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Active Packaging Film of Starch and Chitosan Incorporated with Beetroot Extract for Improvement of Quality and Shelf Life of Chicken Patties

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ABSTRACT

The present work evaluated the effect of edible film of starch and chitosan incorporated with beetroot extract on improving chicken patties' quality and shelf life. The chicken patties coated with an edible film of starch and chitosan (T1) and chicken patties coated with an edible film of starch and chitosan incorporated with beetroot extract (T2) were compared with patties without any coating (control). The results revealed that T_2 had significantly lower (P<0.05) pH, TBARS value, tyrosine value, and DPPH free radical scavenging activity than T_1 and control during a storage period of 20 days. Total plate count, yeast and mold count, and psychrophilic counts were significantly reduced in T_2 than in T_1 and control, while coliforms were absent in all the treatments throughout the storage period. Edible film coating did not show any significant effect (P>0.05) on the sensory characteristics of patties during storage. The application of starch-chitosan edible film and beetroot extract extended chicken patties' shelf-life by 8 to 10 days.

Key words: *Active Packaging, Edible film, Beet Root Extract, Natural Preservation*

INTRODUCTION

With increased awareness about health, consumers demand foods with increased safety, health benefits, and desirable sensory properties (Soro et al. 2021). The concept of food preservation using synthetic preservatives has gone beyond typical preservation to the utilization of novel preservation methods without any harmful substances (Montes and Munoz 2021). Consumers are trending towards naturally preserved, minimally processed meat products containing health-promoting substances. Therefore, producing safe food free from synthetic preservatives has become a significant challenge for food manufacturers (Kumar et al. 2020).

Meat and meat products are susceptible to microbial growth and oxidative changes, the principal cause of their spoilage. Antimicrobial and antioxidative active packaging systems incorporated with natural antimicrobials and antioxidants could be a significant breakthrough in delaying spoilage and extending of shelf life of these products. This type of packaging may be developed either by incorporating the active components into the sachet, direct incorporation of active components in the packaging material, coating of packaging material with a matrix containing antimicrobial properties, or use of antimicrobial polymers for the development of active packaging (Fang et al. 2017).

Various polymers have been utilized for the active packaging of meat products in the form of edible coatings and films. Chitosan is one of the essential natural polymers and has been widely used to form edible coatings and films (Van den Broek et al. 2015). It possesses many desirable physicochemical properties such as biodegradability, biocompatibility with human tissues, non-toxic, and possesses antimicrobial and antifungal properties, making it a suitable contestant for the development of edible films. Various natural antimicrobials and antioxidants such as essential oils, oleoresins, and plant extracts have been incorporated in edible coatings for microbial inhibition, antioxidant activity, improved flavour, etc. Red beets (*Beta vulgaris* L.) is one such food ingredient having an extremely high antioxidant activity (Aykin-Dinçer et al. 2020). This high antioxidant property is related to the antioxidant potential of betalain, which is far more active than anthocyanins, tocopherol, and catechin (Ceclu et al. 2020).

Natural preservation using edible film is a promising area for extending the shelf life of meat products. Active packaging in the form of edible films incorporated with natural antimicrobials can be used as natural preservatives for meat and meat products barring any harmful effects of the synthetic preservatives. Therefore, this study was carried out to develop an active packaging film of starch and chitosan incorporated with beetroot extract to improve the quality and shelf life of chicken patties.

MATERIALS AND METHODS

Preparation of beetroot extract

Beetroot extracts were prepared from beetroot using water as a solvent. For the extract preparation, 25 g of beetroot was cut and minced in a pestle and mortar with 50ml of water for 5 min to obtain a fine paste. Then, 50ml of additional distilled water and 0.2g of citric acid were added to the mixture and mixed properly. Then, the whole mixture was kept overnight in a volumetric flask wrapped with aluminum foil at refrigeration temperature. Finally, the mixture was centrifuged at 1000rpm for 15 min, and the supernatant was collected. The supernatant was filtered through Whatman filter paper No.1 to obtain beetroot extract.

Edible film preparation

The edible film was prepared by mixing 4g starch with 100 ml distilled water and 1.6 ml glycerol. The suspension was agitated using a magnetic stirrer at 500rpm for 30min in the water bath at 90°C and then cooled at room temperature. After that, 2 g chitosan was dissolved in 100 ml distilled water mixed with 1% glacial acetic acid, and the mixture was agitated using a magnetic stirrer at 500rpm for 30 minutes. The starch and chitosan solutions were mixed (100 ml starch-solution+100 ml chitosan solution), and 2.5 ml beetroot extract was added to it and again stirred for 10 min at 500 rpm. The solution was then mixed gently with a magnetic stirrer for 20 minutes at 1000 rpm to release all air bubbles. About 90 ml of the sample was poured onto a petri dish having a diameter of 14.5 cm and allowed to settle down for 15min and then dried at 45°C for 24 hours in an incubator to form edible films.

Preparation of chicken patties and application of edible film

Emulsion for chicken patties was prepared in a bowl chopper (Seydelmann K20, Ras, Germany) using a pre-standardized formulation (Soni et al. 2018). The frozen chicken meat was minced using an 8 mm sieve plate followed by a 4 mm sieve in a plate mincer, which was then admixed with salt and sodium tripolyphosphate in a bowl chopper for 2-3 min. After that condiments, crushed ice, and sodium nitrite were added and chopping was continued for 1-2min. Refined vegetable oil was added while chopping was continued for 2-3min. In the end, binder and spices were added, and the mixture was chopped for 1 min until a thick tacky emulsion was formed, which was transferred to a patty-forming machine to form patties (75g each). The patties were placed in a hot air oven at 180°C and cooked for 10min. Thereafter, patties were turned and cooked further for 10 min. till the internal temperature reached 72°C, measured by a digital probe thermometer (Jiangsu Jingchuang Electronics Co. Ltd., Elitech, China). The patties were cooled, and each patty was enclosed in edible film. For analysis, three samples were made and categorized as Control, T_1 (Chicken patties wrapped with the edible film of chitosan and starch); and T_2 (Chicken patties wrapped with the edible film of chitosan and starch incorporated with beetroot extract). The patties were packaged in low-density polyethylene pouches and stored under refrigerated conditions (4±1°C) for further studies (Plate 1).

Determination of quality and shelf life of chicken patties wrapped with edible film during refrigeration storage (4±1°C)

Physicochemical parameters

The pH of homogenate was recorded by combining glass electrodes of a digital pH meter (Model T-25, Janke and Kenkel, 1KA Labor Technik, Germany) (Trout et al. 1992). Tyrosine value was estimated as per Strange et al. (1977) method and expressed in mg/100g. Thiobarbituric acid reacting substances (TBARS) were estimated by the method described by Tarladgis et al. (1960) and expressed as mg malonaldehyde/kg.

Antioxidant activity

Antioxidant activity was analyzed by DPPH free radical scavenging activity as per Tepe et al. (2005).

Microbiological quality

Microbiological quality was analyzed as per the method described by APHA (2001). The average number of colonies was multiplied with the reciprocal of the respective dilutions and expressed as log_{10} CFU/g.

Sensory evaluation

Sensory evaluation of chicken patties was performed by the method described by Keeton (1983). The samples were served warm (40–60°C) by pre-heating the samples in a microwave oven (L.G., Model MC-7148, MS, 1200 W microwave power, India) for 1 min, and sensory evaluation was conducted around 3.30–4.00 PM every time in sensory evaluation laboratory. Panelists were asked to evaluate each sample based on a standard 8-point hedonic scale (where, 8= extremely liked and 1= for extremely disliked) for appearance and colour, flavour, aftertaste, texture, and overall acceptability. During storage, the sensory analysis was performed on days 1, 5, 10, 15, and 20.

Statistical analysis

The experiments were repeated three times, and the data generated for different quality characteristics were compiled and analyzed using SPSS (version 26.0 for Windows; SPSS, Chicago, III., U.S.A.). The data obtained were subjected to analysis of variance, and the level of significance was reported at a 5% level significance ($P<0.05$).

RESULT AND DISCUSSION

Physicochemical parameters

pH

The pH value had a significant increase ($P<0.05$) in all the treatments with the advancement of the storage period, which could be attributed to the degradation of proteins and the generation of volatile basic components (Table 1). The increase was significantly ($P<0.05$) lower in T_2 than in T_1 and control. This might be due to the antimicrobial effect of chitosan, as reported by Kanatt et al. (2013) and flavanoids and phenolics present in beetroot extract, which could have reduced the microbial load and breakdown of proteins (Vulie et al. 2014). Similarly, Wang et al. (2017), reported that the pH of lean pork slices coated with an edible coating of chitosan containing cinnamon and ginger essential oil was 6.01 on the first-day storage, which increased to 6.75 and 7.53 on the ninth-day refrigeration storage in treatment and control respectively. Further, Aykin-Dinçer et al. (2020) reported that the addition of beetroot extract decreased the initial pH in beef sausage. A similar result was also observed in the present study, which could be attributed to the low pH value of beetroot extract due to the citric acid used in the extraction.

TBARS

TBARS values of control, as well as treatment groups, showed a significant ($P<0.05$) increasing trend with the advancement of the storage period, which could be attributed to the production of oxidative substances during the storage period (Table 1). However, T_2 had a significantly ($P<0.05$) lower TBARS value than control and T_1 during all the stages of the storage, which could be attributed to the antioxidative activity of chitosan (Kanatt et al. 2013), betalains and flavonoids present in the beetroot extract (Aykln-Dinçer et al. 2020). Moreover, Fidelis et al. (2017) also reported that beetroot contains high amount of total phenolics, flavonoids, and other pigments, accounting for intense antioxidant activity. da Silva et al. (2019) also reported that the addition of 2% beetroot pigments reduced the TBARS value in ground pork loin than control.

Table 1. Effect of edible films on physico-chemical parameters and antioxidant activity of chicken patties at refrigeration storage (4±1oC) (Mean±S.E.)*

Treatments	Refrigerated storage period (days)				
	Day 1	Day 5	Day 10	Day 15	Day 20
pH					
Control	$6.16{\pm}0.03^{c1}$	$6.22{\pm}0.13^{c2}$	$6.37{\pm}0.04^{c3}$	$6.44{\pm}0.18^{c4}$	NE
T_1	$6.13{\pm}0.09^{b1}$	$6.19{\pm}0.01^{b2}$	$6.28{\pm}0.17^{b3}$	$6.33{\pm}0.07^{b4}$	$6.34{\pm}0.23^{b5}$
T_2	$6.11{\pm}05^{a1}$	$6.16{\pm}0.02^{a2}$	$6.24{\pm}0.12^{a3}$	$6.27{\pm}0.15^{a4}$	$6.30{\pm}0.08^{a5}$
TBARS (mg malonaldehyde/kg of meat)					
Control	$0.26{\pm}0.02^{c1}$	$0.38{\pm}0.04^{c2}$	$0.49{\pm}0.09^{c3}$	$0.71{\pm}0.03^{c4}$	NE
T_1	$0.18{\pm}0.01^{b1}$	$0.33{\pm}0.08^{b2}$	$0.39{\pm}0.07^{b3}$	$0.64{\pm}0.05^{b4}$	$0.85{\pm}0.11^{b5}$
T_2	$0.16{\pm}0.04^{a1}$	$0.26{\pm}0.03^{a2}$	$0.31{\pm}0.12^{a3}$	$0.55{\pm}0.06^{a4}$	$0.79{\pm}0.09^{a5}$
Tyrosine (mg/100g)					
Control	$17.0{\pm}0.23^{b1}$	$22.5{\pm}0.32^{c2}$	$27.0{\pm}0.26^{c3}$	$33.0{\pm}0.40^{c4}$	NE
T_1	$17.0{\pm}0.12^{b1}$	$18.4{\pm}0.17^{b2}$	$22.5{\pm}0.24^{b3}$	$26.0{\pm}0.19^{b4}$	$30.0{\pm}0.30^{b5}$
T_2	$15.0{\pm}0.18^{a1}$	$17.2{\pm}0.13^{a2}$	$21.0{\pm}0.39^{a3}$	$24.3{\pm}0.27^{a4}$	$27.0{\pm}0.41^{a5}$
DPPH (%)					
Control	$24.36{\pm}0.13^{c1}$	$22.63{\pm}0.11^{c2}$	$20.67{\pm}0.16^{c3}$	$17.88{\pm}0.08^{c4}$	NE
T_1	$25.55{\pm}0.22^{b1}$	$22.32{\pm}0.12^{b2}$	$21.32{\pm}0.07^{b3}$	$18.34{\pm}0.09^{b4}$	$17.12{\pm}0.06^{b5}$
T_2	$27.03{\pm}0.18^{a1}$	$30.34{\pm}0.26^{a2}$	$34.32{\pm}0.16^{a3}$	$32.31{\pm}0.27^{a4}$	$29.01{\pm}0.28^{a5}$

N=6; NE: Not estimated; *Mean±S.E. bearing different superscripts row-wise (small alphabet) and column-wise (numerals) indicate a significant difference (P<0.05) Control: Control sample without any edible film; T_1: Chicken patties coated with edible film of chitosan and starch; T_2: Chicken patties coated with edible film of chitosan and starch incorporated with beet root extract.

Tyrosine value

Tyrosine is released during the protein breakdown of meat, reflecting the degree of protein denaturation. The control, T_1, and T_2 had a tyrosine value of 17.00, 17.12 and 15.00 mg/100g, respectively, on the first day of storage which increased significantly (P<0.05) in all the treatments with the progression of storage period (Table 1). The increase in tyrosine value in all the treatments during the storage period could be due to the degradation of meat proteins producing amino acids. However, T_2 has a significantly (P<0.05) lower tyrosine value than T_1 and control during the storage period which could be due to the antimicrobial action of chitosan and beetroot extract.

The results corroborated with Shukla et al. (2020), who observed a significant reduction in the tyrosine value of chicken patties coated with an edible coating of chitosan than control during refrigeration storage. The significant (P<0.05) increase in tyrosine value during refrigeration storage for all the treatments could be due to hydrolytic changes in meat by inherent tissue enzymes and bacterial proteolysis, as reported by Strange et al. (1977).

Antioxidant Activity

DPPH

All the treatments showed a significant (P<0.05) decrease in DPPH value during the storage period, which could be due to the production of oxidative substances during storage. However, the presence of antioxidative edible film over T_1 and T_2 reduced the oxidative changes (Table 1). Further, beetroot extract containing betalains, phenols, and flavonoids having a known antioxidant activity might have reduced the oxidative changes in T_2. Further, the antioxidant activity of beetroot extract could also be attributed to various flavonoids with antioxidative activity (Vulie et al. 2014). Similar results were also observed by Shukla et al. (2020), who found a significantly higher (P<0.05) DPPH value of chicken patties coated with an edible film of chitosan incorporated with clove essential oil during refrigeration storage than the control.

Total Plate Count (TPC)

The initial TPC of control, T_1 and T_2 were 1.94, 1.92, and 1.83 CFU/g, respectively, which were significantly different

(P<0.05) among themselves. However, all the treatments showed increasing microbial load with the progression of the storage period (Fig. 1). The control crossed the permissible limit of $4\log_{10}$ CFU/g (FSSAI 2016) on the 15th day, but T_1 and T_2 were below the acceptable limit even on the 20th day of refrigeration storage. Among all the treatments, T_2 had the lowest microbial count at all stages of the storage, which could be due to the antimicrobial effect of different types of flavonoids and phenols (Vulie et al. 2014; Chhikara et al. 2019) as well as nitrates (Dominguez et al. 2020) present in beetroot extract. Moreover, the microbial contamination is mainly limited to the surface, and the application of edible film with antimicrobial properties could have limited the microbial growth over the surface.

Chatli et al. (2014) also found that raw chevon chunks wrapped in starch-chitosan edible film and impregnated with nisin and cinnamaldehyde under aerobic conditions for ten days of refrigeration storage had a significantly (P<0.05) lower standard plate count than control. The results in the present study could also be explained by the findings of Kanatt et al. (2013), who observed that chicken meatballs with an edible coating of 2% chitosan had a total plate count of 6.6 $\log_{10}$CFU/g on the 14th day of refrigerated storage. In contrast, the control samples had this value on the sixth day of refrigeration. Similarly, excellent antibacterial activities of beetroot have been reported against *S. aureus, L. monocytogenes, E. coli, and P. aeruginosa* (Salamatullah et al. 2021).

Coliform count

The coliforms were absent during the entire storage period, which could be due to the hygienic measures adopted during the processing and preservation of chicken patties. The absence of coliforms during the storage period could be attributed to the destruction of coliforms during cooking at high temperatures, much above their death point of 57°C. Kanatt et al. (2013) also reported the absence of coliform in various meat products after applying an edible coating of 2% of chitosan coating while the initial load was $2\log_{10}$CFU/g at refrigeration storage. Further, Marrone et al. (2021) reported that beetroot extracts had a dose-dependent antimicrobial effect against coliform, while Salamatullah et al. (2021) also reported intense in-vitro antimicrobial activity of beetroot extract against *E. Coli*.

Psychrophilic count

Psychrophiles were absent for the first five days of the storage period in all the treatments. However, their number increased with the progression of the storage period in all the treatments (Fig. 2).

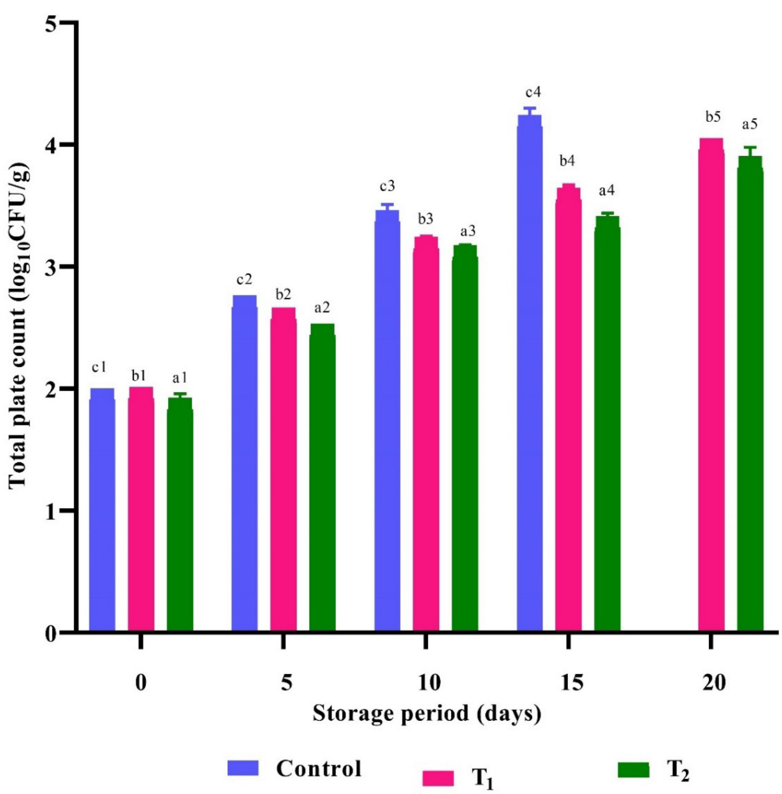

Fig. 1: Effect of edible films on total plate count (log10CFU/g) of chicken patties during refrigeration storage (4±1oC).

The psychrophilic counts in T_2 were significantly (P<0.05) lower than in control due to the effects of betalains and chitosan on bacterial growth. Similarly, Venkatachalam and Lekjing (2020) also reported that pork patties treated with chitosan, clove oil and nicin had an excellent inhibitory effect on the growth of *Pseudomonas* spp. in chilled pork patties during refrigeration storage for 15 days.

Yeast and mold Count

The yeast and mold were not detected in all the treatments during the first five days of the storage period. Control, T_1, and T_2 showed yeast and mold counts of 0.98±0.16, 0.89±0.23 and 0.84±0.09 $\log_{10}$ CFU/g, respectively, on the 10th day (Fig. 3). However, the yeast and mold count increased in all the treatments with the progression of the storage period. Among all the treatments, T_2 had the lowest yeast and mold count at all stages of the storage period, which could be due to the antimicrobial activity of chitosan and beetroot extract. Similarly, Langroodi et al. (2018) and Shahvandari et al. (2021) also reported a reduction in yeast and mold count by applying 2% of chitosan and chitosan-cumin essential oils in beef and chicken respectively during refrigeration storage.

Sensory evaluation

The interaction between treatments and storage time recorded for the sensory evaluation is presented in Table 2. The results showed that edible film did not produce a significant difference (P>0.05) in the appearance and color of chicken patties (Table 2). However, during the storage period, the decrease in colour score was significantly lower (P<0.05) in treatments due to the antioxidant activity of the film, leading to higher scores for colour and appearance than in the control. Venkatachalam and Lekjing (2020) also observed that the pork patties treated with an edible coating of chitosan, clove essential oil, and nicin had a better appearance than the uncoated pork patties during refrigerated storage of 14 days. Similar improvement in sensory scores was also observed on incorporation of beetroot powder in chicken sausage (Swastike et al. 2020).

The results showed that edible film did not produce any significant difference (P>0.05) in the flavour of chicken patties. However, with the advancement of the storage period, T_2 had significantly (P<0.05) higher flavour scores than other treatments, which could be attributed to decreased oxidation and protein degradation compared to other treatments.

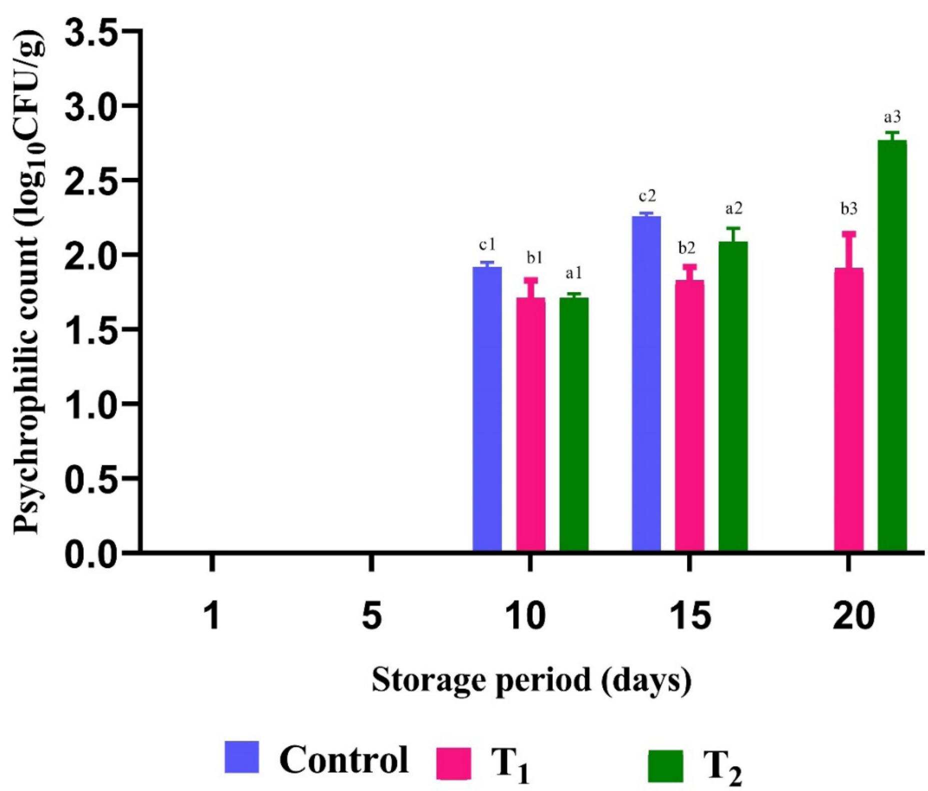

Fig. 2: Effect of edible films on psychrophilic count (log10CFU/g) of chicken patties during refrigeration storage (4±1oC).

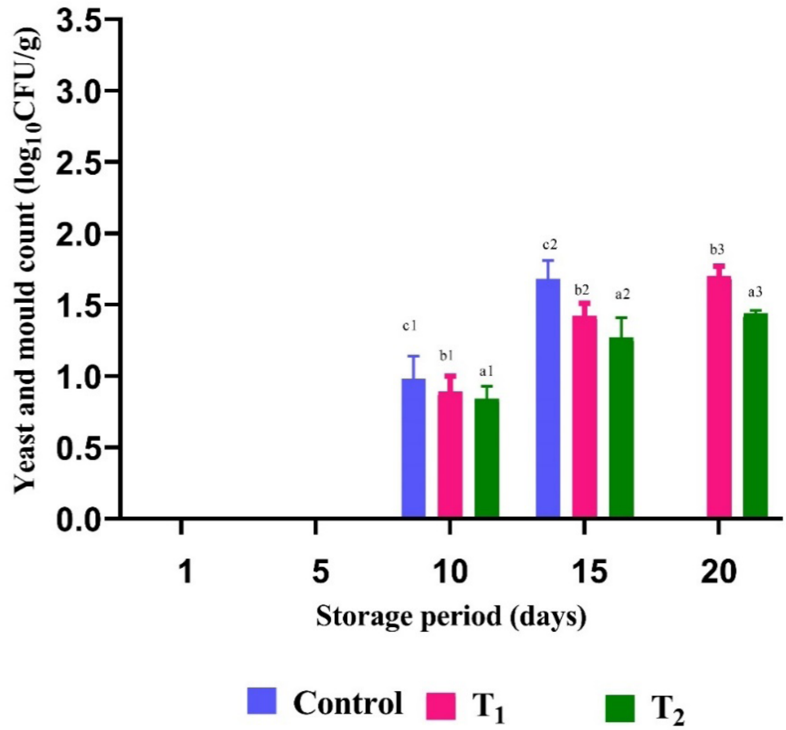

Fig. 3: Effect of edible films on yeast and mold (log10CFU/g) of chicken patties during refrigeration storage (4±1oC)

The after-taste scores of chicken patties had no significant difference (P>0.05) among themselves, which could be due to the presence of thin edible film having no significant effect on the sensory properties. However, during the storage period, the decrease in after-taste score was significantly higher (P<0.05) in treatments than in control. Similarly, no deleterious effect was observed on the texture scores of chicken patties which could be due to the minimal thickness of the edible films and the absence of off-flavor-producing components in the developed edible film.

Table 2: Effect of edible films on sensory quality of chicken patties at refrigeration storage (4±1oC) (Mean±S.E.)*

Treatments	Refrigerated storage period (days)				
	Day 1	Day 5	Day 10	Day 15	Day 20
Appearance/Colour					
Control	7.51±0.09^{c1}	7.23±0.12^{c2}	6.74±.04^{c3}	6.07±0.14^{c4}	NE
T_1	7.53±0.02^{b1}	7.33±0.17^{b2}	6.81±0.23^{b3}	6.27±0.03^{b4}	6.18±0.06^{b5}
T_2	7.58±0.13^{a1}	7.41±0.07^{a2}	6.89±0.02^{a3}	6.36±0.08^{a4}	6.29±0.01^{a5}
Flavour					
Control	7.33±0.04^{c1}	7.28±0.11^{c2}	6.75±0.14^{c3}	6.21±0.06^{c4}	NE
T_1	7.37±0.13^{b1}	7.34±0.03^{b2}	6.67±0.17^{b3}	6.29±0.09^{b4}	5.88±0.15^{b5}
T_2	7.42±0.08^{a1}	7.37±0.16^{a2}	6.81±0.07^{a2}	6.39±0.01^{a3}	5.98±0.12^{a4}
After taste					
Control	7.38±0.03^{c1}	7.09±0.13^{c2}	6.54±0.04^{c3}	6.13±0.15^{c4}	NE
T_1	7.40±0.09^{b1}	7.25±0.11^{b2}	6.68±0.17^{b3}	6.27±0.09^{b4}	6.03±0.12^{b5}
T_2	7.47±0.06^{a1}	7.29±0.01^{a2}	6.79±0.08^{a3}	6.42±0.19^{a4}	6.19±0.05^{a5}

(Table continued)

(Table continued)

Treatments	Refrigerated storage period (days)				
	Day 1	Day 5	Day 10	Day 15	Day 20
Texture					
Control	7.41±0.06^{a1}	7.29±0.12^{b2}	6.64±0.03^{c3}	6.02±0.17^{c4}	NE
T_1	7.47±0.07^{b1}	7.38±0.09^{b2}	6.73±0.11^{b3}	6.26±0.06^{b4}	6.12±0.14^{b5}
T_2	7.51±0.04^{a1}	7.42±0.05^{a2}	6.91±0.15^{a3}	6.46±0.01^{a4}	6.22±0.02^{a5}
Overall Acceptability					
Control	7.50±0.02^{a1}	7.19±0.16^{c2}	7.13±0.07^{c3}	6.18±0.12^{c4}	NE
T_1	7.44±0.09^{a1}	7.36±0.06^{b2}	7.15±0.14^{b3}	6.49±0.17^{b4}	6.21±0.08^{b5}
T_2	7.46±0.11^{a1}	7.38±0.03^{a2}	7.28±0.04^{a3}	6.53±0.01^{a4}	6.43±0.05^{a5}

N=6; NE: Not estimated; *Mean±S.E. bearing different superscripts row-wise (small alphabet) and column-wise (numerals) indicate significant difference ($p<0.05$)
Control: Control sample without any edible film; T_1: Chicken patties coated with edible film of chitosan and starch; T_2: Chicken patties coated with edible film of chitosan and starch incorporated with beet root extract.

The results revealed that edible films did not affect overall acceptability on the initial day of storage. It reflected that edible coating could be easily chewed by the sensory panelists, who did not find any significant difference (P>0.05) between treatments and control. The edible films did not produce any off flavour and taste during chewing, which could have otherwise reduced sensory score. Moreover, overall acceptability scores were significantly higher (P<0.05) in T_2 than in T_1 and control during the storage period, which could be due to due to edible film incorporated with beetroot extract with significant antioxidant activity.

Similar results were also observed by Chatli et al. (2014), who observed that edible film of starch-chitosan supplemented with nisin and cinnamaldehyde applied on chevon chunk had improved sensory characteristics than the control samples throughout the storage period. Similarly, Shukla et al. (2020) also observed a significant improvement in the sensory quality of chicken patties coated with chitosan alone and incorporated with clove essential oil during refrigeration storage.

CONCLUSION

The edible film of chitosan incorporated with beetroot extract significantly improved the quality parameters of chicken patties during refrigerated storage (4±1°C). Among all the treatments, T_2, i.e., chicken patties coated with an edible film of 2% chitosan and 4% starch incorporated with 1.25% beetroot extract, significantly improved (P<0.05) the physicochemical parameters. T_2 also had the lowest oxidative change, protein degradation, and microbial growth, followed by T_1. Although the control crossed the permissible microbial limit between the 10th to 15th days of storage, the values for both T_1 and T_2 were well below the permissible microbial limit of 4log10 CFU/g even on the 20th day of storage. Thus, a shelf-life extension of about 8-10 days was observed in treatments than in control. However, among the treatments, the quality parameters were significantly improved in T_2 than in T_1, signifying the positive effect of beetroot extract incorporation in chitosan-starch edible film coating to improve the quality and shelf life of chicken patties.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

ETHICS STATEMENT

Not applicable

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