containing plant extracts (Saricaoglu, Tural, Gul, & Turhan, 2018; Tural & Turhan, 2017), increase in the thickness of the edible films on addition of natural antioxidant extracts was reported by Akcan et al. (2017). This increase in the thickness of the films (Akcan et al., 2017) was attributed to the changes in rheological properties and was also suggested to have contributed to the barrier properties, thereby increasing the antioxidant potential of the films. Addition of *R. cordifolia* might have increased the density of the film which might have resulted in a decrease in the transparency of the films. Addition of plant extracts has been reported to increase the particle size that can affect the distribution of film forming solutions and can also reduce the solubility of films in water (Saricaoglu et al., 2018). The addition of essential oils or plant extracts can affect the moisture content of the edible films by affecting the hydrophobicity or hydrophilicity of the developed films (Zhang et al., 2020).

3.3. Lipid stability

The mean values of various lipid stability parameters of chicken nuggets packaged in edible films containing different concentrations of *R. cordifolia* viz. control (0.0%), T_1 (0.50%) T_2 (0.75%) and T_3 (1.0%) are presented in Table 1.

3.3.1. Thiobarbituric acid reacting substances

A significant impact of the packaging was observed on the lipid oxidation (TBARS, mg malondialdehyde/kg) and the mean TBARS values of the products packaged in the films containing *R. cordifolia* were significantly ($P<0.05$) lower than control samples. The lowest values were recorded for the products packaged in T_2 and T_3 films. The TBARS analysis is a widely accepted method that is aimed at measuring the levels of malondialdehyde, a secondary metabolite product of lipid oxidation (Bhat, Morton, Mason, & Bekhit, 2020a, b). This effect of the packaging on the lipid oxidation might be attributed to *R. cordifolia* that contains high amounts of bioactive phytochemicals which have potential to inhibit the chain reactions of lipid oxidation and neutralize the free radicals (Chandrashekar et al., 2018; Martelli & Giacomini, 2018). The extract of *R. cordifolia* has strong antioxidant and radical scavenging properties and contains a range of metabolites with antioxidant properties such as anthraquinones, alizarin, hexapeptides, triterpenes, glycosides, quinones, rubiadin, quinine, iridoids, purpurin, munjistin, purpuroxanthin and pseudopurpurin (Chandrashekar et al., 2018; Martelli & Giacomini, 2018). Previous studies and present research suggest that extracts containing high phenolics are beneficial in reducing TBARS values in meat products. For example, Xiong et al. (2020) observed significantly lower TBARS values for the pork samples packaged in the edible films containing grape seed extract during refrigerated storage.

The TBARS values of all the products, both control and treatments, increased significantly ($P<0.05$) with increasing storage time. While the TBARS values of the control samples exceeded the threshold limit of 1 mg malonaldehyde/kg for consumer acceptance (Kalem et al., 2018a, b) on 45th day of storage, the TBARS values of the products packaged inside the *R. cordifolia* films remained below the limit during the entire period of storage. A significant increase in TBARS values was found for meat sausages coated in garlic oil incorporated edible films during refrigerated storage (Esmaeili et al., 2020).

3.3.2. Free fatty acids (% oleic acid)

The free fatty acids (FFA) of the products packaged in the films containing *R. cordifolia* (T_1, T_2 and T_3) were significantly ($P<0.05$) lower than control samples during entire storage time and the lowest values were observed for T_2 and T_3 films. The effect of the packaging on the FFA values might be attributed to the strong antioxidant and antimicrobial properties of *R. cordifolia* due to the presence of several bioactive phytochemicals (Chandrashekar et al., 2018; Ismail, Wedyan, Al Zuabe, & Abderrahma, 2016). The FFA values of all the products, both control and treatments, did not exceed the threshold value of 1.8% for consumer acceptance for cooked meat products during entire storage time (Kalem, Bhat, Kumar, & Desai, 2017). Free fatty acids are produced from polar and neutral lipids due to oxidative, microbial or enzymatic lipolysis during storage and gives information about the stability of lipids in the meat matrix (Noor et al., 2018a). The growth of lipolytic bacteria which produce lipases and/or phospholipases, such as *Pseudomonas*, can cause lipid hydrolysis in meat during storage (Dilnawaz et al., 2017a, b). Significantly lower FFA values have been reported in meat products packaged in edible films containing plant extracts such as *T. cordifolia* and *T. arjuna* during refrigerated storage (Kalem et al., 2018a, b).

3.4. Physicochemical parameters

The mean values of pH and moisture content of chicken nuggets packaged in edible films containing different concentrations of *R. cordifolia* viz. control (0.0%), T_1 (0.50%) T_2 (0.75%) and T_3 (1.0%) are presented in Table 1. Packaging had a significant impact on pH and the products packaged in the edible films containing *R. cordifolia* (T_1, T_2 and T_3) showed significantly ($P<0.05$) lower pH values during storage except on day 0. This effect of the films on the pH might be attributed to the phenolic and acidic compounds present in *R. cordifolia* (Martelli & Giacomini, 2018; Chandrashekar et al., 2018). Previous studies have reported a similar decline in the pH of meat products packaged in edible films containing bioactive plant ingredients (Xiong et al., 2020).

While packaging did not alter the moisture content ($P>0.05$) of the products, there was a significant ($P<0.05$) decrease in the moisture content with storage time which might be attributed to evaporative loss of moisture that goes into the head space or condenses on the packaging itself. Several studies have reported a similar decline in the moisture content of meat products packaged in edible films during storage (Noor et al., 2018a, b).

3.5. Microbial stability

The mean values of various microbial counts of chicken nuggets packaged in the edible films containing different concentrations of *R. cordifolia* viz. control (0.0%), T_1 (0.50%) T_2 (0.75%) and T_3 (1.0%) are presented in Table 2.

3.5.1. Total plate, psychrophilic and anaerobic count (log_{10} cfu/g)

A significant impact of the packaging was recorded on microbial growth and the mean values of total plate, psychrophilic and anaerobic counts of the products packaged in the bioactive edible films (T_1, T_2 and T_3) were significantly ($P<0.05$) lower than the control samples during storage. This effect might be attributed to *R. cordifolia* that is reported to have strong antimicrobial properties against several Gram-positive and Gram-negative bacteria such as *B. subtilis, S. aureus, E. coli, E. faecalis, E. aerogenes, P. mirabilis* and *P. aeruginosa* (Ismail et al., 2016).

A significant impact of storage time was also observed and the microbial counts of all the products showed a significant increasing trend with increasing storage time. However, the maximum permissible limits were not exceeded by any of the samples during the storage and the counts of all the samples were within the limits of 5.33 log cfu/g and 4.6 log cfu/g for total plate and psychrophilic counts, respectively (Dilnawaz et al., 2017a, b). Previous studies have also reported a significant impact of bioactive edible films containing plant ingredients on the microbial characteristics of meat products (Xiong et al., 2020; Kalem et al., 2018a, b).

3.5.2. Yeast and mould and coliform count (log cfu/g)

Significantly ($P<0.05$) lower counts for yeast and moulds were observed for the products packaged in the bioactive edible films (T_1, T_2 and T_3) compared to control. This effect on yeast and moulds might be attributed to *R. cordifolia* that has been reported to have an activity against fungi such as *C. albicans* (Ismail et al., 2016). Significantly ($P<0.05$) lower yeast and mould counts have been reported for meat products packaged in bioactive edible films containing plant extracts during storage (Kalem et al., 2018b; Noor et al., 2018a).

The coliforms were not detected in any of the samples during the entire storage time and might be attributed to the higher cooking temperature and other hygienic practices followed during product preparation and packaging. Several studies have reported zero counts for coliforms for various meat products packaged in the bioactive edible films during storage (Esmaeili et al., 2020; Sharma et al., 2021).

3.6. Sensory quality

The mean values of various sensory attributes of chicken nuggets packaged in edible films containing different concentrations of *R. cordifolia* viz. control (0.0%), T_1 (0.50%) T_2 (0.75%) and T_3 (1.0%) are presented in Table 3. Edible films showed a significant impact on the sensory attributes of the meat nuggets and significantly ($P<0.05$) higher scores were recorded for the products packaged in T_1 and T_2 films for colour, appearance, flavour, juiciness, and overall acceptability compared to control samples. While no effect of bioactive packaging was observed on the texture of the meat products, products packaged in the T_3 films showed significantly lower flavour scores compared to control samples. The positive impact of bioactive edible films on the sensory attributes of meat nuggets might be attributed to the strong antimicrobial and antioxidant properties of *R. cordifolia* which were also reflected in the values for TBARS, FFA and microbial counts. Colour changes observed in the meat products during storage are mainly due to the primary and secondary metabolites produced during lipid and protein oxidation (Bhat, Pathak, & Bhat, 2011a, b; Bekhit et al., 2019). The off-flavour development in the meat products during storage is attributed to several compounds produced during lipid oxidation, lipolysis and proteolysis (Kalem et al., 2018b; Bhat et al., 2019d, e). Comparatively lower scores for the flavour of the meat products packaged in T_3 films might be due to the perception of bitter compounds at this concentration. The barrier properties of the edible films have been reported to improve the moisture retention and juiciness of the meat products (Saricaoglu et al., 2018).

Both positive and negative effects of the bioactive edible films containing plant extracts on the sensory quality of the meat products have been reported in the literature. While a positive impact of garlic oil incorporated edible films was recorded on the scores for colour, odour, taste, and texture of the meat sausages during storage (Esmaeili et al., 2020), a negative impact of whey protein edible films containing plant extracts has been reported on the sensory attributes of the meat balls (Akcan et al., 2017). Several studies have observed a positive impact of bioactive edible films on the sensory quality of the meat products towards the end of the storage time (Sharma et al., 2021; Kalem et al., 2018a).

Conclusions

The present study showed a successful development of a calcium alginate and maltodextrin-based edible film using *R. cordifolia* as a novel bioactive ingredient for meat products. The developed bioactive film significantly improved the lipid and microbial stability of the meat nuggets during refrigerated storage. The oxidative potential (total phenolics and flavonoids) and antimicrobial properties of the film was significantly improved and were confirmed by significantly lower values for TBARS (mg malondialdehyde/kg), FFA (% oleic acid) and microbial counts (log_{10} cfu/g). Thus, it may be concluded that *R. cordifolia* can be used as a novel bioactive ingredient for the development of bioactive edible and biodegradable films for meat products.

Acknowledgement and conflict of interest

The authors declare that there is no conflict of interest.

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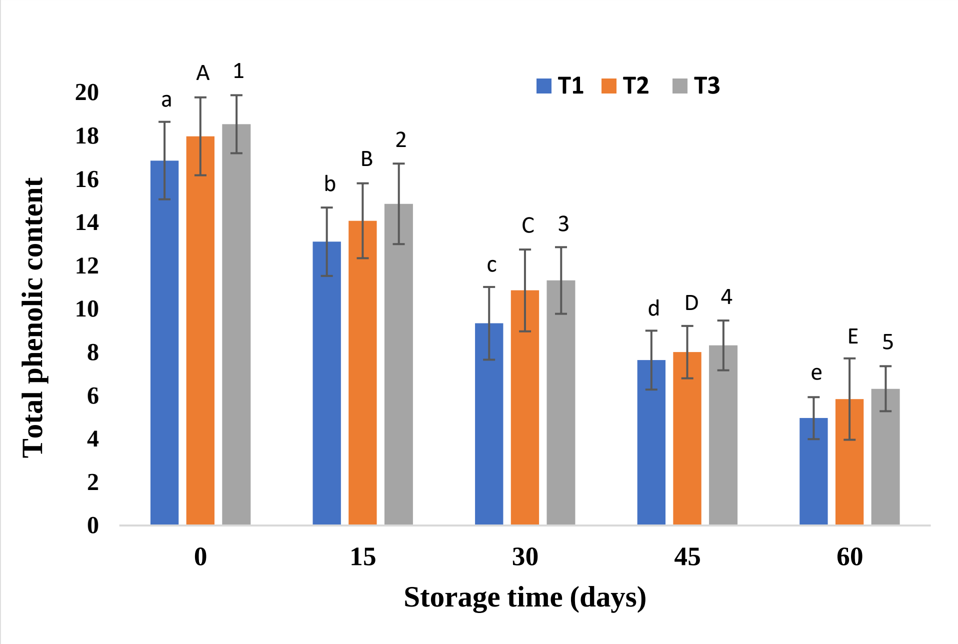


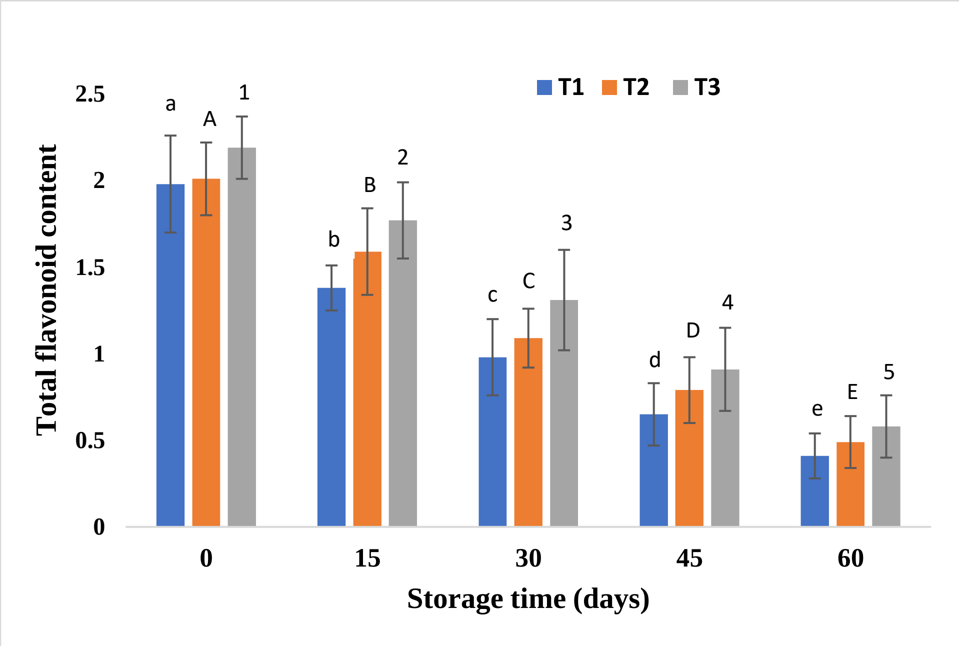
Different superscripts on blue (lower case alphabet), brown (upper case alphabet) and grey (numerals) columns differ significantly (P<0.05)

T_1, T_2, and T_3 films contain 0.50%, 0.75% and 1.0% *R. cordifolia*, respectively

n = 6 for each treatment

Error bars represent the standard errors

Figure 1: Effect of the addition of *R. cordifolia* on total phenolic content (mg Gallic acid equivalents/g) of the edible films



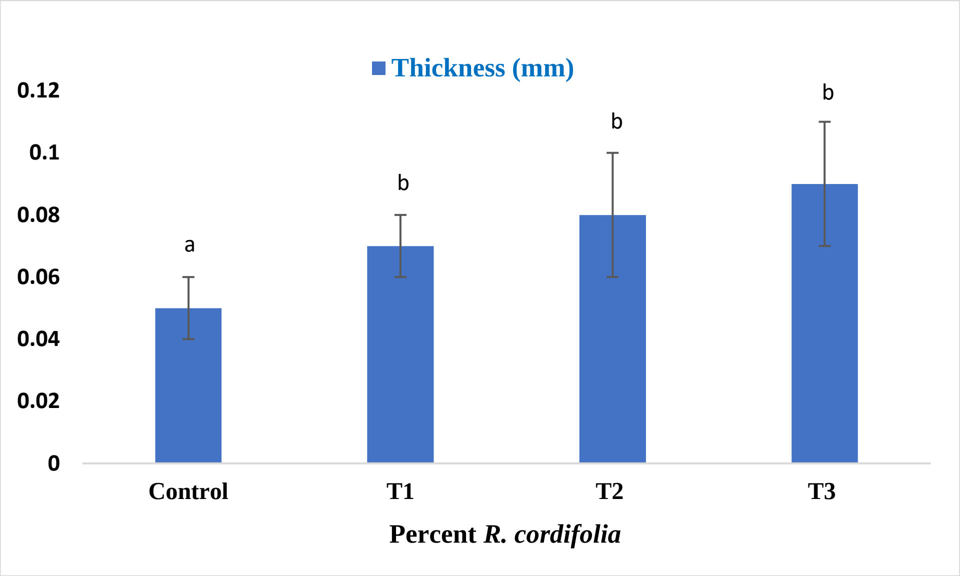
Different superscripts on blue (lower case alphabet), brown (upper case alphabet) and grey (numerals) columns differ significantly (P<0.05)

T_1, T_2, and T_3 films contain 0.50%, 0.75% and 1.0% *R. cordifolia*, respectively

n = 6 for each treatment

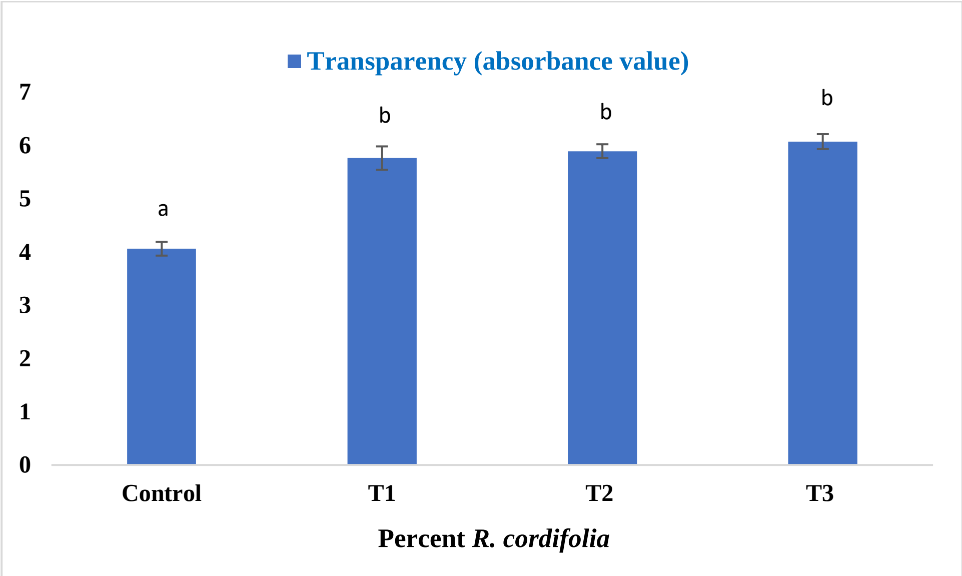
Error bars represent the standard errors

Figure 2: Effect of the addition of *R. cordifolia* on total flavonoid content (mg Quercetin equivalent/g) of the edible films



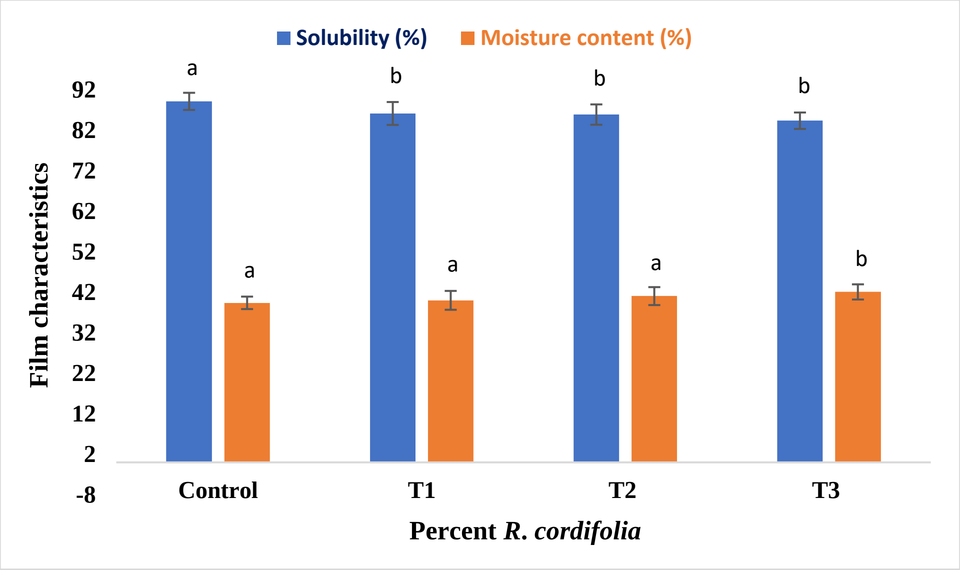
Different superscripts on columns differ significantly (P<0.05)
T_1, T_2, T_3 and control films contain 0.50%, 0.75%, 1.0% and 0.0% *R. cordifolia*, respectively
n = 6 for each treatment
Error bars represent the standard errors

Figure 3: Effect of the addition of *R. cordifolia* on thickness of the edible films



Different superscripts on columns differ significantly (P<0.05)
T_1, T_2, T_3 and control films contain 0.50%, 0.75%, 1.0% and 0.0% *R. cordifolia,* respectively
n = 6 for each treatment
Error bars represent the standard errors

Figure 4: Effect of the addition of *R. cordifolia* on solubility of the edible films



Different superscripts on blue (lower case alphabet) and brown (upper case alphabet) columns differ significantly (P<0.05)

T_1, T_2, T_3 and control films contain 0.50%, 0.75%, 1.0% and 0.0% *R. cordifolia,* respectively

n = 6 for each treatment

Error bars represent the standard errors

Figure 5: Effect of the addition of *R. cordifolia* on solubility and moisture content of the edible films

Table 1: Effect of *R. cordifolia* incorporated edible films on lipid stability and physicochemical properties of chicken nuggets during refrigerated (4±1 ºC) storage

Treatments	Storage time (days)				
	0	15	30	45	60
TBARS (mg malondialdehyde/kg)					
Control	0.311±0.011^{e}	0.436±0.007^{Ad}	0.672±0.013^{Ac}	1.025±0.019^{Ab}	1.120±0.019^{Aa}
T_1 (0.50%)	0.250±0.010^{e}	0.334±0.006^{Bd}	0.563±0.010^{Bc}	0.894±0.015^{Bb}	0.934±0.013^{Ba}
T_2 (0.75%)	0.183±0.011^{e}	0.234±0.009^{Cd}	0.482±0.011^{Cc}	0.767±0.005^{Cb}	0.863±0.012^{Ca}
T_3 (1.0%)	0.166±0.010^{e}	0.224±0.008^{Cd}	0.461±0.012^{Cc}	0.757±0.007^{Cb}	0.855±0.016^{Ca}
FFA (% oleic acid)					
Control	0.110±0.0017^{Ae}	0.125±0.0008^{Ad}	0.212±0.0014^{Ac}	0.299±0.0012^{Ab}	0.312±0.0021^{Aa}
T_1 (0.50%)	0.103±0.0013^{Ae}	0.111±0.0012^{Bd}	0.181±0.0016^{Bc}	0.202±0.0010^{Bb}	0.234±0.0018^{Ba}
T_2 (0.75%)	0.077±0.0014^{Be}	0.085±0.0011^{Cd}	0.122±0.0014^{Cc}	0.141±0.0012^{Cb}	0.153±0.0015^{Ca}
T_3 (1.0%)	0.070±0.0011^{Be}	0.079±0.0014^{Cd}	0.116±0.0016^{Cc}	0.137±0.0014^{Cb}	0.147±0.0019^{Ca}
pH					
Control	5.94±0.008^{Ae}	5.86±0.011^{Ad}	6.00±0.012^{Ac}	6.11±0.013^{Ab}	6.25±0.013^{Aa}
T_1 (0.50%)	5.84±0.010^{Ae}	5.71±0.014^{Bd}	5.94±0.011^{Bc}	6.16±0.043^{Bb}	6.21±0.037^{Ba}
T_2 (0.75%)	5.70±0.016^{Ae}	5.57±0.008^{Cd}	5.68±0.010^{Cc}	6.00±0.058^{Cb}	6.07±0.043^{Ca}
T_3 (1.0%)	5.65±0.016^{Ae}	5.59±0.013^{Dd}	5.69±0.009^{Dc}	5.72±0.028^{Db}	5.93±0.026^{Da}
Moisture (%)					
Control	62.28±0.67^{a}	61.14±0.65^{ab}	60.36±0.69^{ab}	59.26±0.68^{b}	57.23±0.68^{b}
T_1 (0.50%)	62.42±0.35^{a}	61.21±0.33^{ab}	60.44±0.33^{bc}	59.34±0.32^{c}	57.32±0.31^{cd}
T_2 (0.75%)	62.44±0.37^{a}	61.25±0.37^{ab}	60.47±0.37^{bc}	59.39±0.35^{c}	57.36±0.33^{cd}
T_3 (1.0%)	62.93±0.52^{a}	61.76±0.51^{ab}	60.95±0.51^{bc}	59.77±0.50^{c}	57.77±0.51^{cd}

Mean ± SE with different superscripts in a row (lower case alphabet) and column (upper case alphabet) differ significantly ($P<0.05$)

T_1, T_2, T_3 and control films contain 0.50%, 0.75%, 1.0% and 0.0% *R. cordifolia,* respectively

n = 6 for each treatment

Table 2: Effect of *R. cordifolia* incorporated edible films on microbial stability of chicken nuggets during refrigerated (4±1 ºC) storage

Treatments	Storage time (days)				
	0	15	30	45	60
Total plate count (log_{10} cfu/g)					
Control	$2.53±0.015^{Ae}$	$3.31±0.011^{Ad}$	$3.51±0.017^{Ac}$	$4.15±0.014^{Ab}$	$4.49±0.012^{Aa}$
T_1 (0.50%)	$2.30±0.012^{Be}$	$3.11±0.012^{Bd}$	$3.11±0.013^{Bc}$	$3.75±0.017^{Bb}$	$3.82±0.016^{Ba}$
T_2 (0.75%)	$1.73±0.013^{Ce}$	$2.44±0.011^{Cd}$	$2.71±0.014^{Cc}$	$3.14±0.019^{Cb}$	$3.29±0.015^{Ca}$
T_3 (1.0%)	$1.63±0.014^{Ce}$	$2.38±0.011^{Cd}$	$2.60±0.013^{Cc}$	$3.02±0.011^{Cb}$	$3.12±0.012^{Ca}$
Psychrophilic count (log_{10} cfu/g)					
Control	ND	ND	ND	$1.22±0.011^{Ab}$	$1.61±0.018^{Aa}$
T_1 (0.50%)	ND	ND	ND	$0.93±0.027^{Bb}$	$1.29±0.021^{Ba}$
T_2 (0.75%)	ND	ND	ND	$0.61±0.021^{Cb}$	$0.80±0.011^{Ca}$
T_3 (1.0%)	ND	ND	ND	$0.51±0.018^{Cb}$	$0.66±0.010^{Ca}$
Yeast and mould count (log_{10} cfu/g)					
Control	ND	ND	ND	$2.21±0.012^{Ab}$	$3.09±0.015^{Aa}$
T_1 (0.50%)	ND	ND	ND	$2.00±0.013^{Bb}$	$2.01±0.014^{Ba}$
T_2 (0.75%)	ND	ND	ND	$1.20±0.017^{Cb}$	$1.46±0.018^{Ca}$
T_3 (1.0%)	ND	ND	ND	$1.11±0.018^{Cb}$	$1.32±0.016^{Ca}$
Anaerobic count (log_{10} cfu/g)					
Control	ND	ND	ND	$2.09±0.013^{Ab}$	$2.89±0.016^{Aa}$
T_1 (0.50%)	ND	ND	ND	$1.84±0.012^{Bb}$	$1.91±0.014^{Ba}$
T_2 (0.75%)	ND	ND	ND	$1.21±0.016^{Cb}$	$1.49±0.017^{Ca}$
T_3 (1.0%)	ND	ND	ND	$1.13±0.013^{Cb}$	$1.31±0.016^{Ca}$
Coliform count (log_{10} cfu/g)					
All samples	Not detected throughout the storage				

Mean ± SE with different superscripts in a row (lower case alphabet) and column (upper case alphabet) differ significantly ($P<0.05$)

T_1, T_2, T_3 and control films contain 0.50%, 0.75%, 1.0% and 0.0% *R. cordifolia*, respectively

n = 6 for each treatment, ND = Not detected (Detection limit <10 cfu/g)

Table 3: Effect of *R. cordifolia* incorporated edible films on sensory quality of chicken nuggets during refrigerated (4±1 ºC) storage

Treatments	Storage time (days)				
	0	15	30	45	60
Colour and appearance					
Control	6.49±0.07^{Da}	6.13±0.09^{Db}	5.68±0.10^{Dc}	4.99±0.13^{Dd}	4.55±0.13^{De}
T_1 (0.50%)	7.02±0.10^{Ba}	6.59±0.10^{Bb}	6.11±0.08^{Bc}	5.49±0.13^{Bd}	5.07±0.13^{Be}
T_2 (0.75%)	7.47±0.10^{Aa}	7.19±0.11^{Ab}	6.76±0.09^{Ac}	6.07±0.08^{Ad}	5.69±0.08^{Ae}
T_3 (1.0%)	6.70±0.10^{Ca}	6.51±0.11^{Cb}	6.01±0.11^{Cc}	5.56±0.13^{Cd}	5.22±0.13^{Ce}
Flavour					
Control	6.99±0.11^{Ba}	6.31±0.11^{Bb}	5.78±0.11^{Bc}	5.26±0.13^{Bd}	4.83±0.13^{Be}
T_1 (0.50%)	7.22±0.07^{Aba}	6.52±0.09^{ABb}	5.99±0.09^{ABc}	5.51±0.08^{ABd}	4.97±0.08^{Abe}
T_2 (0.75%)	7.37±0.09^{Aa}	6.68±0.09^{Ab}	6.17±0.10^{Ac}	5.72±0.10^{d}	5.13±0.10^{Ae}
T_3 (1.0%)	6.16±0.09^{Ca}	5.81±0.11^{Cb}	5.19±0.13^{Cc}	4.57±0.08^{Cd}	4.41±0.08^{Ce}
Texture					
Control	7.38±0.05^{a}	6.98±0.11^{b}	5.57±0.09^{c}	4.61±0.12^{d}	4.36±0.12^{e}
T_1 (0.50%)	7.50±0.08^{a}	6.98±0.08^{b}	5.55±0.07^{c}	4.66±0.11^{d}	4.41±0.11^{e}
T_2 (0.75%)	7.62±0.07^{a}	7.09±0.08^{b}	5.73±0.12^{c}	4.78±0.12^{d}	4.52±0.12^{e}
T_3 (1.0%)	7.36±0.07^{a}	6.86±0.09^{b}	5.45±0.09^{c}	4.49±0.12^{d}	4.33±0.12^{e}
Juiciness					
Control	6.63±0.08^{Ba}	6.15±0.10^{Bb}	5.40±0.14^{Bc}	4.76±0.09^{Bd}	4.75±0.09^{Be}
T_1 (0.50%)	6.86±0.09^{ABa}	6.37±0.13^{ABb}	5.69±0.13^{ABc}	4.93±0.10^{ABd}	4.88±0.09^{Abe}
T_2 (0.75%)	6.98±0.10^{Aa}	6.49±0.10^{Ab}	5.78±0.11^{Ac}	5.04±0.08^{Ad}	4.84±0.09^{Ae}
T_3 (1.0%)	7.09±0.08^{Aa}	6.66±0.11^{Ab}	6.08±0.09^{Ac}	5.24±0.06^{Ad}	5.08±0.09^{Ae}
Overall acceptability					
Control	6.80±0.18^{BCa}	5.91±0.12^{BCb}	5.64±0.13^{BCc}	5.17±0.10^{BCd}	5.02±0.10^{BCe}
T_1 (0.50%)	6.94±0.10^{Ba}	6.07±0.09^{Bb}	5.88±0.12^{Bc}	5.44±0.08^{Bd}	5.45±0.09^{Be}
T_2 (0.75%)	7.31±0.09^{Aa}	6.45±0.11^{Ab}	6.29±0.11^{Ac}	5.77±0.10^{Ad}	5.78±0.10^{Ae}
T_3 (1.0%)	6.56±0.10^{Ca}	5.68±0.11^{Cb}	5.47±0.14^{Cc}	5.11±0.10^{Cd}	4.97±0.10^{Ce}

Mean ± SE with different superscripts in a row (lower case alphabet) and column (upper case alphabet) differ significantly (P<0.05)

T_1, T_2, T_3 and control films contain 0.50%, 0.75%, 1.0% and 0.0% *R. cordifolia*, respectively

Ten members of a trained panel replicated the experiment thrice based on a 9-point hedonic scale, wherein 9 denoted “extremely liked” and 1 denoted “extremely disliked”