

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.808.046>

Enhancement of Quality and Shelf Life of Chicken Patties Using an Edible Coating of Chitosan

Vivek Shukla^{1*}, S.K. Mendiratta², R.J. Zende³, Ravikant Agarwal² and S.R. Badhe¹

¹Department of Livestock Products Technology, ³Department of Veterinary Public Health,
Mumbai Veterinary College, Parel, Mumbai-12, India

²Division of Livestock Products Technology, ICAR-IVRI, Izatnagar-243122, India

*Corresponding author

A B S T R A C T

Effect of edible coating of chitosan was evaluated on quality and shelf-life of chicken patties during refrigerated storage ($4\pm1^{\circ}\text{C}$). Chicken patties were divided into three groups of which T_1 was kept as control, T_2 was dipped in 1% glacial acetic acid and T_3 was dipped into 1.5% chitosan dissolved in 1% glacial acetic acid. All the samples were analyzed for physico-chemical parameters, microbiological quality and sensory analysis during refrigeration storage ($4\pm1^{\circ}\text{C}$) at an interval of 5 days. The results revealed that T_3 had significantly ($P<0.05$) lower pH, TBARS, Tyrosine and TVBN value as compared to other treatments while microbiological values were also significantly ($P<0.05$) reduced. T_3 was found to be the most stable among all samples and had shelf-life of 15 days at refrigeration temperature ($4\pm1^{\circ}\text{C}$) as compared to a control sample with a shelf-life of 10 days. Thus, a shelf-life extension of 5 days was observed in chicken patties dipped in 1.5% chitosan dissolved in 1.0% glacial acetic acid as compared to control at refrigeration storage.

Keywords

Chicken patties,
Chitosan, Edible
coating, Shelf-life,
Natural preservation

Article Info

Accepted:
04 July 2019
Available Online:
10 August 2019

Introduction

The increased awareness regarding the use of synthetic preservatives in meat products has augmented the usage of natural preservatives which have contributed to the rise of an increased preference to safer food, which is also referred to as "green consumerism". This

has become the major driving force for the development of unconventional or natural methods for food preservation (Imran *et al.*, 2012). Microbial growth on meat surfaces is one of the major causes of spoilage which could be minimized by the use of antimicrobial coating over them. The efficiency of various substances used for

edible coating is related to barrier properties and ability to retard spoilage due to antioxidant or antibacterial capacity (Kanatt *et al.*, 2013). Chitosan is non-toxic, biodegradable and biocompatible and approved GRAS by the USFDA with broad-spectrum antimicrobial activity against both Gram-positive, Gram-negative bacteria as well as fungi (Harish Prashanth and Tharanathan, 2007). Moreover, FSSAI has also listed chitosan as a nutraceutical.

Potential applications of chitosan as a bio-preserved have been investigated in various meat products either alone or in combination with other natural preservatives (Soullos *et al.*, 2008). There has been a growing demand for natural preservatives with antimicrobial and antioxidant activity for enhancement of quality and safety of meat products. However, limited work has been done on the effect of edible coating on the quality and shelf-life of meat products. Therefore, the present study was undertaken to determine the effect of edible coating of chitosan on quality and shelf-life of chicken patties during refrigerated storage.

Materials and Methods

Raw materials

Chicken meat required for the experiments was procured from freshly slaughtered poultry at a selected meat shop in Mumbai city. The meat was purchased and immediately brought to the laboratory under refrigeration condition ($4\pm1^{\circ}\text{C}$) and further processed. Chitosan, in powder form (Medium molecular weight, >75% de-acetylation) was obtained from Hi-Media, Mumbai. All other reagents used were of analytical grade and procured from Qualigens Fine Chemicals (Mumbai, India) and Sisco Research Lab (Mumbai, India). All other non-meat ingredients were purchased from a supermarket in Mumbai city.

Preparation of edible coating solution

1.5g of chitosan was mixed with 100 ml of distilled water having 10ml of glacial acetic acid. The whole mixture stirred for 30 minutes at 10000 rpm using magnetic stirrer (Spinit Digital Magnetic Stirrer, Tarson) to make 1.5% solution of chitosan to be used as an edible coating for chicken patties.

Preparation of samples

Meat emulsion for chicken patties was prepared in bowl chopper (Seydelmann K20, Ras, Germany) using following formulation: Lean meat:70%, ice flakes:10%, refined vegetable oil:8%, condiment mix: 5% (onion and garlic in 3:1 ratio), salt: 1.6%, sodium tripolyphosphate: 0.4%, refined wheat flour as binder: 3.5%, sodium nitrite: 150ppm, spice mix: 1.5% and the procedure for the preparation of chicken patties used as below:

Chilled chicken meat ($4\pm1^{\circ}\text{C}$) was minced using 8 mm sieve plate followed by 4 mm sieve in a plate mincer. Minced meat was mixed with salt and sodium tripolyphosphate and chopped in bowl chopper for 2-3 min. Then condiments, crushed ice, and sodium nitrite were added and chopping was done for 1-2min. Refined vegetable oil was added while continuous chopping for 2-3min. Refined vegetable flour as binder and spice mix was then added and the mixture was chopped for 1 min until a thick tacky emulsion was formed. The emulsion was transferred to patty forming machine to form chicken patties (70g each).

Experimental setup

Based on preliminary trials, a 1.5% solution of chitosan was selected for the edible coating of chicken patties. Chicken patties were dipped in 1.5% chitosan for 5 minutes and kept over perforated mesh for 10 minutes to drain the

excess of chitosan. Chicken patties were then packed in LDPE bags (50μ) and kept at refrigeration temperature for 2 hours for proper adherence and solidification of chitosan. For the purpose of analysis, samples were categorized as T₁ (control), T₂ (chicken patties dipped in 1% glacial acetic acid), and T₃ (chicken patties dipped in 1.5% chitosan dissolved in 1% glacial acetic acid). All the samples were packed in LDPE bags (50μ) and analyzed at 5 days interval at refrigeration temperature (4 ± 1 °C) using physico-chemical parameters, microbiological qualities and sensory analysis.

Physico-chemical parameters

The pH of chicken patties was analyzed by combined glass electrodes of digital pH meter (Model T-25, Janke and Kenkel, 1KA Labor Technik, Germany) in homogenate (Troutt *et al.*, 1992). Thiobarbituric Acid Reactive Substances (TBARS) value was estimated as per the method of Tarladgis *et al.*, (1960) and expressed in mg malonaldehyde/kg of meat. TVBN content was estimated by the micro-diffusion technique described by Pearson (1968) and expressed in mg/100g. Tyrosine value was estimated by the method of Strange *et al.*, (1977) and expressed in mg/100g.

Microbiological quality

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

Sensory analysis

The method as described by Keeton (1983) using an 8-point descriptive scale was used for sensory evaluation, where 8 was given for extremely liked product and 1 for extremely

disliked products. The samples were served warm (40–60 °C) by pre-heating in a microwave oven (LG, Model MC-7148, MS, 1200 W microwave power, India) for 1 min and sensory evaluation was conducted in sensory evaluation laboratory using five sensory attributes viz. appearance and colour, flavour, texture, after taste and overall acceptability.

Statistical analysis

Four sample packages of each treatment (T₁, T₂ and T₃) were taken at the appropriate time and analyzed for physico-chemical parameters, microbiological quality and sensory analysis. Each sample determination was replicated three times (12 determinations in total per test condition). The average value of the three determinations was used per sample so that the statistics describe the variation between samples with n=4. Sensory evaluation was conducted thrice with 10 sensory panellists so that n=30 was used. The data generated for different quality characteristics were compiled and analysed using SPSS (Statistical Package for Social Sciences, version 20.0 for Windows; SPSS, Chicago, IL, USA) with randomized block design and subjected to analysis of variance.

Results and Discussion

Physico-chemical parameters

pH

The pH of T₁, T₂ and T₃ had no significant ($P>0.05$) difference among themselves on the first day of storage but a significant ($P<0.05$) increase in pH was observed in control and treatments through-out the storage period. T₁, T₂ and T₃ had a mean pH of 6.11 ± 0.02 , 6.08 ± 0.10 and 6.08 ± 0.09 on the first day of storage which increased significantly ($P<0.05$) to 6.35 ± 0.09 , 6.30 ± 0.11 and 6.23 ± 0.05 on the

10th day for T₁, T₂ and 15th day for T₃ (Table 1), the days till which respective samples were found acceptable. T₁ and T₂ were examined until the 15th day of storage while T₃ was examined until the 20th day of refrigeration storage due to the presence of evident signs of spoilage on these days. The increase in pH could be attributed to the accumulation of metabolites of bacterial action on meat and deamination of meat proteins having basic nature (Jay, 1996).

T₃ had non-significant ($P>0.05$) lower pH as compared to other samples from the 5th day onwards which was due to inhibition of microbial action on meat proteins by the antimicrobial activity of chitosan maintaining pH to a significantly ($P<0.05$) lower level as compared to other treatments. Antimicrobial activity of chitosan against foodborne microbes has been observed in various studies (Kanatt *et al.*, 2013; Langroodi *et al.*, 2018). Kanatt *et al.*, (2013) observed that chicken meatball with an edible coating of 2% of chitosan had a total plate count of $6.6 \log_{10}$ CFU/g on the 14th day of refrigerated storage while the control samples achieved this count on the 6th day of refrigeration storage. The initial lower pH of T₂ and T₃ as compared to T₁ was due to glacial acetic acid used in the dipping solution.

Thiobarbituric Acid Reactive Substances (TBARS)

The oxidative rancidity increased in all the treatments during the storage period. The initial average TBARS value of T₁, T₂ and T₃ was observed as 0.21 ± 0.02 , 0.19 ± 0.01 and 0.17 ± 0.01 which increased significantly ($P<0.05$) to 0.56 ± 0.01 , 0.40 ± 0.02 and 0.38 ± 0.08 mg malonaldehyde/kg, respectively on the 10th day of refrigerated storage (Table 1). The TBARS value when the signs of spoilage were observed was 0.89 ± 0.03 , 0.85 ± 0.02 and 0.91 ± 0.01 mg

malonaldehyde/kg in T₁, T₂ and T₃ respectively.

The chitosan is known to have its metal chelating activity and is a major cause of the antioxidant activity of chitosan (Vilela *et al.*, 2017). Inhibition of oxidation in chicken patties was also ascribed to the formation of chitosan layer which prevented the entry of oxygen, thus, reduced the oxidation of meat. Langroodi *et al.*, (2018) reported a significant reduction in TBARS value of beef coated with 2% of chitosan during the storage period of 20 days at refrigeration temperature as compared to control. The control and treatment had an initial TBARS value of 0.28 ± 0.02 and 0.27 ± 0.02 which increased to 2.65 ± 0.36 and 1.58 ± 0.34 mg malonaldehyde/Kg of meat, respectively on the 20th day of refrigeration storage.

The antioxidant activity of chitosan is probably due to the formation of stable fluorosphere by primary amino group of chitosan with a volatile aldehyde such as malondialdehyde derived from the breakdown of fat during oxidation (Weist and Karel, 1992). Edible coating made with 2% of chitosan produced a significant reduction in TBARS value in chicken meatball as compared to the control during refrigeration storage of 14 days (Kanatt *et al.*, 2013)

Total Volatile Basic Nitrogen (TVBN)

Edible coating of chitosan had a significant ($P<0.05$) effect on TVBN concentrations of chicken patties (Table 1). Freshly prepared T₁, T₂, and T₃ had a mean TVBN concentration of 6.84 ± 0.05 , 5.90 ± 0.16 and 5.69 ± 0.12 which increased to 28.88 ± 0.17 , 24.48 ± 0.21 and 18.75 ± 0.14 mg/100g, respectively on the 10th day of refrigeration storage which was due to the degradation of meat proteins producing volatile bases and other nitrogenous components in all the treatments. T₃ had

significantly ($P<0.05$) lower TVBN value as compared to other treatments throughout the storage period, which was due to the antimicrobial effect of chitosan, which prevented degradation of proteins and decreased TVBN. Langroodi *et al.*, (2018) also reported a significant decrease in TVBN concentration in beef treated with 2% of chitosan as compared to the control and ascribed it to the reduction in total plate count of treated samples than control with a simultaneous reduction in protein degradation.

Tyrosine value

Changes in tyrosine value were time-dependent and reported a significant ($P<0.05$) increase during refrigeration storage for all treatments which was attributed to hydrolytic changes in meat by inherent tissue enzymes and bacterial proteolysis (Strange *et al.*, 1977). The mean initial tyrosine value of 15.67 ± 0.15 , 14.24 ± 0.08 and 13.27 ± 0.12 for T₁, T₂ and T₃ increased significantly ($P<0.05$) to 28.88 ± 0.17 , 24.48 ± 0.21 and 21.14 ± 0.06 mg/100g, respectively on the 10th day of refrigerated storage (Table 1). The tyrosine value at the time of spoilage i.e. on the 15th day in T₁ and T₂ and 20th day in T₃ was 35.10 ± 0.15 , 34.17 ± 0.24 and 37.50 ± 0.22 mg/100g, respectively. Significantly ($P<0.05$) lower tyrosine value of T₃ as compared to T₁ and T₂ from 5th day onwards was due to the antimicrobial activity of chitosan which inhibited the microbial proteolysis. Both TVBN and tyrosine are related to protein degradation and followed a similar pattern during the storage period.

Microbiological quality

Total Plate Count (TPC)

Average total plate count (\log_{10} CFU/g) of control and treatments increased significantly ($P<0.05$) during refrigeration storage (4 ± 1

°C). T₃ did not report any microbial activity on the first day of refrigerated storage and had significantly ($P<0.05$) lower total plate count as compared to other treatments at each interval of storage study (Table 2). The average total plate count (\log_{10} CFU/g) of T₁ and T₂ was 1.91 ± 0.02 and 1.69 ± 0.05 on first day which increased to 4.47 ± 0.21 and 4.31 ± 0.19 on 15th day of refrigeration storage (4 ± 1 °C) while T₃ did not report any TPC on the first day which increased to 4.03 ± 0.18 \log_{10} CFU/g on 20th day.

The significant ($P<0.05$) reduction in microbial load of T₃ as compared to T₁ and T₂ was due to the antimicrobial nature of chitosan. The significant ($P<0.05$) difference between T₁ and T₂ was due to the antimicrobial effect of glacial acetic acid. T₃ crossed the microbial limit of $4 \log_{10}$ CFU/g (FSSAI, 2016) on the 20th day of refrigerated storage while T₁ and T₂ crossed the limit of microbial spoilage on the 15th day of storage.

About $2 \log_{10}$ CFU/g reduction in TPC of chicken meatball coated with 2% chitosan was observed during refrigeration storage for 14 days as compared to control (Kanatt *et al.*, 2013). Langroodi *et al.*, (2018) observed that beef coated with an edible coating of 2.0% chitosan and control had a TPC of 7.01 ± 0.13 and 8.95 ± 0.14 at the end of refrigeration storage of 20 days while the initial TPC in control and treated sample was 4.63 ± 0.07 and $4.59\pm0.09 \log_{10}$ CFU/g, respectively.

The chitosan works by changing the membrane properties of microbes which provoke the osmotic imbalance and inhibit the microbial growth (Sahidi *et al.*, 1991). It also acts by hydrolysing the peptidoglycan wall in the microorganisms which release the intracellular electrolytes like potassium ions and other low molecular compounds such as nucleic acid, proteins, glucose etc. (Devlieghere *et al.*, 2004).

Table.1 Effect of edible coating of chitosan on physico-chemical parameters of chicken patties at refrigeration storage ($4\pm1^{\circ}\text{C}$)
(Mean \pm S.E.)*

Sr. No.	Refrigerated storage period (days)					
1	pH					
	Treatments	Day 1	Day 5	Day 10	Day 15	Day 20
	T ₁	6.11 \pm 0.02 ^{aC}	6.20 \pm 0.12 ^{aBC}	6.35 \pm 0.09 ^{aAB}	6.42 \pm 0.07 ^{aA}	NE
	T ₂	6.08 \pm 0.10 ^{aC}	6.18 \pm 0.11 ^{aBC}	6.30 \pm 0.11 ^{abAB}	6.34 \pm 0.04 ^{abA}	NE
	T ₃	6.08 \pm 0.09 ^{aD}	6.14 \pm 0.05 ^{aCD}	6.19 \pm 0.13 ^{bBC}	6.23 \pm 0.05 ^{aB}	6.32 \pm 0.10 ^A
2	TBARS (mg malonaldehyde/Kg)					
	T ₁	0.21 \pm 0.02 ^{aD}	0.39 \pm 0.04 ^{aC}	0.56 \pm 0.01 ^{aB}	0.89 \pm 0.03 ^{aA}	NE
	T ₂	0.19 \pm 0.01 ^{aD}	0.31 \pm 0.03 ^{abC}	0.40 \pm 0.02 ^{aB}	0.85 \pm 0.02 ^{aA}	NE
	T ₃	0.17 \pm 0.01 ^{aE}	0.23 \pm 0.01 ^{bD}	0.38 \pm 0.05 ^{bC}	0.65 \pm 0.04 ^{bB}	0.91 \pm 0.01 ^{aA}
3	Tyrosine (mg/100g)					
	T ₁	15.67 \pm 0.15 ^{aD}	20.80 \pm 0.17 ^{aC}	28.88 \pm 0.17 ^{aB}	35.10 \pm 0.15 ^{aA}	NE
	T ₂	14.24 \pm 0.08 ^{bD}	17.88 \pm 0.08 ^{bC}	24.48 \pm 0.21 ^{bB}	34.17 \pm 0.24 ^{aA}	NE
	T ₃	13.27 \pm 0.12 ^{cE}	14.94 \pm 0.09 ^{cD}	21.14 \pm 0.06 ^{cC}	27.48 \pm 0.21 ^{bB}	37.50 \pm 0.22 ^A
4	TVBN (mg/100g)					
	T ₁	6.84 \pm 0.05 ^{aD}	15.47 \pm 0.07 ^{aC}	25.27 \pm 0.19 ^{aB}	30.30 \pm 0.26 ^{aA}	NE
	T ₂	5.90 \pm 0.16 ^{bD}	15.18 \pm 0.20 ^{aC}	23.36 \pm 0.25 ^{bB}	28.57 \pm 0.18 ^{bA}	NE
	T ₃	5.69 \pm 0.12 ^{bE}	13.67 \pm 0.30 ^{bD}	18.75 \pm 0.14 ^{cC}	23.15 \pm 0.13 ^{cB}	24.65 \pm 0.24 ^A

*n=4, Mean \pm S.E. with different superscripts row wise (capital alphabet) and column wise (small alphabet) differ significantly ($P<0.05$)

NE: Not Examined. T₁: Control, T₂: 1% glacial acetic acid, T₃: 1.5% chitosan dissolved in 1% glacial acetic acid

Table.2 Effect of edible coating of chitosan on microbiological qualities (\log_{10} CFU/g) of chicken patties at refrigeration storage ($4\pm1^{\circ}\text{C}$) (Mean \pm S.E.)*

Sr. No.	Refrigerated storage period (days)					
1	Total plate count (TPC)					
	Treatments	Day 1	Day 5	Day 10	Day 15	Day 20
	T ₁	1.91 \pm 0.02 ^{aD}	2.51 \pm 0.12 ^{aC}	3.89 \pm 0.12 ^{aB}	4.47 \pm 0.21 ^{aA}	NE
	T ₂	1.69 \pm 0.05 ^{bD}	2.39 \pm 0.09 ^{bC}	3.71 \pm 0.11 ^{bB}	4.31 \pm 0.19 ^{bA}	NE
	T ₃	ND	1.97 \pm 0.07 ^{cD}	2.70 \pm 0.08 ^{cC}	3.31 \pm 0.12 ^{cB}	4.03 \pm 0.18 ^{aA}
2	Yeast and mould count					
	T ₁	ND	ND	1.73 \pm 0.08 ^{cA}	2.93 \pm 0.13 ^{bB}	NE
	T ₂	ND	ND	1.51 \pm 0.07 ^{bA}	2.89 \pm 0.02 ^{bB}	NE
	T ₃	ND	ND	1.17 \pm 0.11 ^{aA}	1.98 \pm 0.04 ^{aB}	2.72 \pm 0.09 ^{cC}
3	Psychrophilic count					
	T ₁	ND	ND	2.15 \pm 0.02 ^{c1}	3.01 \pm 0.07 ^{b2}	NE
	T ₂	ND	ND	2.01 \pm 0.03 ^{b1}	2.97 \pm 0.08 ^{b2}	NE
	T ₃	ND	ND	1.71 \pm 0.03 ^{a1}	2.09 \pm 0.09 ^{a2}	2.77 \pm 0.05 ^{c3}

*n=4, Mean \pm S.E. with different superscripts row wise (capital alphabet) and column wise (small alphabet) differ significantly ($P<0.05$)

NE: Not Examined. T₁: Control, T₂: 1% glacial acetic acid, T₃: 1.5% chitosan dissolved in 1% glacial acetic acid

Table.3 Effect of edible coating of chitosan on sensory qualities of chicken patties at refrigeration storage ($4\pm1^{\circ}\text{C}$) (Mean \pm S.E.) *

Sr. No.	Refrigerated storage period (days)					
1	Appearance and colour					
	Treatments	Day 1	Day 5	Day 10	Day 15	Day 20
	T ₁	7.38±0.21 ^{aA}	6.51±0.12 ^{aB}	5.05±0.05 ^{aC}	3.90±0.04 ^{aD}	NE
	T ₂	7.32±0.17 ^{aA}	6.76±0.11 ^{bB}	5.31±0.02 ^{bC}	3.98±0.03 ^{bD}	NE
	T ₃	7.29±0.23 ^{aA}	7.20±0.09 ^{cB}	7.00±0.08 ^{cC}	6.41±0.03 ^{cD}	4.21±0.02 ^E
2	Flavour					
	T ₁	7.32±0.13 ^{aA}	7.20±0.04 ^{aB}	6.49±0.03 ^{aC}	4.16±0.05 ^{aD}	NE
	T ₂	7.29±0.12 ^{aA}	7.21±0.14 ^{aB}	6.71±0.01 ^{bC}	4.23±0.02 ^{bD}	NE
	T ₃	7.25±0.04 ^{aA}	7.15±0.13 ^{aB}	7.01±0.09 ^{cC}	5.73±0.09 ^{cD}	4.71±0.13 ^E
3	Texture					
	T ₁	7.43±0.22 ^{aA}	7.01±0.08 ^{aB}	6.07±0.04 ^{aC}	4.67±0.02 ^{aD}	NE
	T ₂	7.40±0.20 ^{abA}	7.19±0.08 ^{bB}	6.11±0.03 ^{bC}	4.28±0.03 ^{bD}	NE
	T ₃	7.37±0.13 ^{bA}	7.17±0.08 ^{cB}	6.87±0.09 ^{cC}	5.96±0.09 ^{cD}	4.99±0.23 ^E
4	After taste					
	T ₁	7.31±0.09 ^{aA}	7.10±0.19 ^{aB}	6.57±0.26 ^{aC}	4.10±0.06 ^{aD}	NE
	T ₂	7.28±0.11 ^{aA}	7.11±0.12 ^{bB}	6.76±0.23 ^{bC}	4.31±0.04 ^{bD}	NE
	T ₃	7.25±0.12 ^{aA}	7.15±0.18 ^{cB}	6.96±0.09 ^{cC}	5.81±0.13 ^{cD}	5.21±0.28 ^E
5	Overall acceptability					
	T ₁	7.34±0.41 ^{aA}	7.08±0.13 ^{aB}	6.51±0.23 ^{aC}	4.73±0.11 ^{aD}	NE
	T ₂	7.30±0.24 ^{aA}	7.17±0.19 ^{bB}	6.67±0.17 ^{bC}	4.99±0.12 ^{bD}	NE
	T ₃	7.28±0.16 ^{aA}	7.17±0.18 ^{bB}	6.89±0.09 ^{cC}	6.27±0.08 ^{cD}	4.97±0.09 ^E

*n=30, Mean ± S.E. with different superscripts row wise (capital alphabet) and column wise (small alphabet) differ significantly ($P<0.05$)

NE: Not Examined. T₁: Control, T₂: 1% glacialacetic acid, T₃: 1.5% chitosan dissolved in 1% glacia lacetic acid

Psychrophilic count

Psychrophiles were first detected on the 10th day of refrigerated storage which was due to the inhibitory effect of cooking and slower growth rate of psychrophiles. However, the number of psychrophiles increased significantly ($P<0.05$) after the 10th day of storage (Table 2). The mean psychrophilic count in T₁, T₂, and T₃ on the 10th day of refrigeration storage was 2.15 ± 0.02 , 2.01 ± 0.03 and 1.71 ± 0.03 which increased to 3.01 ± 0.07 , 2.97 ± 0.08 and $2.77\pm0.05 \log_{10}$ CFU/g at the end of storage period. T₃ had significantly ($P<0.05$) lower psychrophilic count as compared to T₁ and T₂ which was due to the antimicrobial activity of chitosan.

Edible coating of 2% chitosan completely inhibited the 10^6 CFU/ml of *Pseudomonas aeruginosa* inoculated in chicken kebab during refrigerated storage of 14 days (Kanatt *et al.*, 2013). Edible coating of 2% of chitosan reduced the *Pseudomonas* count by $0.80 \log_{10}$ CFU/g in beef as compared to control during refrigerated storage of 20 days. The control sample had a *Pseudomonas* count of 5.96 ± 0.00 while the treatment with an edible coating of 2% chitosan had a *Pseudomonas* count of $5.16 \pm 0.04 \log_{10}$ CFU/g at the end of 20 days of refrigerated storage (Langroodi *et al.*, 2018).

Yeast and mould count

Yeast and mould count increased gradually in all the treatments starting from the 10th day of refrigerated storage. The mean yeast and mould count in T₁, T₂ and T₃ on the 10th day of refrigerated storage was 1.73 ± 0.08 , 1.51 ± 0.07 and 1.17 ± 0.11 which increased significantly ($P<0.05$) to 2.93 ± 0.13 , 2.89 ± 0.02 and $1.98\pm0.04 \log_{10}$ CFU/g, respectively on the 15th day of refrigerated storage (Table 2). The absence of yeast and mould count during the first five days of

refrigerated storage was due to the inhibitory effect of cooking on the growth of yeast and mould. The yeast and mould count of T₃ was significantly ($P<0.05$) lower as compared to T₁ and T₂ throughout the storage period, which was ascribed to the inhibitory effect of chitosan on growth of yeast and mould. The acetic acid hindered the growth of yeast and mould in T₂ as compared to T₁ throughout the storage.

Chitosan prevented fungal growth by inhibition of spore germination, germ tube elongation and radial growth of fungus (Ghaouth *et al.*, 1992). Langroodi *et al.*, (2018) also reported a reduction in yeast and mould count by application of 2% of chitosan in beef during refrigeration storage of 20 days. The initial yeast and mould count in control and treatment were 3.03 ± 0.06 and 3.14 ± 0.03 which increased to 8.57 ± 0.04 and $8.13 \pm 0.01 \log_{10}$ CFU/g, respectively at the end of the storage period of 20 days at refrigeration storage. The antifungal activity of chitosan could be due to the diffusion of oligomers of chitosan inside fungal hyphae and interfering the enzymatic activity responsible for microbial growth (Eweis *et al.*, 2006).

Sensory evaluation

Results of the sensory characteristics during refrigerated storage of chicken patties are given in Table 3. Appearance and colour scores for all the treatments were non significantly ($P>0.05$) different in the freshly prepared product but it was significantly ($P<0.05$) higher in T₃ from the 5th day of refrigerated storage as compared to other treatments. The higher scores for T₃ during refrigerated storage was due to the inhibition of lipid peroxidation by chitosan. Lipid oxidation is the major reasons affecting the general appearance of the product (Sharma *et al.*, 2015). Inhibition of oxidation by chitosan

is due to its metal quenching ability (Kanatt *et al.*, 2013).

Flavour scores of all the treatments reported a significant ($P<0.05$) decrease during refrigerated storage ($4\pm1^{\circ}\text{C}$). During the first five days of refrigerated storage, there was no significant difference ($P>0.05$) among all the treatments. However, from 10th day onwards, the flavour score for T₃ was significantly ($P<0.05$) higher as compared to T₁ and T₂. The decreased flavour during storage period was due to oxidation of chicken patties as observed by a simultaneous increase in TBARS value. There is a strong correlation between the decrease in flavour and increase in TBARS and free fatty acids in meat and meat products under aerobic conditions (Tarlaldgis *et al.*, 1960).

Texture followed a gradual decrease during refrigerated storage but T₃ had significantly ($P<0.05$) higher score throughout the storage as compared to T₁ and T₂. T₃ reported slightly undesirable texture on the 20th day but T₁ and T₂ had slightly undesirable texture on the 15th day of refrigerated storage. There is a direct correlation of texture with protein degradation which was inhibited in T₃ due to antimicrobial activities of chitosan. Change in the texture of the meat is mainly associated with protein degradation due to chemical and enzymatic activity (Diaz *et al.*, 2008).

Aftertaste scores for control as well as treatments decreased significantly ($P<0.05$) during refrigeration storage ($4\pm1^{\circ}\text{C}$). Aftertaste score was non-significantly ($P>0.05$) higher for T₁ as compared to other treatments on the first day of storage which became higher in T₃ with the progress of storage period. The significantly ($P<0.05$) higher aftertaste score in T₃ was due to the inhibition of protein degradation and oxidative rancidity by an edible coating of chitosan.

Overall acceptability decreased significantly ($P<0.05$) as expected during refrigerated storage ($4\pm1^{\circ}\text{C}$) but no significant ($P>0.05$) difference was observed among all the treatments on the first day of refrigerated storage. Overall acceptability of T₃ was comparable to the control till the 5th day of storage period after which T₃ had significantly ($P<0.05$) higher overall acceptability as compared to other treatments. T₃ was acceptable till the 15th day of refrigerated storage but T₁ and T₂ were acceptable till the 10th day of refrigerated storage. Overall acceptability is the cumulative outcome of different sensory attributes and follows a similar trend.

Edible coating of chitosan enhanced the quality and shelf-life of chicken patties as compared to control. T₃ i.e. chicken patties with an edible coating of 1.5% chitosan dissolved in 1.0% glacial acetic acid had a shelf life of 15 days as depicted by physico-chemical parameters, microbiological quality and sensory analysis. The edible coating of chitosan produced the least microbial counts, oxidative rancidity, lowest protein degradation and the highest sensory scores. Bland nature of chitosan did not produce any sensory discrimination and its antimicrobial property inhibited the microbial growth. Although the acetic improved the storage characteristics of T₂ as compared to control, its shelf life was limited to 10 days only, similar to that of control. Thus, it was concluded that an edible coating of 1.5 % chitosan dissolved in 1% glacial acetic acid had a significant effect on the quality and shelf-life of chicken patties and can be successfully used as a natural method of preservation for meat and meat products.

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How to cite this article:

Vivek Shukla, S.K. Mendiratta, R.J. Zende, Ravikant Agarwal and Badhe, S.R. 2019. Enhancement of Quality and Shelf Life of Chicken Patties Using an Edible Coating of Chitosan. *Int.J.Curr.Microbiol.App.Sci*. 8(08): 406-417.
doi: <https://doi.org/10.20546/ijcmas.2019.808.046>