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Effect of Chitosan Coating Enriched with Cinnamon Oil (*Cinnamomum zeylanicum*) on Storage Stability of Refrigerated Chicken Meat Nuggets

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ABSTRACT

The purpose of this paper was to evaluate the shelf-life of chitosan and cinnamon oil coated chicken meat nuggets under refrigeration conditions. Three types of coated nuggets were developed viz., Meat coated with Chitosan (1%) and Cinnamon oil (0.05%) (T_1), direct addition of Chitosan and Cinnamon oil in emulsion (T_2) and nuggets dipped in Chitosan and Cinnamon oil (T_3) and were aerobically packaged in low-density polyethylene pouches and assessed for various storage quality parameters under refrigerated (4±1°C) conditions during 28 days of storage. T_2 had slightly higher emulsion stability and yield although no significant difference (P>0.05) in between samples. T_1 exhibited better storage stability as indicated by lower lipid oxidation than other treatments and the control. Texture profile studies indicated that T_2 sample had higher hardness value than other samples. SPC significantly (P<0.05) increased in all the samples with control having the highest value followed by T_1, T_3 and T_2 throughout the storage period. T_2 sample exhibited lowest sensory scores and were not accepted by sensory panellist while T_1 and T_3 samples had better acceptability. It was concluded that chitosan in combination with cinnamon oil had synergistic effect to extend the shelf-life of products (T_1 and T_3)

Keywords: Chitosan, cinnamon oil, edible coating, nuggets, chicken breast/fillets.

Chicken breast (fillet) is favoured by consumers worldwide; its consumption has increased over the last few decades. The reason for the popularity is due to relatively low cost of production, low fat content, more tenderness and juiciness and high nutritional value. Considering the fact that poultry belongs to perishable foods, the main concern of industries is to extend the shelf-life of poultry products. In order to achieve this goal, hurdle technology concept should be applied with use of natural food preservatives in order to maintain minimal processing and also to ensure protection from both spoilage and pathogenic microorganisms (Chouliara and Kontominas 2006). Furthermore, as consumer's demand for more "healthier" meals (free of conventional chemical preservatives) has increased in the last decade, novel packaging (e.g. active) and processing technologies, in some cases, combined with "natural" antimicrobials such as essential oils and chitosan have been suggested (Giatrakou and Savvaidis, 2012).

Edible films and coatings have been particularly considered in food preservation, because of their capability for improving global food quality (Chillo *et al*. 2008). Edible films provide replacement and/or fortification of natural layers to prevent moisture losses, while selectively allowing for controlled exchange of important gases.

Chitosan is a linear polymer made of 2-amino-2-deoxy-β-D-glucan, is a deacetylated form of chitin, a naturally occurring cationic biopolymer. It is Generally Recognised as Safe (GRAS) by the US-FDA (2001). It has wide range of antimicrobial activity and also possesses other functional properties like water binding, emulsifying capacity and ability to bind with intestinal lipids and act as hypocholesterolemic agent.

Cinnamomum zeylanicum (L.), commonly known as cinnamon is rich in cinnamaldehyde as well as b-caryophyllene, linalool and other terpenes. It is used worldwide as a food additive and flavouring agent and it is listed as "Generally Recognized as Safe" (GRAS) by US FDA (2001). It has been found that cinnamaldehyde and eugenol inhibit production of an essential enzyme by the bacteria and/or cause damage to the cell wall of bacteria. Application of cinnamon oil as antimicrobial and antioxidant with hydrocolloids on meat surface could be a tool to increase the shelf life of muscle foods and to delay the rancidity and discoloration in meat as well as reducing the microbial loads. In present study nuggets prepared from chitosan and cinnamon oil coated meat, nuggets prepared from emulsion directly added with coating solution and nuggets dipped into coating solution were evaluated for storage stability under refrigerated conditions.

MATERIALS AND METHODS

Chemicals and other materials

Meat samples required for the experiments were obtained from broilers slaughtered as per standard procedure in the experimental slaughterhouse of Department of Livestock Products Technology (Meat Science) at Madras Veterinary College, Chennai-7, Tamil Nadu. The breast portion of the dressed carcasses (Boneless skinless breast) after removal of all separable connective tissues, fat, skin, fascia, and blood vessels were used for edible coating. Sunflower Oil (Gold Winner-Kaleesuwari Refinery Pvt. Ltd. Chennai) Iodised sodium chloride (Tata Chemicals Ltd., Mumbai, India), tetra sodium pyrophosphate (HiMedia Laboratories Pvt., Ltd., Mumbai, India), spices used in product preparation procured from local market of Chennai, Tamil Nadu, India. Low density polyethylene (LDPE) and polyester propylene laminated plastic bags of 200 Gauge in natural colour were procured from reputed firms and used for aerobic packaging of coated meat nuggets. Analytical grade chemicals and media, and high purity standards required for analyzing the products were procured from standard firms like SRL, Fisher Scientific, CDH, HiMedia, Sigma-Aldrich etc. Cinnamon bark oil was procured from Plant Lipids Pvt. Cochin, Kerala.

Standardization of cinnamon oil and method of application

Four level of cinnamon oil (0.05%, 0.1%, 0.2% and 0.3%) were used in 1% chitosan coating solution. Chicken breast meat coated with coating solution of all the concentrations was used with three types of modes of application viz., (spraying, brushing and dipping) making it into 12 combinations. All the combinations were stored for 6 days at refrigeration temperature and analysed by boiling test and sensory evaluation at 24 hrs interval and found that 0.05% level was optimum based on boiling test and sensory test. Spraying method of application was selected as best among three methods based on preliminary trials and studies conducted in earlier experiment.

Preparation of coating solution

Chitosan coating solution (1000 ml) was prepared by dissolving 1% (w/v) chitosan solution in 1% v/v acetic acid and this coating solution was heated upto 60°C before application. This coating solution was followed with 0.05% cinnamon oil addition.

Spraying was performed using hand sprayer, 50-100 ml coating solution was filled in sprayer then it was uniformly sprayed all over the breast (500-600 gm).

Chicken nuggets formulation

The formulation was as follows:

S. No.	Name of ingredients	Parts/Percentage
1.	Chicken Breast (g)	90
2.	Sunflower oil (g)	10
		100*
Other Ingredients*		
3.	Sodium tetra polyphosphate (g)	0.3
4.	Salt (g)	2
5.	Water (g)	10
6.	Spice mix (g)	2
7.	Condiments (g)	4
8.	Sodium nitrite (ppm)	120
9.	Refined wheat flour (g)	10

*Chicken Breast and sunflower oil consisting 100 % of formulation, and over and above this various additives were added

Preparation of chicken meat nuggets and coating of prepared nuggets

Coated meat were kept at 4 °C for an hour prior to use and were then cut into small pieces to facilitate easy mixing. Thawed meat were minced twice using 4.5mm plate in a meat mincer (Omas, Model No. 169789, Electrolux Food Service, Italy). The minced meat was chopped manually to form a coarse emulsion by following the schedule as shown in the flowchart (Fig.1). The temperature of emulsion was maintained at 10-12 °C during process of chopping by adding slushed ice. About 700 g of emulsion was prepared for every batch of nuggets along with control and three treatments. Three treatments include; T_1: Product from coated meat [chitosan (1 per cent) and cinnamon oil (0.05 per cent)]; T_2: Product from emulsion in which chitosan and cinnamon oil were directly added [water is replaced with 1 per cent chitosan; cinnamon oil is added 0.05 per cent level (w/w) of emulsion weight] and T_3: Dipping of nuggets in coating solution (same coating solution used for coating of meat) for 30 seconds and allowed to drain for few minutes.

Cooking of Nuggets

Emulsion of 700g each was filled in aluminium moulds and were cooked for 30 minutes using steam to an internal temperature of 90 ± 1 °C as indicated by temperature probe. The meat blocks thus formed were immediately chilled and sliced into nuggets of uniform size for physicochemical characteristics and organoleptic evaluation.

Packaging of Nuggets

The sliced nuggets were allowed to cool at room temperature and packed in LDPE bags and stored in a refrigerator (4 ± 1°C) until further analysis. Nuggets weighing five grams were packaged in lockable polythene bags and stored at refrigerated temperature for microbial analysis.

Analytical procedures

pH

The pH of chicken meat (n=6) was determined (Trout *et al.* 1992) with digital pH meter equipped with a combined glass electrode (Digisun electronics system Model No. 2001).

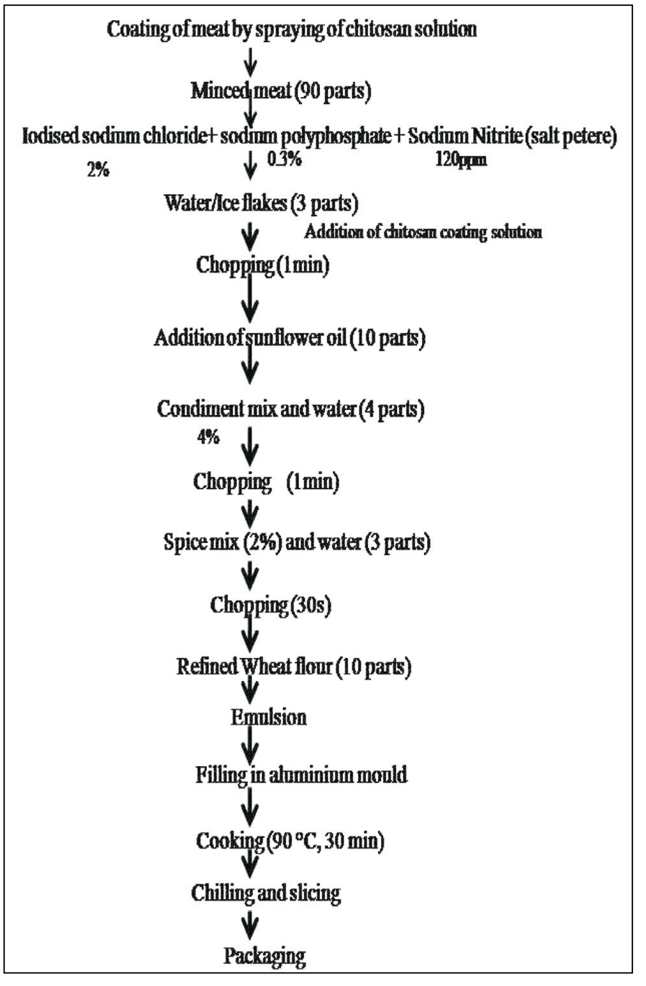

Fig. 1: Flowchart showing steps of preparation of nuggets

Boiling Test

The flavour of fresh meat were checked by boiling small meat samples (approx. 10 pieces of 1 cm^3 each) in preheated water of 80 °C for about five minutes in a vessel covered with lid. The odour of the cooking broth taken immediately by removing lid from vessel and the taste of the warm meat samples will indicate whether the meat was fresh or in deterioration or subject to undesired influences (rancidity and foreign odour).

Hunter colour values (Instrumental colour values)

Colour changes were measured using a MiniScan XE Spectrophotometer (Hunter Associates Laboratory, Reston, Virginia, USA). Three readings were taken for each sample, and the mean values were automatically calculated and recorded by the colorimeter. Colour difference ΔE was calculated according to Eq.

$$\Delta E = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \quad \text{Eq. 1}$$

Where, L_2-L_1, A_2-A_1, and B_2-B_1 are the changes in *L*, *a*, and *b*, respectively, between the interval of interest and baseline, and ΔE is the colour difference.

Cooking Yield

The weight of the raw product and cooked product was recorded and yield was calculated by using the formula.

$$\text{Product Yield (\%)} = \frac{\text{(Weight of cooked product)}}{\text{(Weight of raw product)}} \times 100$$

Emulsion stability

Emulsion stability was estimated as per the method outlined by Baliga and Madaiah (1971).

$$\text{Emulsion stability (\%)} = \frac{\text{(Weight of cooked emulsion)}}{\text{(Weight of raw emulsion)}} \times 100$$

Texture Profile Analysis

Texture profile analysis was conducted using a Stable Microsystems Texturometer (Stable Microsystems Ltd., England, UK) model TA-HD plus texture analyser attached to software, texture expert. The texture profile was analysed as per Bourne, (1978).

Thiobarbituric acid reacting substances (TBARS) numbers

Distillation method described by Tarladgis *et al*. (1960) was used with suitable modifications for determination of TBARS numbers or values in chicken meat nuggets. TBA value was calculated as mg malonaldehyde per kg of sample by multiplying O.D. value with K factor of 7.8.

Free fatty acids (FFAs)

The method as described by Koniecko, (1979) was followed for quantification of free fatty acids. The quantity of potassium hydroxide required for titration was recorded and calculated as follows:

$$\text{Free fatty acid (FFA) \%} = \frac{0.1 \times \text{ml } 0.1\text{N alcoholic KOH} \times 0.282}{\text{Sample weight (g)}} \times 100$$

Peroxide value

Peroxide values were measured as per procedure described by Koniecko, (1979) with suitable modifications. The peroxide value (meq/kg of the meat) was calculated as per the following formula:

$$\text{PV (meq/kg sample)} = \frac{0.1 \times \text{ml } 0.1\text{N sodium thiosulphate}}{\text{Wt. of sample (g)}} \times 1000$$

Assay for microbiological quality

Standard plate counts (SPC), total coliforms counts (CC) and yeast and mold counts (Y&M) in the samples were enumerated following the methods as described by American Public Health Association (APHA, 1984).

Sensory evaluation of chilled nuggets

A six member experienced panel of judges consisting of faculty and postgraduate students of Madras Veterinary College, Chennai-7 evaluated the samples for the attributes of appearance and colour, texture, flavour, juiciness and overall acceptability using an 9 point descriptive scale (Keeton, 1983), where 9=extremely desirable and 1=extremely undesirable. Three sittings (n=18) were conducted for each replicate and at each storage time on samples warmed in a microwave oven for 20 sec.

Statistical analysis

Data were analyzed statistically on 'SPSS-16.0' software package as per standard methods (Snedecor and Cochran, 1994). Samples were drawn for each parameter and the experiment was replicated six times (n=6). Sensory evaluation was performed by a panel of six trained panellist. Data were subjected to one way analysis of variance, homogeneity test and Duncan's Multiple Range Test (DMRT) for comparing the means to find the effects between treatment and between storage periods.

Ethical approval

Permission of Animal Ethics Committee of Madras Veterinary College was taken for slaughter of experimental birds.

RESULTS AND DISCUSSION

Physicochemical properties

There was no significant difference (P>0.05) in yield and emulsion stability in between the treatments but slightly higher yield and emulsion stability were observed in T_2 compared to control, T_1 and T_3. This might be due to addition of hydrophilic hydrocolloids (chitosan) which prevents moisture loss (Varela and Fizsman, 2011). Higher emulsion stability might be attributed to formation of stronger protein gel networks on addition of additives such as calcium compounds, chitosan and oxidizing agents (Martin-Sanchez *et al*. 2009). Similarly, Deliza *et al*. (2002) also revealed that addition of water binders such as soy protein had similar or higher cooking yield than that of control due to more water binding during cooking.

pH of coated nuggets showed a highly significant difference (P<0.01) in between treatments throughout the storage periods whereas no significant difference (P>0.05) was observed in between storage periods in all treatments. A significant increase in pH was observed in control during storage period except on the 7th day where a slight decrease was observed. There was a gradual increase in pH in all samples during storage, probably due to the accumulation of basic compounds such as ammonia, derived from microbial action (Nychas *et al*. 1998). There was no significant difference between control, T_1 and T_2 on day 0 of storage whereas, T_3 had significantly lower (P<0.05) pH value than other treatments and control throughout the storage which could be attributed to acidic pH of the dipping solution.

Oxidative stability

Thiobarbituric acid reactive substances (TBARS)

Microbial spoilage along with chemical deterioration especially lipid oxidation is considered as one of the main factor limiting the shelf-life of muscle foods. Lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS). Results revealed that there was no significant difference in between samples during initial days of storage but as storage interval progressed the TBARS value also increased significantly (P<0.05) in all the treatments.

The control samples showed a significantly (P<0.05) higher TBARS values as compared to all the treatments. These results indicated a strong pro-oxidant effect of salt and anti-oxidative effect of chitosan and cinnamon oil in coated nuggets. There was no significant difference (P>0.05) observed in between the treatments throughout storage period. However, at the end of storage period; nuggets from coated meat (T_1) had slightly lower value followed by T_3 and T_2. This was in accordance with findings of Biswas *et al.* (2012) who also found significantly lower TBARS value in chicken meat balls, patties and nuggets added with chitosan and eugenol compared to their corresponding controls. The present finding is in agreement with the finding of Rao *et al*. (2005) in intermediate moisture (IM) mutton kababs who had reported that chitosan coating of IM mutton kabab lowered the TBARS values by 30%.

The findings of the present study revealed that chitosan inhibited lipid oxidation in all the meat products. The mechanism of antioxidant activity of chitosan may be due to primary amino group of chitosan forming a stable fluorosphere with volatile aldehydes such as malondialdehyde, which is derived from breakdown of fat during oxidation (Weist and Karel 1992). The antioxidant properties of phenolic compounds were very well documented. A significant relation in between phenolic content and antioxidant effect of chitosan and cinnamon oil has been reported by Perdones *et al*. (2014). Similarly, Devatkal *et al*. (2010) observed a positive correlation between phenolic content of plant by-products extract (*kinnow rind*, *pomegranate rind* and *seed powders*) and reduction of TBARS in cooked goat meat patties.

Free Fatty acid (FFA)

Free fatty acid content is a measure of quality deterioration of meat products and it is produced due to bacterial enzymatic lipolysis through the breakdown of fat into fatty acids and triglycerides. Results obtained in this study revealed that FFA content increased significantly (P<0.01) with storage period irrespective of treatments. There was no significant difference in between treatments throughout the storage period. During the initial 14 days of storage there were no significant difference between samples but higher values were recorded for control compared to other treatments. There was a significant difference in FFA value in between treatments on 21st day of storage.

Table 1: Mean ± S.E values of physicochemical properties (Yield, Emulsion stability pH TBARS, FFA and Peroxide value of chitosan and cinnamon oil coated chicken meat nuggets stored at 4 ± 1°C.

Days	Control	T_1	T_2	T_3	F Value
			Yield		
0	94.91±0.68	95.96±0.233	96.19±0.207	95.63±0.34	90.18^{NS}
			Emulsion stability		
0	95.05±0.87	96.06±0 .675	96.59±0.551	95.45±0.45	1.07^{NS}
pH					
0	6.47±0.031^{abB}	6.44±0.056^{B}	6.36±0.048^{B}	6.17±0.031^{aA}	1.165**
7	6.45±0.075^{aB}	6.44±0.130^{B}	6.35±0.105^{AB}	6.19±0.136^{aA}	4.398*
14	6.50±0.034^{abB}	6.50±0.044^{B}	6.38±0.038^{AB}	6.24±0.045^{aA}	9.092**
21	6.54±0.053^{abB}	6.48±0.027^{B}	6.40±0.028^{AB}	6.25±0.037^{aA}	10.876**
28	6.67±0.058^{bB}	6.57±0.057^{B}	6.48±0.064^{B}	6.26±0.017^{aA}	11.165**
F Value	2.713*	1.257^{NS}	1.306^{NS}	1.077 ^{NS}	
			TBARS (mg malonaldehyde/kg)		
0	0.31±0.062^{a}	0.18±0.035^{a}	0.16±0.040^{c}	0.19±0.032^{a}	2.384^{NS}
7	0.37±0.064^{abB}	0.21±0.013^{abA}	0.22±0.023^{bcA}	0.24±0.036^{abA}	3.681*
14	0.49±0.066^{abA}	0.32±0.067^{abcA}	0.30±0.048^{abA}	0.32±0.051^{abcA}	2.276^{NS}
21	0.63±0.053^{bcB}	0.40±0.062b^{cA}	0.40±0.044^{aA}	0.43±0.055^{bcAB}	4.051*
28	0.84±0.069^{cB}	0.47±0.050^{cA}	0.53±0.048^{aA}	0.52±0.058^{cA}	8.894**
F Value	11.421**	6.287**	12.299**	7.903**	
FFA (%)					
0	0.07±0.005^{aA}	0.05±0.004^{aA}	0.05±0.006^{aA}	0.05±0.004^{aA}	0.978^{NS}
7	0.10±0.009^{ab}	0.08±0.006^{ab}	0.07±0.005^{ab}	0.09±0.008^{ab}	2.555^{NS}
14	0.12±0.009^{bc}	0.09±0.009^{a}	0.10±0.005^{bc}	0.11±0.008^{bc}	1.941^{NS}
21	0.15±0.009^{cdA}	0.12±0.007^{dA}	0.12±0.006^{dA}	0.14±0.006^{cdAB}	2.916*
28	0.18±0.012^{d}	0.15±0.013^{cd}	0.15±0.015^{cd}	0.17±0.015^{d}	1.268^{NS}
F Value	24.953**	0.635**	22.317**	22.861**	
			Peroxide value (meq/kg)		
0	1.08±0.091^{aB}	0.90±0.037^{aAB}	0.86±0.049^{aA}	0.88±0.017^{aAB}	3.441*
7	1.53±0.084^{aB}	1.20±0.073^{aA}	1.03±0.056^{aA}	1.30±0.068^{aAB}	8.645**
14	2.13±0.084^{bB}	1.81±0.054^{bAB}	1.66±0.066^{aB}	1.85±0.108^{bAB}	5.767**
21	2.90±0.153^{cB}	2.40±0.154^{cAB}	2.13±0.098^{cA}	2.33±0.133^{cA}	5.673**
28	3.40±0.155^{dB}	2.63±0.158^{cA}	2.57±0.174^{dA}	2.87±0.152^{dAB}	5.570**
F Value	64.893**	47.346**	51.842**	54.280**	

Means bearing different superscript between rows a, b, c and between columns A, B, C differ significantly ($p<0.05$)

*Indicates significant value ($P<0.05$); ** Highly Significant value; ($P<0.01$); NS - Non significant

T1= Nuggets from chitosan and cinnamon coated chicken meat (spraying of chicken breast), T2=Chitosan and cinnamon added chicken meat nuggets, T3=Coated chicken meat nuggets (Dipping of nuggets)

On the last day of storage (28th day) control samples exhibited highest value of 0.18 (Table 1) followed by T_3 (0.17), T_2 (0.15) and T_1 (0.15). Similar results were revealed by Ninan *et al*. (2010) who observed that FFA did not differ significantly on coating shrimp with three different hydrocolloids. However, coated meat had lower values than uncoated/control. Sahoo and Anjaneyulu (1997) reported that the FFA of buffalo meat nuggets increased with storage due to growth of some lipolytic microorganisms. A lower FFA value in treatments may be attributed to antioxidant and antimicrobial effect of chitosan and cinnamon oil.

Peroxide value

Peroxide value of control and treatments showed a highly significant difference (P<0.01) in between the treatments and in between the storage period. Peroxide value increased significantly with storage period in all the samples and at the end of storage period on (28th day), control having the highest value followed by T_3, T_1 and T_2 (Table 1). Similar, results were reported by Gheisari, (2011), who found a significant increase in peroxide value with the storage period in chicken meat stored under refrigeration temperature.

Instrumental/Hunter color

The mean values of Instrumental/Hunter color of chitosan and cinnamon oil coated chicken meat nuggets stored at 4 ± 1°C are presented in Table 2. The results of present study indicated that *L** value did not differ significantly in between storage period and in between treatments. *L** value of treatments had slightly higher value than control. The *L** values of all the samples did not differ significantly on day 0 but during subsequent storage days *L** value of treatments were higher which was in agreement with Jo *et al*. (2001) who also found similar results in pork sausages added with chitosan oligomer. Coated nuggets (T_3) had comparatively higher *L** value than T_2 and T_1 which could be attributed to better penetration of coating solution (chitosan and cinnamon oil) during dipping. *L** values in meat and meat products are related to surface water, water vapour exchanges between the products and the environment and modifications of the different stages of the haempigments (Fernandez-Lopez *et al*. 2005). However, Biswas *et al*. (2012) reported a higher lightness values in control samples compared to chitosan and eugenol incorporated chicken meat products.

Table 2: Mean ± S.E values of Instrumental/Hunter color of chitosan and cinnamon oil coated chicken meat nuggets stored at 4 ± 1 °C

Days	Control	T_1	T_2	T_3	F Value
			L* value		
0	60.01±2.11^{aA}	60.05±1.34^{aA}	62.56±0.45^{aA}	60.17±1.87^{aA}	2.400^{NS}
7	58.99±0.68^{aA}	61.43±2.25^{aA}	60.18±1.54^{aA}	63.73±1.95^{aA}	1.400^{NS}
14	59.74±0.61^{aA}	62.10±0.62^{aA}	60.79±0.9^{aA}	62.43±1.27^{aA}	1.898^{NS}
21	59.04±2.23^{aA}	61.04±0.74^{aA}	61.95±1.15^{aA}	61.85±1.55^{aA}	0.783^{NS}
28	62.95±1.33^{aA}	59.96±1.65^{aA}	59.80±1.40^{aA}	61.81±1.20^{aA}	1.137^{NS}
F Value	1.091^{NS}	0.391^{NS}	0.356^{NS}	0.317^{NS}	
			a* value		
0	8.14±0.25^{bAB}	8.57±0.36^{bAB}	8.82±0.35^{bB}	7.47±0.33^{aA}	3.070^{NS}
7	7.73±0.271^{abA}	7.73±0.39^{abA}	7.77±0.37^{abA}	7.37±0.30^{aA}	0.330^{NS}
14	7.84±0.316^{abA}	7.64±0.17^{abA}	7.76±0.17^{abA}	7.02±0 .26^{aA}	2.457^{NS}
21	7.53±0.17^{abA}	7.13±0.34^{abA}	7.73±0.22^{abA}	6.82±0.26^{aA}	2.446^{NS}
28	7.97±0.299^{aB}	7.27±0.32^{aAB}	7.50±0.21^{aAB}	6.75±0.34^{aB}	2.913^{NS}
F Value	0.762^{NS}	2.673*	3.753**	1.148^{NS}	

			***b** value**		
0	26.50±1.12^{aA}	26.95±0.87^{aA}	27.42±0.75^{aA}	26.56±0.53^{aA}	0.254^{NS}
7	25.67±0.96^{aA}	26.01±0.71^{aA}	25.31±0.95^{aA}	26.58±1.07^{aA}	0.331^{NS}
14	25.37±1.20^{aA}	25.82±0.31^{aA}	26.38±0.73^{aA}	25.69±1.05^{aA}	0.223^{NS}
21	24.57±0.70^{aA}	24.75±0.82^{aA}	26.11±0.41^{aA}	25.11±0.49^{aA}	1.190 ^{NS}
28	26.54±0.77^{aA}	24.80±0.98^{aA}	25.74±0.74^{aA}	25.22±0.79^{aA}	0.808 ^{NS}
F Value	0.717^{NS}	1.404^{NS}	1.157^{NS}	0.728^{NS}	
			ΔE		
0	66.13±2.28^{aA}	66.41±1.42^{aA}	66.58±1.83^{aA}	68.38±0.56^{aA}	0.381^{NS}
7	64.83±0.83^{aA}	67.18±0.22^{aA}	64.90±2.20^{aA}	67.91±0.14^{aA}	0.650^{NS}
14	63.90±0.77^{aA}	66.85±0.75^{aA}	65.06±1.04^{aA}	65.95±1.65^{aA}	1.267^{NS}
21	64.40±2.29^{aA}	66.28±0.78^{aA}	67.83±1.36^{aA}	66.60±1.56^{aA}	0.789^{NS}
28	67.12±1.16^{aA}	65.30±1.89^{aA}	65.42±1.43^{aA}	65.56±1.53^{aA}	0.311^{NS}
F Value	0.665^{NS}	0.211^{NS}	0.576^{NS}	0.601^{NS}	

Means bearing different superscript between rows a, b, c and between columns A, B, C differ significantly (p<0.05)

*Indicates significant value (P<0.05); ** Highly Significant value; (P<0.01); NS - Non significant

T1= Nuggets from chitosan and cinnamon coated chicken meat (spraying of chicken breast), T2=Chitosan and cinnamon added chicken meat nuggets, T3=Coated chicken meat nuggets (Dipping of nuggets)

There was no significant difference in *a** values in between storage period in control and T_3 though slight decrease was observed in both the samples. T_1 and T_2 showed significant decrease with storage period. Several authors have studied the effect of various antioxidants on the colour of meat and meat products (Morrissey *et al*. 1998) and they have reported that antioxidants stabilizes color values. Decrease in color values of control samples were probably due to lipid oxidation and microbial spoilage. Yellowness (*b**) and total color change value did not differ significantly (P<0.05) between storage period and between treatments. In general yellowness of treatments was slightly higher than control which could be due to presence of cinnamon oil in coating solution. Total color change was higher in treatments due to higher lightness, yellowness and redness values of treatments.

Texture profile analysis

The mean values of Texture profile analysis of chitosan and cinnamon oil coated chicken meat nuggets stored at 4 ± 1°C are presented in Table 3. A significant (P < 0.05) difference in hardness value was observed in between treatments throughout the storage period except on 21st day. Hardness value of control sample decreased significantly with storage period. However, no significant decrease (P>0.05) in hardness was observed between storage periods in all treatments. This decrease could be due to increase in moisture content and disintegration of protein molecules by bacterial proliferation. In general T_2 sample had significantly higher value than other treatments and control. This might be attributed to direct addition of chitosan into emulsion by replacing water, as hydrophilic hydrocolloid leads to absorption of moisture and makes product harder on cooking.

Youn *et al*. (1999) reported that hardness of the sausage was increased with increase in molecular weight of chitosan and sausages added with high molecular weight chitosan were harder compared to low molecular weight chitosan added sausages. Kataoka *et al*. (2007) also observed that the surimi gel added with 1.5% chitosan had nearly double hardness value than control. However, Garcia *et al*. (2010) reported no significant difference in hardness value on addition of chitosan in pork sausages. T_3 had least hardness followed by T_1, control and T_2 due to moisture of coating solution gained during dipping of nuggets. However, T_1 and T_3 did not differ significantly P>0.05) in between storage period.

The lower hardness value of nuggets from chitosan and cinnamon oil coated meat was due to presence of chitosan which slightly reduced the myosin heavy chain content via its polymerization, thereby, enhancing the formation of cross-linked myosin heavy chain components, simultaneously. Springiness, chewiness, gumminess, cohesiveness and resilience ratio did not differ significantly (P>0.05) in between storage period and between treatments. These results were in agreement with Biswas *et al*. (2012) who observed that inclusion of chitosan and eugenol in chicken meat products did not have significant effect on texture profile scores except hardness. Martin-Sanchez *et al*. (2009) opined that additives such as calcium compounds, chitosan and oxidizing agents in meat products had ability to form protein gel networks resulted in higher hardness scores. Chewiness, gumminess, cohesiveness and resilience ratio varied accordingly with increase and/or decrease with hardness value as all parameters were secondary parameters of textural values.

Microbiological quality

The mean values of microbiological quality of chitosan and cinnamon oil coated chicken meat nuggets stored at 4±1°C are presented in Table 4. The results of the study revealed a highly significant (P<0.01) difference in standard plate count between storage period and between treatments and standard plate count increased significantly (P<0.01) with storage period. The SPC of the control and treatments on 0th day was nearly 1.8 log_{10}cfu/gm. During 21st day of storage SPC was very high for all the samples (3.89-4.49), with lower values in T_2, T_1, T_3 and control. Similar results were reported by Biswas *et al*. (2012) who also observed that the SPC of chitosan and eugenol incorporated chicken meat product on 20th day of storage at refrigeration temperature was 4 log_{10}cfu/g. Banwart, (1989) suggested that aerobic plate counts of 4-5 log_{10}cfu/g as microbiological specifications for cooked poultry products.

Table 3: Mean ± S.E values of Texture profile analysis of chitosan and cinnamon oil coated chicken meat nuggets stored at 4 ± 1°C

Days	Control	T_1	T_2	T_3	F Value
			Hardness (Kgf)		
0	36.59±1.245cB	28.74±1.849aA	37.68±1.484aB	26.83±0.776aA	15.409**
7	34.82±0.998bcB	30.65±1.424aB	34.72±0.996aB	26.05±1.170aA	2.826**
14	$32.37±0.946^{abcB}$	$27.74±1.654^{aAB}$	$33.15±1.664^{aB}$	$25.39±1.457^{aA}$	6.478**
21	$28.22±0.916^{aA}$	$29.622±2.039^{aA}$	$33.48±2.777^{aA}$	$26.19±1.384^{aA}$	2.592^{NS}
28	$30.502±0.136^{abAB}$	24.61±1.820aA	34.88±1.936aB	26.51±1.801aA	5.575**
F Value	6.282**	1.705NS	0.914NS	0.156^{NS}	
			Springiness (mm)		
0	0.51±0.028aA	0.50±0.059aA	0.44±0.038aA	0.51±0.030aA	0.879^{NS}
7	0.54±0.073aA	0.56±0.049aA	0.56±0.019aA	0.56±0.033aA	0.032NS
14	0.60±0.037aA	0.60±0.028aA	0.58±0.032aA	0.63±0.047aA	0.349NS
21	0.57±0.05aA	0.59±0.056aA	0.60±0.039aA	0.61±0.042aA	0.204NS
28	0.59±0.055aA	0.61±0.050aA	0.54±0.062aA	0.63±0.044aA	0.528NS
F Value	0.4 NS	0.789 NS	2.37^{NS}	1.597NS	
			Cohesiveness (ratio)		
0	0.56±0.040aA	0.59±0.062aA	0.68±0.068aA	0.64±0.065aA	0.785NS
7	0.56±0.066aA	0.51±0.047aA	0.49±0.040aA	0.47±0.024aA	0.590NS
14	0.51±0.039aA	0.52±0.083aA	0.47±0.06aA	0.47±0.028aA	0.214NS
21	0.62±0.043aA	0.48±0.024aA	0.49±0.046aA	0.54±0.070aA	1.630NS
28	0.58±0.083aA	0.57±0.054aA	0.65±0.093aA	0.52±0.069aA	0.439NS
F Value	0.417 NS	0.565 NS	2.405NS	1.515NS	

			Gumminess (N)		
0	17.24±1.524aA	14.68±1.071aA	15.35±2.550abA	14.84±1.550aA	0.446NS
7	17.19±1.771aA	14.73±0.862aA	14.16±1.672aA	12.85±1.096aA	1.679NS
14	14.53±1.642aA	16.22±1.990aA	14.54±1.433abA	12.95±0.907aA	0.749NS
21	17.11±1.740aA	15.01±0.800aA	15.02±0.788abA	14.36±1.379aA	0.922NS
28	17.64±1.794^{aA}	18.40±1.394aA	21.46±1.566^{bA}	16.09±1.786aA	1.886NS
F Value	0.543NS	1.473NS	3.181*	0.968NS	
			Chewiness (Kgf/mm)		
0	8.22±1.126aA	7.58±0.610aA	7.98±0.658^{aA}	8.30±1.792^{aA}	0.059^{NS}
7	8.76±1.126^{aA}	8.23±0.705^{aA}	8.43±0.701^{aA}	8.05±1.055^{aA}	1.032^{NS}
14	8.75±1.419aA	10.45±0.820aA	10.85±0.822aA	9.19±1.613aA	0.534NS
21	10.25±0.707aA	10.27±1.218aA	10.47±1.498aA	11.04±1.377aA	0.489NS
28	10.64±0.844aA	8.11±0.413aA	8.51±0.423aA	10.64±1.022aA	1.872NS
F Value	1.108NS	2.140 NS	2.140NS	0.922NS	
			Resilience (ratio)		
0	0.24±0.034aA	0.26 ± 0.046aA	0.31±0.046bA	0.28±0.041aA	0.54NS
7	0.18±0.023aA	0.17± 0.014aA	0.17±0.029aA	0.17±0.015aA	0.56NS
14	0.19±0.020abA	0.19±0.037aA	0.19±0.032abA	0.17±0.020aA	0.70NS
21	0.22±0.015aA	0.19±0.027aA	0.18±0.02aA	0.23±0.04aA	0.71^{NS}
28	0.23±0.034abA	0.21±0.037aA	0.22±0.023abA	0.19±0.035aA	0.29NS
F Value	0.945NS	0.926 NS	3.405*	2.337NS	

Means bearing different superscript between rows a, b, c and between columns A, B, C differ significantly (p<0.05)

*Indicates significant value (P<0.05); ** Highly Significant value; (P<0.01); NS - Non significant

T1= Nuggets from chitosan and cinnamon coated chicken meat (spraying of chicken breast), T2=Chitosan and cinnamon added chicken meat nuggets, T3=Coated chicken meat nuggets (Dipping of nuggets)

At 28th day of storage all the samples exceeds the permissible level as reported by Banwart, (1989) with control having the highest value of 5.55 followed by T_3 (4.83), T_1 (4.67) and T_2 (4.65). There was no significant difference in between treatments on last day of storage. These results were in agreement with Pranoto and Rakshit, (2008) who observed a SPC level of 5 log_{10}cfu/g in control and below 5 log_{10}cfu/g in chitosan coated meat balls during 20th day of storage at refrigeration temperature. Lower values of treatments could be due to antimicrobial activity of chitosan and cinnamon oil. Antimicrobial activity of chitosan is due to interaction between positively charged chitosan molecules and negatively charged microbial cell membranes leading to the leakage of proteinaceous and other intracellular constituents.

Cinnamaldehyde acts by inhibiting the amino acid decarboxylase in target bacteria (Ouattara *et al*. 1997). Therefore acidified chitosan (acetic acid) and cinnamon oil act synergistically against microbial growth.

In the present study no coliform were detected throughout the storage and yeast and mould were not detected during initial 7 days of storage. This could be due to thermal processing, hygienic practices followed during processing and synergistic antibacterial effects of chitosan and cinnamon oil (Geornaras *et al*. 2006). However, yeast and mould count increased significantly (P<0.01) during subsequent days of storage and on 14th day control sample had 0.95 log_{10}cfu/g and it doubled on 28th day of storage. Yeast and mould count in T_1 and T_2 sample increased significantly (P<0.01) upto 21st day of storage. However, no significant difference was observed on 28th day of storage. There was no significant difference (P<0.01) in

Table 4: Mean ± S.E values of microbiological quality of chitosan and cinnamon oil coated chicken meat nuggets stored at 4 ± 1°C

Days	Control	T_1	T_2	T_3	F Value
			Standard Plate Count (log_{10}cfu/gm)		
0	$1.89±0.042^{aC}$	$1.65±0.082^{aAB}$	$1.51±0.026^{aA}$	$1.78±0.040^{aAB}$	9.518**
7	$2.72± 0.045^{bC}$	$2.53±0.03^{bB}$	$2.36±0.034^{bA}$	$2.62±0.044^{bB}$	15.040**
14	$2.95±0.036^{bB}$	$2.72± 0.082^{bcAB}$	$2.56±0.082^{bA}$	$2.76±0.073^{bcAB}$	4.976**
21	$4.49±0.123^{cB}$	$4.03± 0.09^{cA}$	$3.89±0.12^{bA}$	$4.06±0.042^{cA}$	6.526*
28	$5.55±0.128^{dB}$	$4.67± 0.131^{dA}$	$4.65±0.244^{cA}$	$4.83±0.128^{dA}$	6.407**
F Value	132.010**	65.646**	36.685**	100.747**	
			Yeast and mould count (log_{10}cfu/gm)		
0	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND
14	$0.95±0.203^{Ba}$	$0.72±0.232^{abAB}$	$0.55±0.250^{bAB}$	$1.08±0.078^{cB}$	1.374**
21	$1.35±0.081^{bcA}$	$1.02±0.217^{bB}$	$1.17±0.076^{cAB}$	$1.17±0.076^{cAB}$	1.234**
28	$1.77±0.358^{cA}$	$1.03±0.239^{bAB}$	$1.23±0.117^{cB}$	$1.05±0.246^{cAB}$	1.704**
F Value	18.156**	8.595**	22.001**	25.012^{NS}	

Means bearing different superscript between rows a, b, c and between columns A, B, C differ significantly (p<0.05)

*Indicates significant value (P<0.05); ** Highly Significant value; (P<0.01); NS - Non significant

T1= Nuggets from chitosan and cinnamon coated chicken meat (spraying of chicken breast), T2=Chitosan and cinnamon added chicken meat nuggets, T3=Coated chicken meat nuggets (Dipping of nuggets)

*Coliform count not determined throughout storage study.

*ND-Not determined

T_3 sample throughout the storage period. Increase in yeast and mould count during storage could be due to chilling temperature and relative humidity which favours mould growth.

Sensory attributes

The mean values of sensory attributes of chitosan and cinnamon oil coated chicken meat nuggets stored at 4±1 °C are presented in Table 5**.** Color and appearance scores did not differ significantly (P>0.05) in between samples during initial 7 days. It decreased significantly (P<0.01) with storage period. There was highly significant difference (P<0.01) observed during 14th and 21st day of storage. However, no significant (P>0.05) difference was observed in between treatments during 28th day. Control and T_2 had significantly (P<0.01) lower scores compared to other treatments throughout storage period. The significant decrease (P<0.05) in appearance and colour value might be due to rapid oxidation of myoglobin and increase of moisture concentration. However, higher scores of treatments might be attributed to antioxidant potential of chitosan and cinnamon oil which minimizes the pigment oxidation. Texture scores also follow similar trend with no significant difference (P>0.05) on initial day of storage and with storage period scores decreased significantly (P<0.01). At the end of storage period control and T_2 had lowest value followed by T_1 and T_3. These lowest and highest values of T_2 and T_3 might be positively but inversely correlated to their hardness values. However, slight contradictory result revealed by Jo *et al*., (2001) who found that addition of chitosan oligomer in the emulsion-type pork sausage did not change its color, flavor, texture and overall acceptance. T_2 had lowest flavour scores throughout storage period due to intense cinnamon oil flavour which was added in emulsion.

Flavour scores of T_1 and T_3 did not differ significantly (P>0.05) throughout storage period and scores of all the samples decreased significantly (P<0.01) with storage period. Similar variation was also observed for juiciness scores also but T_3 had comparatively higher scores due to additional moisture gained during dipping of nuggets

Table 5: Mean ± S.E values of sensory attributes of chitosan and cinnamon oil coated chicken meat nuggets stored at 4 ± 1 °C

Days	Control	T_1	T_2	T_3	F Value
			Colour and Appearance		
0	8.83±0.167^{cA}	9.00±0.100^{cA}	8.83±0.167^{cA}	9.00±0.100^{cA}	0.667^{NS}
7	7.67±0.211^{bA}	8.17±0.167^{bcAB}	7.67±0.211^{bA}	8.67±0.211^{cB}	5.690^{NS}
14	6.00±0.258^{aA}	7.50±0.500^{bB}	6.00±0.258^{aA}	7.50±0.224^{bB}	6.923**
21	5.67±0.211^{aA}	6.33±0.211^{aA}	5.67±0.211^{aA}	6.50±0.224^{aA}	4.192**
28	5.50±0.224^{aA}	6.17±0.167^{aA}	5.50±0.224^{aA}	5.83±0.167^{aA}	2.619^{NS}
F Value	45.417**	20.754**	45.417**	53.629**	
			Texture		
0	9.00±0.100^{dA}	9.00±0.100^{dA}	9.00±0.100^{dA}	8.83±0.167^{cA}	1.000^{NS}
7	7.33±0.211^{cA}	7.33±0.211^{cA}	7.33±0.211^{cA}	7.67±0.211^{bA}	0.625^{NS}
14	6.33±0.21^{cA}	6.33±0.211^{cA}	6.33±0.211^{cA}	6.00±0.258^{aA}	0.556^{NS}
21	4.67±0.211^{bA}	4.67±0.211^{bA}	4.67±0.211^{bA}	5.67±0.211^{aB}	5.625**
28	3.33±0.422^{aA}	3.53±0.311^{aA}	3.33±0.210^{aA}	5.50±0.224^{aB}	8.048**
F Value	79.107**	79.107**	79.107**	45.41**	
			Flavour		
0	8.67±0.211^{dB}	9.00±0.000^{dB}	5.67±0.211^{dB}	9.00±0.000^{cB}	117.917^{NS}
7	7.83±0.167^{dB}	8.17±0.167^{cB}	5.00±0.258^{cA}	8.50±0.224b^{cB}	59.946**
14	5.83±0.167^{cB}	6.67±0.211^{bB}	4.67±0.211^{cA}	6.17±0.401^{bB}	10.400**
21	4.33±0.333^{bB}	6.33±0.211^{bC}	2.67±0.211^{bA}	3.83±0.307^{aB}	31.792*
28	3.33±0.21^{aB}	4.50±0.224^{aC}	1.67±0.211^{aA}	4.17±0.167^{aB}	38.444**
F Value	99.728**	91.083**	58.409**	85.417**	
			Juiciness		
0	8.83±0.167^{dB}	9.00±0.167^{dB}	6.33±0.333^{bB}	8.83±0.167^{cB}	28.714^{NS}
7	8.00±0.000^{dC}	8.30±0.000^{dC}	5.67±0.615^{bA}	7.67±0.211^{cB}	23.509**
14	6.33±0.211^{cB}	6.33±0.211^{cB}	4.83±0.401^{bA}	6.67±0.211^{bB}	9.151**
21	4.33±0.211^{bA}	5.33±0.211^{bA}	4.00±0.447^{bA}	5.00±0.632^{aA}	0.215^{NS}
28	2.83±0.167^{aA}	3.83±0.408^{aA}	2.83±0.167^{aA}	4.83±0.307^{aB}	22.500**
F Value	229.519**	69.19**	9.858**	18.795**	
			Overall acceptability		
0	8.67±0.211^{dB}	8.83±0.167^{cB}	6.17±0.307^{dB}	8.67±0.211^{dB}	31.053^{NS}
7	8.17±0.307^{dB}	8.50±0.224^{cB}	5.83±0.307^{dA}	7.67±0.211^{cB}	19.967**
14	5.83±0.167^{cB}	6.50±0.428^{bB}	4.50±0.224^{cA}	6.50±0.224^{bB}	11.429**
21	3.67±0.333^{bAB}	4.83±0.401^{aBC}	3.33±0.211^{bC}	5.33±0.211^{aA}	9.923**
28	2.17±0.167^{aA}	4.33±0.211^{aB}	2.17±0.167^{aA}	5.33±0.211^{aC}	70.256**
F Value	128.955**	45.179**	45.446**	47.073**	

Means bearing different superscript between rows a, b, c and between columns A, B, C differ significantly ($p<0.05$)

*Indicates significant value ($P<0.05$); ** Highly Significant value; ($P<0.01$); NS - Non significant

T1= Nuggets from chitosan and cinnamon coated chicken meat (spraying of chicken breast), T2=Chitosan and cinnamon added chicken meat nuggets, T3=Coated chicken meat nuggets (Dipping of nuggets) in coating solution. Overall acceptability scores did not differs significantly during initial day of storage. T_2 had lowest score throughout storage period and this sample was not accepted by sensory panel due to intense cinnamon oil smell and harsh hardening effect of chitosan. Control had reached unacceptable level on 21st day of storage. However, T_1 and T_3 sample had rated significantly higher ($P<0.01$) for overall acceptability score and both samples reached lowest/unacceptable score on 28th day of storage.

CONCLUSION

Chitosan in combination with cinnamon oil had synergistic effect in extending shelf-life of chicken meat nuggets developed from coated meat and nuggets dipped in solution containing chitosan and cinnamon oil had better acceptability and higher shelf-life upto 28 days at refrigerated temperature (4±1 °C) than product developed from emulsion directly added with coating solution.

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