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Prevention of Protein Oxidation in Chicken Meat during Chilled Storage Using Chitosan Gelatin Films with Green Tea Extracts

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Abstract: In present study, the usability of chitosan and gelatin (1:1) films incorporated with green tea extract (GTE) to improve the shelf life of the chicken meat stored in chilled condition was evaluated. The extract of green tea was evaluated for its phenolic content, antioxidant activity and ferric ion chealating ability. The ChGel films possessed antimicrobial activity and inactivated approximately 3 log cfu/ ml of *K. pneumoniae, S. typhi var. Weltevreden, S. typhi var. Oslo, Y. enterocolitica, E. feacalis, B. cereus, E. coli and S. aureus* in 3 hours. Incorporation of GTE influenced the optical and mechanical properties of the films. Chicken samples without films were observed to be microbiologically safe for not more than 6 days, while ChGel and ChGel-GTE films improved the microbial safety of chicken samples till day 13. ChGel-GTE films also prevented lipid peroxidation in samples as evident by TBARS value (day 10: control: 1.14; ChGel-GTE: 0.21 mg MDA eq/kg). Protein oxidation during chilled storage of chicken was also prevented by ChGel-GTE by inhibiting protein carbonylation, loss of free thiols groups in protein and lowering the number of disulphde bonds. This study supports use of ChGel films with GTE for enhancing the safety of stored chicken meat not only by maintaining the microbial quality of the samples but also preventing oxidative changes which can hamper the functional, nutritional and sensorial properties.
Keywords: Green tea; Active films; Chitosan-Gelatin Films; Protein oxidation; Lipid oxidation; Shelf life extension.

1. Introduction

Packaging is an integral component of the food processing sector. It is a combination of art, science and technology of enclosing a product for achieving hygienic condition and safe transportation leading to increment in self life [1]. The food packaging technologies are improving consistently in response to demand from modern society. Petroleum based synthetic polymers are unique for food packaging due to low cost, excellent physical properties like density and molecular weight, mechanical properties, transmission properties of various gases. They not only increase the shelf-life of the product [2], but also add functionality in terms of convenience and attractiveness to the consumers. Synthetic polymers are resistant to degradation in the environment [1] and hence, bio-polymer films made from natural polymers for food packaging have attracted attention. Packaging based on bio-polymers can be prepared from plant or animal based proteins, starches, cellulose and its derivatives, chitin/chitosan, gums, lipids, or their mixtures [3]. In recent times, food packaging films may have antimicrobial releasing systems, gas scavengers or emitters, gas flusher, moisture absorbent, ultraviolet light barriers or antioxidants [4] in or on the packaging material or the package head-space, to augment the performance of the packaging system [5]. These packaging materials not only extend the shelf life of food product but also monitor the freshness, and display information about the product, its quality and safety; thus making the packaging active, intelligent or smart.

Meat being a good source of proteins and essential amino acids along with vitamins, minerals and fats, is an important part of balanced diet [6]. It is highly perishable food product due to its high moisture and fat content and result in protein degradation, lipid peroxidation and putrefaction caused by microbial and endogenous enzymes. The water activity and the pH of the meat is favourable for the microbal spoilage and hence proper processing, packaging and storage is required for improving shelf life and safety of the meat products. Also, oxidative changes are known to occur during processing and storage of meat and meat products, resulting in rancidity and reduction in quality, nutrition and taste. Even toxic products may be produced which could be hazardous to human health. After slaughtering of animal, during postmortem aging and refrigerated storage, oxidation of proteins occur due to decreased ability of the muscle to maintain its antioxidant defense system resulting in deterioration of meat quality [7]. Protein oxidation is known to impair the protein functionality, nutritional value and sensory attributes

like tenderness, flavour and juiciness of meat [8]. Structural changes in protein resulting from oxidative changes may affect the recognition sites for protease, thus decreasing its susceptibility to proteolysis and hence digestibility. Antioxidants are known to prevent the undesirable oxidative changes, improving the shelf life of food and also preserving the nutritional and sensory qualities. Natural antioxidants like essential oils, plant extracts, Millard reaction products are widely being considered to overcome the use of synthetic antioxidants which are now known to be toxic and carcinogenic.

Green tea is widely recognized for its rich antioxidant content. The main catechins of green tea are: (+)-catechin (C), (-)-epicatechin (EC), (-)-gallocatechin (GC), (-)-epicatechingallate (ECG), (-)-epigallocatechin (EGC), (-)-epigallocatechin gallate (EGCG) [9]. Extracts of green tea are reported to scavenge radicals DPPH and superoxide, prevent bleaching of β-carotene in β-carotene-linolate systems and prevent oxidative changes in various food model systems. Green tea incorporated in packaging material is known to posses antioxidant activity [10], prevent lipid peroxidation [11, 12] in flesh foods. The present study delineates the synthesis of active bio-polymer films of chitosan and gelatin loaded with extracts of green tea leaves and its effect on microbial quality and oxidative changes of minced chicken meat packed in the films.

2. Material and methods

2.1 Material

Dried green tea leaves (*Camellia sinensis*) (GT) of brand Tettley were procured from local market. Chitosan with deacetylation range of 78-82% (manufacturer's data) was supplied as gift by Mahatani Chitosan Pvt. Ltd. (Veraval, India). All other reagents of analytical grade were obtained from Sigma-Aldrich (Munich, Germany) except gelatin and plate count agar from Himedia (Mumbai, India) and Folins- ciocalteu reagent from SRL (Mumbai, India).

2.2 Extract preparation and characterization

The green tea leaves (5 g) was extracted with 100 ml of distilled water with constant shaking for 2 hours and filtered extracted was stored at 4˚ C till further use. The extract was characterized by estimating phenolic content (by Folins- ciocalteu reagent), DPPH radical scavenging activity, antioxidant activity coefficient as measured using β- carotene bleaching assay and ferric ion reducing capacity [13].

2.3 Preparation and characterization of chitosan and gelatin films

Chitosan- gelatin (Ch-Ge) films were prepared by casting method as described by [14]. Equal volumes of aqueous solution of gelatin (2%, w/v) and chitosan (2% w/v in 1% acetic acid) were mixed and plasticized with glycerol (20% w/w of solid content of film). The film forming solution was degassed by application of vacuum to obtain smooth bubble free films and casted by drying 70 ml of solution in Teflon plates of area 100 cm^2 at 50˚ C and 50% relative humidity. For active films, green tea extract (GTE, 30 ml/ 600 ml of film forming solution, corresponding to active components from 175 mg of green tea leaves in every film of 100 cm^2) was added to film forming solution. The total volume was adjusted by dissolving gelatin in lesser volume of distilled water.

Thickness was measured using micrometer gauge. Tensile strength (TS) and elongation were measured using Texture Analyzer TA-HD plus (Stable Micro Systems, Surrey, UK). The films were cut into strips of 2.5 cm x 10 cm pieces and conditioned for 24 hrs at 25° C and 50 % relative humidity. The samples were subjected to extension at speed of 2 mm/ sec for distance of 50 mm. The maximum force and distance covered before breakage of the film was noted. Initial grip separation of 50 mm was maintained. Tensile strength and elongation were expressed in MPa and percentage, respectively. Tensile strength and elongation was calculated by following expression:

$$\text{Tensil Strength (Pa)} = \frac{\text{Force } (N)}{2.5 \times 10^{-3} \times \text{thickness of film } (m)} \quad (1)$$

$$\text{Percent Elongation} = \frac{l\ (\text{cm})}{5} \times 100 \quad (2)$$

Where l is the distance covered by the grip of instrument before breaking of the film.

The films were characterized for opacity and transparency by measuring the % transmittance of films at 600 nm and absorbance of films at 500 nm by placing films in the sample holder of UV-Vis spectrophotometer test cell. The measurements were performed using air as the reference. Following expression were used to obtain transparency and opacity [15].

$$\text{Transperancy} = \frac{\text{Transmittance (600 nm)}}{\text{Thickness of film (mm)}} \quad (3)$$

$$\text{Opacity} = \text{Absorbance (500 nm)} \times \text{Thickness (mm)} \quad (4)$$

The colour of the films were analyzed with a CR-400 Konica Minolta Chroma Meter (NJ, USA). The instrument was calibrated for white and black colour using white tile and zero box, respectively. L*, a* and b*values were measured and colour difference between the control and active films were calculated as

$$\Delta E^* = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b*)^2} \quad (5)$$

where ΔL, Δa and Δb are the difference between L, a and b values of control and active films.

Moisture content, swelling and solubility of the film was determined according to Uranga et al. (2019). Pre-weighed films (2 cm x 2 cm) were dried in a hot air oven at 110° C till constant weight and dry weight noted. Moisture content (%) of the films was calculated using following expressions.

$$\text{Moisture content (\%)} = \frac{\text{difference in weight of film before and after drying}}{\text{weight of film before drying}} \times 100 \quad (6)$$

For swelling, the films (2 cm × 2 cm) were weighed (W_0) and then immersed into 50 ml distilled water and incubated 80 rpm shaking condition for different time interval (24, 48 and 72 hrs) and weighed (W_1) was noted after removal of excess water. The swelling was calculated as:

$$\text{Swelling} = \frac{W_1 - W_0}{W_0} \times 100 \quad (7)$$

To determine the solubility of the films, the films (2 cm x 2cm) were immersed in 50 ml distilled water for 24, 48 and 72 hrs and dried after removal of excess water in hot air oven at 110° C till constant weight ($W_{residual}$) was obtained. Solubility of the films was calculated as

$$\text{Solubility (\%)} = \frac{\text{dry weight of film - } W_{residual}}{\text{dry weight of film}} \times 100 \quad (8)$$

The antimicrobial activity of the films was tested against different microorganism (*Klebsiella pneumoniae, Salmonella typhi.* var. weltevreden, *Yersenia enterocolitica, Enterococcus feacalis, Salmonella typhi* var. oslo, *Bacillus cereus, Escherichia coli, Staphylococcus aureus*). Overnight grown cultures were washed twice with sterile saline and control film samples (1 cm × 1 cm) were incubated (3 hrs, 37° C) in culture suspended in saline. The initial cell count of approximately 10^3 cfu/ ml was maintained. The cultures were serially diluted and plated on plate count agar after completion of incubation time to enumerate the number of cells survived.

2.4 Evaluation of shelf life extension of chicken meat packed in active films

Chicken meat was bought from local market. The meat was washed thoroughly 3 times with tap water and cut in chunks. 20-25 gm of meat was packed in films and stored at 4-6° C. Meat without films served as control. Sample was collected at different time point for spoilage analysis.

2.5 Analysis of microbial quality

10 gm of chicken was weighed aseptically and homogenized in sterile saline by using Stomacher. The homogenate was subjected to serial decimal dilution and total viable count was enumerated by spreading 100 µl of diluted homogenate on plate count agar (PCA).

2.6 Estimation of TCA soluble protein in meat samples

Chicken meat (2 gm) was homogenized with 18 ml of 5 % TCA for 1 min and homogenate was incubated at 4° C for 1 hr, followed by centrifugation at 10,000 rpm, 4° C for 10 minutes. 2 ml of alkaline copper sulphate was added to 0.2 ml of filtrate an incubated at room temperature for 5 minutes. 0.2 ml of 1 N Folin- ciocalteu reagent was added to above reaction mixture and incubated at 50° C in water-bath for 30 minutes. The colour formed was measured at 600 nm.

2.7 Evaluation of oxidative changes in meat

2.7.1 Estimation of lipid peroxidation

Lipid peroxidation was quantified by measuring the TBA value as described by [16]. Briefly, chicken samples (4 gm) was homogenized with 5% trichloro acetic acid (16 ml) by using Polytron tissue homogenizer and homogenate was filtered through whatman no. 4 filter paper. Equal amount of filtrate was added to 0.02 M

thiobarbituric acid solution and incubated in boiling water bath for 30 minutes. After cooling, absorbance was measured at 532 nm. The amount of thiobarbituric acid reactive substances (TBARS) was expressed as mg malondialdehyde per kg of meat.

2.7.2 Evaluation of protein oxidation by Estimating Free & Total thiols

1) Sample preparation

Chicken meat (4 gm) was homogenized in 20 ml of (100 Mm) tris buffer (pH 8.0) for 1 minutes by Polytron tissue homogenizer. Homogenized sample was centrifuged at 8000 rpm for 10 min at 4° C and 2 ml of filtrate was diluted with 8 M urea containing Tris buffer (pH 8.0). The diluted homogenate was mixed vigorously and used for estimation of free thiols and protein concentration. For estimation of total thiols, the disulphide bonds in the diluted filtrate was subjected to reduction.

2) Reduction of disulphide bond

16.7 µl of octanol and 33.4 µl of freshly prepared sodium borohydride (30% in 1 M NaOH) was added in 1 ml of filtrate. After vortexing, mixture was incubated at 50°C for 30 min in water bath for reduction of disulphide bond to free thiols. 450 µl of 6 M HCl was added and stirred for 10 mins to neutralize excess sodium borohydride.

3) Estimation of thiols by 4-DPS assay

250 µl of filtrate was added in 1 ml of 0.1 M citric acid buffer with 8 M urea, and 250 µl of Aldrithiols (4-DPS). The reaction mixture was vortexed and incubated at room temperature for 30 min in dark. The concentration of thiols was measured at 324 nm in spectrophotometer with 0.1 M citric acid buffer with 8 M urea used as reference. Standard curve of thiols was plotted using 2.5 – 500 µM of L-cysteine in 8 M urea in 0.1 M citric acid buffer. The thiols concentration was expressed in nmol/mg protein.

4) Protein estimation

The concentration of protein in filtrate was determined by measuring absorbance at 280 nm. Bovine serum albumin in 100 mM Tris buffer pH 8 was used for plotting standard curve.

2.7.3 Evaluation of Protein oxidation by estimating carbonyls

Chicken meat (4 gm) was homogenized in 20 ml of (100 Mm) tris buffer (pH 8.0) for 1 minutes by Polytron tissue homogenizer. 50 µl of filtrate was added to 300 µl of urea (8 M) containing 20 mM sodium phosphate buffer (pH 6.0). 50 µl of 20 mM sodium phosphate buffer (pH 6.0) and 400 µl of DNPH (10 mM in 1 M H_3PO_4) was added and incubated at 50° C for 30 min. After incubation, 200 µl of 6 M NaOH was added and incubated for 10 min at room temperature. The carbonyl content was measured at 450 nm in spectrophotometer. Concentration of protein was determined in filtrate by measuring absorbance at 280 nm. Bovine serum albumin in 20 mM sodium phosphate buffer (pH 6) containing 8 M urea was used to plot standard curve. The carbonyl content was calculated in nmol/mg protein using absorption coefficient of DNPH is 22,000 $M^{-1}cm^{-1}$ [17].

2.8 Statistical analysis

All experiments were carried out in triplicate. Mean and standard errors were calculated using MS Excel 2007. Statistical significance was tested by Student's t test using web version of GraphPad's t test calculator (www.graphpad.com). A statistical difference at $p \leqslant 0.05$ were considered significant.

3. Results and discussion

3.1 Characterization of plant extracts

Total phenolic content in GTE was estimated to be 103.62 mg GAE/ g dry weight and 22.00 µg/ml of GTE was required to neutralize 50% radicals of DPPH (Table 1). The antioxidant effect of the samples cannot be attributed to single compound and may be a synergistic action of various components presents in the extract. GTE is widely known to consist of catechins and its derivatives. Antioxidant activity coefficient as estimated by β-carotene bleaching assay was 88.40 for 200 µg/ml of GTE. Also, the extract exhibited good ferric ion reducing capacity. Plant extracts are known to act as electron donors and they react with free radicals converting them into stable products and terminate the radical chain reaction [18]. Antioxidant effect of the extracts is exponentially correlated with the reducing power due to its hydrogen donating ability.

Table 1. Characterisation of antioxidant activities of green tea leaves extract (GTE).

Total Phenolic Content (mg GAE/g dry weight)	103.62 ±12.72
DPPH Radical Scavenging Activity (IC_{50} in µg/ml)	22.00
Antioxidant Activity Coefficient (at 200 µg/ml)	88.40 ± 0.21
Ferric ion reducing capacity (at 200 µg/ml)	0.04 ± 0.01

3.2 Physical properties of the films

Smooth and homogeneous films of chitosan and gelatin (ChGel) were obtained. Incorporation of GTE in the films (ChGel-GTE) did not alter the smoothness or homogenicity of the films but significant change in the colour of the film was observed.

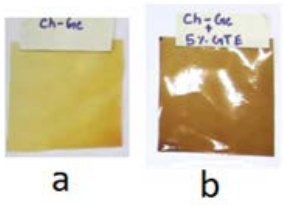

Fig 1. image of (a) Control film: ChGel and (b) active film: ChGel-GTE.

Hunter's Lab colour variable for films containing GTE varied significantly from control ChGel films (Table 2). L and a values indicate that, addition of extract in the films lowered the luminosity, increased the greenness of the films. The b values of the film indicated that GTE increased the blueness of the films. All the films exhibited similar moisture content (21.00 to 25.00%). The ChGel films posses mean tensile strength of 5.26 MPa which was improve by addition of GTE (11.38 MPa). Various polyphenols are known to crosslink the free amino groups of gelatin [19] resulting in stronger and more firm films. Mean percent elongation was unaffected by addition of GTE in the ChGel films. Also addition of GTE increased the opacity of the films while decreasing the transparency in comparison to ChGel films.

Table 2. Optical and mechanical properties (mean ± SD) of control (ChGel) and active (ChGel-GTE) films. Values with different small letter in a row indicate significant difference ($p < 0.05$) in mean.

	Control	ChGel-GTE
Colour (units)		
L	41.68 ± 0.82 a	34.20 ± 3.45 b
a	-0.66 ± 0.55 a	-5.87 ± 2.88 b
b	5.00 ± 0.63 a	-2.07 ± 1.18 b
ΔE*	-	11.54
Opacity (units)	2.41 ± 0.21 a	3.89 ± 0.12 b
Transparency (units)	7.85 ± 1.00 a	5.82 ± 0.18 b
Moisture Content (%)	21.49 ± 1.28 a	24.087 ± 0.211 a
Tensile (MPa)	5.26 ± 1.326 a	11.38 ± 1.380 b
Elongation (%)	34.81 ± 15.42 a	44.45 ± 7.60 a

The water solubility percentages (weight loss) and swelling percentage of the films is reported in table 3. After day 1, the ChGel films were (44.22 %) more soluble than ChGel-GTE films (28.55 %). Solubility of all the films increased by day 2 and remained unchanged thereafter. Gelatin being soluble in water dissolves partially resulting in loss of the fibrous structure of the films. Polyphenols from GTE may cause crosslinking of the free amino groups of gelatin [19] in the films resulting in lower solubility in comparison to ChGel films. The exact composition of the polyphenols and their roles in altering the fibrous structure and crosslinking of various functional groups of the films needs to be evaluated. Rui *et. al.* [20] has reported increase in the solubility of chitosan-gelatin films upon incorporation of gallic acid, both in free and grafted forms, but with increase in the degree of substitution of gallic acid in grafted forms, the solubility of films decreased. This can be ascribed to the formation of larger molecular weight and longer chains causing poor solubility [20]. ChGel films showed minimum swelling (339.75 %) after day 1, while ChGel-GTE showed significantly higher swelling of 560.12 %. At all the time points evaluated the ChGel films showed significantly lower swelling than active films. Also, it should be noted that ChGel absorbed water and continued to swell till day 2 (457.46 %) while ChGel-GTE films continued to swell till day 3 (618.91 %). Gelatin being hydrophilic in nature is expected to absorb water molecules. The swelling properties also represent the porosity of the films, thus suggesting more porous nature of ChGel-GTE films. Also, presence of phenolic compounds may interrupt the intra- and inter-molecular hydrogen networks and provide sites for covalent bonding, resulting in loose and enormous network of chitosan and/or gelatin [20]. Hydrophilicity of the phenolic compounds may render the films with improved ability to interact with and hold more water resulting in increased swelling in ChGel-GTE without increasing the solubility.

Antimicrobial activity of ChGel films against important food spoilage organisms and food-borne pathogens suspended in neutral saline is represented Table 4. The antimicrobial activity of the films was tested with approximately 3 log cfu/ml of *K. pneumoniae, S. typhi var. Weltevreden, S. typhi var. Oslo, Y. enterocolitica, E. feacalis, B. cereus, E. coli and S. aureus*. In absence of film, the cell count after 3 hours was unchanged but no

viable cells were detected when cultures were incubated in presence of ChGel films. The antimicrobial activity of these films may be attributed to the positively charged amino groups of chitosan that can interact with the negatively charged molecules on the cell surface causing leakage of intracellular constituents of the micro organisms.

Table 3. Solubility and Swelling ability of control and active films of ChGel.

Time (Days)	Solubility (%)		Swelling (%)	
	ChGel	ChGel-GTE	ChGel	ChGel-GTE
1	44.22 ± 1.12 Aa	28.55 ± 0.21 Ab	339.75 ± 8.60 Aa	560.12 ± 16.30 Ab
2	49.01 ± 1.97 Ba	35.42 ± 0.14 Bb	457.46 ± 20.16 Ba	609.14 ± 11.60 ABb
3	50.11 ± 0.37 Ba	38.13 ± 3.38 Bb	472.80 ± 26.32 Ba	618.91 ± 30.8 Bb

*Mean ± SD of water solubility (%) and swelling (%) of control (ChGel) and active (ChGel-GTE) films. Values with different capital letters in a column and different small letters in row indicate significant difference ($p < 0.05$) in mean.

Table 4. Antimicrobial activity of control and active films of ChGel.

Organism	0 hours log (cfu/ml)	3 hours log (cfu/ml)	
		No film	ChGel films
Klebsiella pneumoniae	3.08	3.29	Nil
Salmonella typhi var. *weltevreden*	3.19	2.93	Nil
Yersenia enterocolitica	2.97	2.82	Nil
Enterococcus feacalis	2.24	2.25	Nil
Salmonella typhi var. *oslo*	3.05	2.9	Nil
Bacillus cereus	2.0	1.9	Nil
Escherichia coli	3.09	2.81	Nil
Staphylococcus aureus	2.8	2.5	Nil

3.3 Microbial quality of stored chicken meat

Total viable count (TVC) of the chicken cubes stored in chilled condition is represented in Fig. 2. the initial TVC of the chicken sample was noted to be 5.84 log cfu/g of meat which increased to 6.69 and 7.11 log cfu/g on day 6 and day 10, respectively. Thus demonstrating shelf life of chicken chunks to be not more than 6 days at chilled conditions. Application of ChGel films and ChGEl-GTE films maintained the microbial quality of the chicken samples till day 13 with mean TVC of 5.95 and 5.83 log cfu/g, respectively. Similar improvement in the microbial quality in minced trout was reported by Kakaei & Shahbazi [14], where wrapping of samples in films of chitosan and gelatin resulted in shelf life of 7 days in comparison to 4 days for control samples. Composite and bilayer films of chitosan and gelatin are also demonstrated to improve the shelf life of rainbow trout by maintaining TVC below 7 log cfu/g till day 16 in chilled condition [21].

3.4 TCA soluble protein (TSP)

TCA soluble protein of chicken samples is represented in Fig. 2. On day 0, it was estimated to be 8.14 mg BSA eq/ gm meat, which increased in all the samples during the storage period. The increase in TSP during the initial storage period is attributed to the endogenous enzymes like cathepsin, neutral and alkaline proteinases that cause degradation of protein. Till day 3, there was no difference in the TSP amongst any of the samples but further storage caused in significant increase in TSP in chicken without ChGel films. The increase in the TSP in later stages of storage is also a result of action of microbial proteases. The TSP increased to 33.77 mg BSA eq/ gm on day 10 in control samples which can be attributed to increase in microbial count and hence there proteolytic activity. Similar increase in TSP of various fish samples was reported by Rodrigues [22]. For all other samples wrapped in films the TSP estimated was significantly lower than the control samples on day 6, 10 and 13. Antimicrobial activity of ChGel films may be, in part, be responsible for lower protein degradation and lesser loss in fish quality.

3.5 Evaluation of lipid peroxidation

Lipid peroxidation expressed as TBARS value is represented in Fig. 2. Initial TBARS value of 0.19 mg MDA eq/ kg meat was noted in the chicken samples. Storage at 4˚C resulted in steady increase in the lipid peroxidation and increase in TBARS values was observed as a function of time in all samples except chicken samples wrapped in ChGel-GTE films. Maximum increase was noted in samples not wrapped in any films, where these samples exhibited TBARS value of 1.14 mg MDA eq/ kg meat on day 10. Wrapping chicken samples in ChGel films did not lower the TBARS values till day 6 in comparison to control but significantly lowers TBARS value was noted

on day 10. These observations are in agreement with that reported in rainbow trout, where samples packed in chitosan gelatin films had lower TBARS values than control at all time points [21]. In present study it was observed that incorporation of GTE in ChGel films significantly reduced the lipid peroxidation as evident by lower TBARS values at all the time points evaluated. On day 13, TBARS value of only 0.28 mg MDA eq/ kg meat was observed in chicken samples wrapped with ChGel-GTE in comparison to 1.10 mg MDA eq/ kg meat in samples wrapped with ChGel. Although GTE infused in raw chicken was reported to prevent increase in the TBARS value in chicken samples in chilled storage [18, 23]. Present study suggests that even incorporation of GTE in packaging material is sufficient to give similar results.

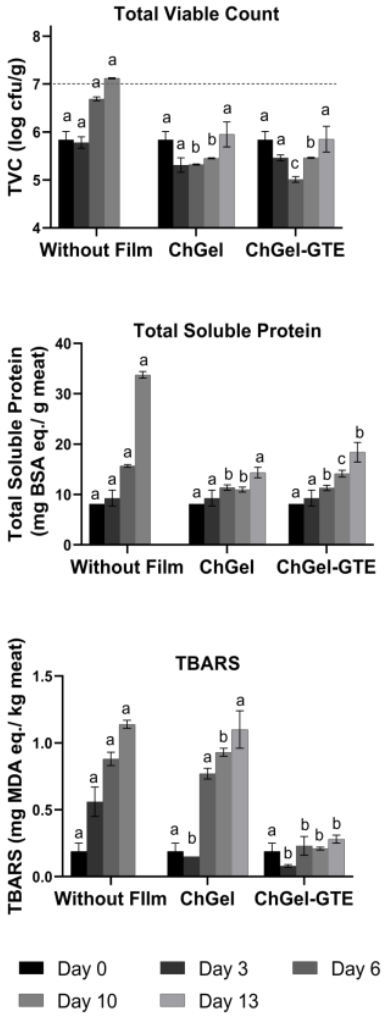

Fig 2. Mean Total viable count (TVC), Total soluble protein (TSP) and Lipid peroxidation (TBARS) values of chicken sample stored at 4˚ C. Different small letters at given time point indicate significant difference ($p< 0.05$) in the mean between the samples.

3.6 Evaluation of Protein oxidation

Loss of thiol groups in the meat samples may result from oxidative modification of cysteine and methionine and can be used to evaluate the extent of oxidative damage [7]. Free thiols and total thiols in chicken samples wrapped in ChGel films with or without GTE and stored at 4˚ C is represented in Fig 3. Most often oxidation of thiol groups is evaluated by quantification of the loss of thiol groups [8]. On day 0, free thiol concentration of 315.94 nmol L-cys eq /mg protein and 869.22 nmol/mg protein of total thiols was estimated in the chicken samples. This significantly higher concentration of total thiols indicate the presence of disulphide bonds (262.41 nmol/mg) in the samples at initial time point. In control samples, free thiols reduced to 262.14 and 183.93 nmol L-cys eq /mg protein on day 6 and day 10, respectively when stored at 4˚C and this loss can be attribute to oxidative changes in protein. Significant reduction in total thiols of control sample was noted on day 6 (546.85 nmol/ mg) which further increased to 760.87 nmol/g on day 10. Approximately, 46% of free thiols groups were lost during storage period of 10 days in chilled condition. This loss of thiol group may be result of reversible oxidation process like

disulphide formation or irreversible oxidation process like sulfonic acid formation. It should be noted that sharp decrease in the total thiol concentration on day 6 may represent conversion of thiols not only to sulphenic acid and disulphides (species that can be reduced by borohydride to thiol groups) but further to species that cannot be reduced to thiols and not be estimated in the assay. Exact reaction and the end products formed during the storage duration need to be evaluated to understand the sudden increase in the total thiol groups in the samples during further storage. Oxidation of thiols is a complex reaction and produce numerous products like sulfenic acid, sulfinic acid, sulfonic acids and thiosulfinates [8] and the loss of thiol from cysteine may result in formation of disulphides, oxyacids or thiyl peroxyl radicals [7] while that from methionine may result in formation of sulfoxide and sulfone.

For samples wrapped in ChGel films without or without GTLE, decrease in concentration of thiol groups was also evident. On day 10 the free thiol content in samples packed in ChGel was 211.29 nmol L-cys eq /mg protein which decreased to 155.71 nmol L-cys eq /mg protein on day 13. However, the loss of free thiols in sample packed in ChGel-GTE films was significantly lesser (258.37 nmol L-cys eq /mg protein on day 13). Similarly, concentration of total thiols in samples wrapped with ChGel and ChGel-GTE was significantly lesser than control after day 6. It was also noted that concentration of disulphide bonds in samples wrapped in ChGel-GTE films (180.87 nmol/ mg) was significantly lower than in samples wrapped in ChGel films (251.27 nmol/mg) at the end of storage period. Such protection from loss of thiols in meat samples was by application of GTE [24], mulberry extract [25] and black currant extract [26] is also known. Although the present study show that the ChGel-GTE films can prevent loss of thiols and formation of species that can be reduced by borohydride, but the exact mechanism and reaction occurring further in the chain of oxidation in presence of GTE as antioxidant needs to be evaluated.

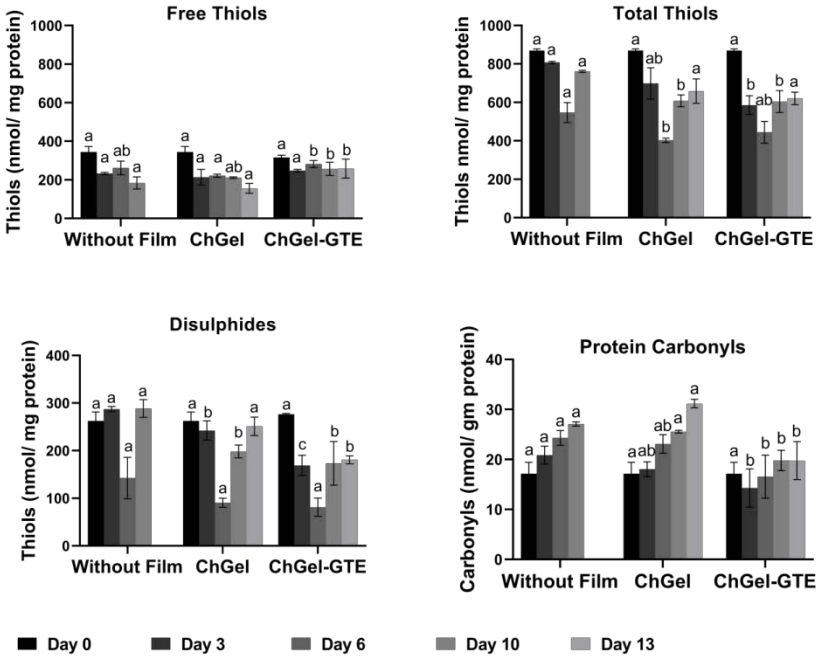

Fig 3. Mean Free thiols, total thiols, Disulphides and protein carbonyl values of chicken sample stored at 4˚ C. Different small letters at given time point indicate significant difference ($p < 0.05$) in the mean between the samples.

3.7 Protein carbonylation

Generation of carbonyls is the most common damage caused due to oxidation of the proteins. Protein carbonylation detected as function of time in stored chicken samples is represented in Fig. 3. On day 0, initial concentration of 17.14 nmol carbonyls per gram of protein was detected in the chicken samples. This increased to 27.08 nmols/g in control samples on day 10, but significantly lower concentration was detected in chicken samples packed in ChGel-GTE (19.76 nmol/g) on day 13. Similar protection against carbonylation of protein group in chicken samples by application of mulberry extract [25], pomegranate [27] and extracts of dog rose, hawthorn, black berry and strawberry [28] during chilled storage is reported. Protein carbonyls can be formed due to fragmentation of protein backbone, binding of non-protein carbonyls from lipid peroxidation to amino acid side chains, direct oxidation of amino acid side chains or addition of reactive carbonyl derivatives generated by reducing sugars and their oxidation products formed after reacting with lysine. Metal ion-catalyzed oxidation of myofibrillar protein is most common cause of protein carbonylation [29]. Hence application of antioxidants like

GTE in meat or in packaging material that can chealate metal ions is promising methods of preventing carbonyl formation in the food.

4. Conclusion

Application of ChGel films improved the microbial safety of the chicken and enhanced its shelf life to 13 days. GTE infused or mixed with meat was previously reported to prevent oxidation of lipids and protein, present study exhibit the efficacy of presence of the GTE in packaging films to get similar protection against oxidative changes. These results strongly suggest that GTE can be incorporated in chitosan-gelatin films and these films have potential application in improving the shelf life of chicken by enhancing the microbial safety as well as preserving the sensorial, nutritional and functional properties.

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