Journal of Animal Research: v.9 n.4, p. 511-519. August 2019
DOI: 10.30954/2277-940X.04.2019.3

Storage Quality of Functional Meat Nuggets with Biocontrol Films, Coliphages and Different Packaging Conditions

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Received: 12 May, 2019 **Revised:** 22 July, 2019 **Accepted:** 28 July, 2019

ABSTRACT

Functional spent hen meat nuggets (SMN) both unwrapped and wrapped in bioactive biodegradable films were evaluated for their storage stability at refrigeration temperature (4 ±1ºC) under different packaging conditions. Standardized zein films formulated with 5% zein proteins and 7% glycerol and incorporated with 5% coliphages having concentration of 10^{10} pfu/ml were utilized in this study. Different treatments such as T1 (product packaged in aerobic conditions), T2 (product packaged in modified atmospheric packaging [MAP] conditions), T3 (product packaged in vacuum), T4 (product wrapped in developed films and packaged in aerobic conditions), T5 (product wrapped in developed films and packaged in MAP conditions), T6 (product wrapped in developed films and packaged in vacuum conditions) were evaluated at weekly interval for 5 weeks for their physico-chemical, sensory and microbiological parameters. Results depicted that T5 (product wrapped in developed films and packaged in MAP conditions) had comparatively lower value for TBARS, Free fatty acids, peroxide value and standard plate count than other treatments. Psychrophiles, yeast and mold, *E.coli*, coliforms were completely absent in the T5 throughout the storage period. All vacuum and MAP packaged products had better sensory attributes throughout storage than aerobic packaged products. It was thus concluded that developed SMN can be successfully stored for 35 days in bioactive biodegradable wrap under MAP conditions at 4±1ºC.

Keywords: Coliphages, Modified Atmospheric Packaging, Vacuum Packaging, Storage Life.

Shelf-life and sensory quality of meat is highly influenced and limited by the perishable nature of meat. Control of spoilage and delivery of meat safely to consumers can be accomplished by means of packaging. Thus packaging is a vital component in the food manufacturing process which has depicted an appreciable growth in the recent years. MAP in which initial atmosphere is created by desired gas mixture is one of the packaging technologies that has led to creation of fresh and minimally processed method for preservation of food (Skandamis and Nychas, 2002). The packaging material commonly used in food industry is of synthetic nature and the continuous use of such material has led to serious environmental issues. Therefore, there has been renewed interest in formulation of biodegradable and edible films (Kristam *et al*., 2016).

Biodegradable packaging is an effective solution to food safety, storage stability and consumer acceptability (Sanchez-Gonzalez *et al*., 2011). These biodegradable films enhance the quality of food, act as barrier for gas and moisture, provides protection to food product after opening of primary package (Kim and Ustunol, 2001). Various sources are being used in the production of biodegradable films like polysaccharides, proteins, lipids or their combinations. Protein based biodegradable films are found to be attractive with better mechanical and gas barrier properties (Ou *et al*., 2004) in comparison to lipids and polysaccharides. Proteins like whey, soy have been used for film formation (Bourtoom, 2008) but Zein has excellent film forming properties. Zein is protein rich

How to cite this article: Mahajan, K., Chatli, M.K., Mehta, N. and Malav, O.P. (2019). Storage quality of functional meat nuggets with biocontrol films, coliphages and different packaging conditions. *J. Anim. Res.,* **9**(4): 01-09.

in glutamic acid, leucine, proline and alanine. Presence of nonpolar amino acid like glycine, alanine in high proportion govern the solubility behavior of Zein (Shukla and cheryan, 2001) and therefore films made from an alcohol soluble Zein have high water vapor barrier properties compared to other proteins. The development of hydrophobic, hydrogen and disulfide bonds occurs between Zein chains for film formation (Guilbert, 1986). Zein films are tough, glossy and grease-proof coatings (Matthews *et al*., 2011). There has been growing interest in formulation functional Zein films for food application by incorporation of antimicrobial substances (Lungu and Johnson, 2005).

Antimicrobial packaging is an upcoming technology that has great effect on shelf life extension and safety of food. By means of antimicrobials in packaging that target and control microbes, high quality of food products can be attained (Perez-Perez *et al*., 2006). The use of bacteriophages to control pathogens is promising and is now becoming a reality. Bacteriophage are the viruses specifically infecting bacteria but are not harmful to animals, humans. Phages have high specificity for host cells and not affect the remaining microbiota. Phages withstand food processing environmental stresses and act as biocontrol agents (Sillankorva *et al.,* 2012). Concept of removing undesirable bacteria using bacteriophages is now being used for foodstuffs (Gill and Young, 2011) and in packaging films. Incorporation of phages in packaging films is beneficial step in terms of food preservation.

Coliphages are antimicrobial agents that specifically targets *Escherichia coli* such as *E.coli* O157:H7. This bacteria is one of the most grave food borne pathogen due to severity of illness produced and its low infective dose (less than 10 cells) (Kiranmayi *et al*., 2010). The bacteriophages infecting and targeting *E. coli*, Enterococcus, and various Bacteroides are considered as indicators of fecal contamination (Vijayavel *et al*., 2010; Purnell *et al*., 2011). Thus extracting the benefits of pathogen specific coliphages that target harmful pathogen and are indicator of faecal contamination has become need of the hour.

A search for natural antimicrobials as a substitute to synthetic additives to reduce microbial spoilage and extend storage life of meat products is being carried out by meat industry. The present study was thus conducted to evaluate the storage stability of the functional spent hen meat nuggets by coating it with coliphage impregnated zein films in supplementation to different packaging conditions.

MATERIAL AND METHODS

Development of bioactive biodegradable Zein protein films

The bioactive biodegradable zein films were formulated with a standardised formulation of 5% zein and 7% glycerol as per the method standardised in our laboratory (Mahajan, 2017). The zein and glycerol were dissolved in solvent absolute alcohol. The mixture solution was heated for suitable time temperature combination and thereafter on cooling was incorporated with coliphages at 5% level having concentration of 10^{10} pfu/ml. The solution was then casted on plastic petri plates (90x100mm) and dried for 8 hours. The developed films were stored in a chamber at 50% RH and 25˚C. The physico-chemical parameters of developed films are thickness (µm) 201.66±0.49, extensibility (N) 6.40±0.08, penetrability (N) 3.35±0.04, moisture(%) 18.71±0.09, Lightness (*L*)* 76.88±0.23, Redness (*a**) 6.38±0.18, yellowness (*b**) 49.14±0.15.

Preparation of spent hen meat nuggets

Spent hen meat nuggets were developed using pre-standardized formulation and process protocol in the laboratory. Frozen deboned spent hen meat pieces were thawed at 4±1ºC and the meat was double minced in mincer through plates of size 4mm. Meat was chopped in bowl chopper while adding additives as per formulation to obtain desired (Mahajan, 2017). Remaining ingredients i.e refined oil 7.5%; salt 1.5%; spices 2.5%, condiments 3%, chilled water 7.5%,refined wheat flour 3%, TSPP 0.3%, sugar 0.2%, egg albumen 5%, nitrite 120ppm, amla fruit juice powder 1.5% were then incorporated and chopped to obtain emulsion of desired characteristics. The emulsion placed into moulds was steam cooked in autoclave at 121ºC at 15 psi for 15 min and was cut into rectangular pieces (Approx. 2×3 inches). The developed SMN has 58.48% moisture, 15.81 % protein, 14.47% fat, 1.34% fibre, 6.0% ash, and 208.54 kcal (per 100 g) calories.

Storage conditions

The developed SMN were wrapped in developed antimicrobial films and control was taken as unwrapped. Unwrapped and wrapped batches were packed in Low Density Polyethylene film bags for aerobic packaging and laminate pouches (polyester/polyethylene; 100/100u) for modified atmosphere and vacuum packaging. Aerobic packages were sealed using impulse sealer whereas MAP (50:50 CO_2/N_2 gas mixture) and vacuum packaging was carried out using Roschermatic packaging machine (Type 19/S/CL, Germany). The treatments were T1 (product packaged in aerobic conditions), T2 (product packaged in MAP conditions), T3 (product packaged in vacuum), T4 (product wrapped in developed films and packaged in aerobic conditions), T5 (product wrapped in developed films and packaged in MAP conditions), T6 (product wrapped in developed films and packaged in vacuum conditions). The samples were drawn at weekly interval for 5 weeks. The storage quality was evaluated on the basis of physico-chemical (pH, Thio-Barbituric Acid Reactive Substances etc), sensory and microbiological (Standard Plate, Psychrophilic count) parameters (APHA, 1984).

Analytical techniques

pH

The pH of cooked SMN (n=6) was determined as per the method of Trout *et al*. (1992) using digital pH meter equipped with a combined glass electrode.

Peroxide value (PV)

The procedure illustrated by Koniecko (1979) with slight modifications was followed. The peroxide value (PV) was calculated in meq/kg of the meat as per following formula:

PV (meq/kg sample) =

$$\frac{0.1 \times \text{ml } 0.1\text{N sodium thiosulphate}}{\text{Sample weight (g)}} \times 100$$

Thiobarbituric Acid Reactive Substances (TBARS)

The extraction method illustrated by Witte *et al.* (1970) for the calculation of TBARS value in SMN was followed with suitable modifications. TBARS number was calculated as mg malonaldehyde per kg of sample by multiplying O.D. value with a factor 5.2.

Free fatty acids (FFA)

The free fatty acid was calculated by method described by Koniecko (1979). Percent free fatty acid content was calculated as follows:

Free fatty acid (FFA) % =

$$\frac{0.1\text{mL} \times 0.1\text{ N alcoholic KOH} \times 0.282}{\text{Sample weight (g)}} \times 100$$

Water activity (a_{w})

a_w was determined using hand held portable digital water activity meter (Rotonix HYGRO Palm AW1 Set/40, Serial no. 60146499).

Microbiological quality parameters

Standard Plate Counts (SPC), Psychrophilic, Coliform, *Escherichia coli* and Yeasts and mold counts of the samples were enumerated following the methods as described by American Public Health Association (APHA, 1984).

Preparation of sample and serial dilutions

The SMN sample packs were opened in presterlised laminar flow (Model: RH-58-03. Rescholar equipments, Ambala, India). 10 g of meat specimen was placed in mortar containing 90 ml of sterile 0.1% peptone water. The sample was homogenized with pestle for uniform dispersion and to get a 10^{-1} dilution. Serial dilutions were made as per requirement.

Sensory analysis

Seven experienced panelists were chosen from the scientific staff of GADVASU, Ludhiana. Protocol for sensory analysis was approved by the departmental research committee, Department of Livestock Products Technology, GADVASU, Ludhiana. Sensory evaluators were selected on the basis of previous experience in consuming meat products, availability and willingness.

An 8-point descriptive scale was used for different sensory quality attributes viz. appearance and color, flavor, tenderness, juiciness and overall acceptability (Keeton, 1983) where 8=extremely desirable and 1=extremely undesirable. All batches were subjected to sensory evaluation in laboratory according to the international standards (ASTM, 1986). Rectangular pieces (approx. 2×3 inches) of nuggets were offered to panelist. The samples presented to each panelist were coded with randomized numbers. Total no. of observations taken were 21 (n= 21).

Statistical analyses

The data obtained from different set of each experiment were subjected to statistical analysis (Snedecor and Cochran, 1994) for two way Analysis of Variance (ANOVA) on Completely Randomized design (CRD) and Duncans multiple range test (DMRT) to compare the means with standard error (SE) by using SPSS-20 (SPSS Inc. Chicago IL, USA). The whole set of experiments were repeated three times. Samples for every parameter were drawn in duplicate leading to observation 6 (n=6)) and seven panelist analyzed the samples for sensory attributes (n=21). Statistical difference was expressed at 5% level of experiment.

RESULTS AND DISCUSSION

Physico-chemical quality parameters

Effect of different storage conditions on physico-chemical quality characteristics of SMN during refrigerated storage are depicted in Table 1. pH followed a decreasing trend in aerobic packaged product upto 14 day. However, pH increased thereafter with progress of storage period. In vacuum and MAP packaged products, pH declined irrespective of supplementation of the bioactive biodegradable films (BBF) and respective packaging conditions. The gradual decline in pH could be due to lactic acid produced by growing lactic acid bacteria in samples. Breakdown of meat protein and production of amines during storage is described to be reason for increase in pH value of stored product. Giriprasad *et al*. (2015) also recorded decrease in pH during storage of meat products. Jin *et al.* (2015) recorded rise in pH of the sausages during refrigerated storage. The intial decrease and subsequent maintainance of pH in MAP packaged product might be due to buffering by the carbonic acid produced due to the influx of carbon dioxide gas in the package (Ashie *et al.,* 1996).

SMN wrapped in Zein based BBF were having higher a_w as compared to the unwrapped ones. It might be due to the water vapor barrier properties of the protein films, which effectively controlled the water vapor transport and water balance between food system and its surroundings. The highest a_w was measured in MAP and Vacuum packaged products, which was significantly ($P<0.05$) higher than aerobic packaged products. It is due to usage of water permeable LDPE films in aerobic package in place of laminated double layered water resistant films in MAP and vacuum packaged products. Lawton (2004) also observed that Zein films owing to their hydrophobicity can efficiently sustain a_w of wrapped food products by inhibiting water vapor condensation in package.

Peroxides are main components of oxidation of poly unsaturated fatty acids in meat system. PV of all samples increased significantly with advancement of storage period regardless of treatments and packaging conditions. Treatment which were wrapped in films had significantly ($P<0.05$) less PV than unwrapped ones. Lower PV in present study can be attributed to antioxidant compound enriched amla fruit juice powder incorporated in SMN.

TBARS values of all groups increased significantly during storage. Wrapped products maintained significantly lower values than unwrapped products. TBARS value was lower than threshold value for both wrapped and unwrapped vacuum and MAP packaged product even on last day of storage studies. TBARS value in T2, T3, T5, T6 is attributed to the antioxidant characteristic of Zein (Gucbilmez *et al.,* 2007) and absence of air in MAP and vacuum that prevented lipid oxidation. Baryia *et al.* (2016) in goat meat patties and Chatli *et al.* (2016) in pork nuggets reported similar findings.

FFA (% oleic acid) showed gradual increase ($P<0.05$) during storage. FFA values were comparable in all treatments on first day of storage. As storage period increased, it followed an increasing trends in all treatments regardless of supplementation of BBF and packaging conditions. FFA value was significantly ($P<0.05$) higher in aerobic products in comparison to MAP and vacuum packaged products.

Table 1: Effect of different packaging methods on the physicochemical parameters of spent hen meat nuggets during storage at refrigeration temperature(4±1°C) (Mean±S.E)*

Treatment/Days	1	7	14	21	28	35
pH						
T1	6.38±0.02^{Ab}	6.31±0.06^{Bb}	6.16±0.01^{Aa}	6.49±0.06^{Cc}	6.53±0.07^{Cc}	N.P.
T2	6.39±0.03^{Ab}	6.18±0.04^{Aa}	6.12±0.07^{Aa}	6.16±0.05^{Aa}	6.14±0.03^{Aa}	6.17±0.06^{Aa}
T3	6.37±0.01^{Ab}	6.29±0.02^{Ba}	6.24±0.05^{Ba}	6.29±0.02^{Ba}	6.22±0.01^{Ba}	6.26±0.01^{Ba}
T4	6.37±0.01^{Ac}	6.30±0.03^{Bb}	6.17±0.01^{Ba}	6.44±0.02^{Cc}	6.52±0.03^{Cd}	6.65±0.02^{Ce}
T5	6.39±0.02^{Ab}	6.19±0.03^{Aa}	6.15±0.02^{Aa}	6.12±0.01^{Aa}	6.11±0.02^{Aa}	6.14±0.02^{Aa}
T6	6.38±0.04^{Aa}	6.32±0.02^{Ba}	6.29±0.02^{Ba}	6.27±0.07^{Ba}	6.23±0.04^{Ba}	6.27±0.01^{Ba}
a_w						
T1	0.95±0.01^{Abc}	0.91±0.06^{Ab}	0.82±0.03^{Aa}	0.81±0.01^{Aa}	0.81±0.04^{Aa}	N.P.
T2	0.94±0.03^{Ac}	0.92±0.04^{Ab}	0.91±0.02^{Bab}	0.91±0.01^{Bab}	0.89±0.06^{Ba}	0.88±0.04^{Ba}
T3	0.93±0.02^{Ab}	0.93±0.01^{Ab}	0.91±0.01^{Bab}	0.89±0.04^{Ba}	0.89±0.07^{Ba}	0.88±0.02^{Ba}
T4	0.93±0.01^{Ab}	0.91±0.03^{Ab}	0.91±0.06^{Bb}	0.88±0.03^{Bab}	0.84±0.06^{ABa}	0.81±0.01^{Aa}
T5	0.94±0.02^{Ab}	0.92±0.03^{Aab}	0.91±0.01^{Bab}	0.89±0.02^{Ba}	0.86±0.01^{ABa}	0.88±0.03^{Ba}
T6	0.95±0.01^{Ab}	0.91±0.02^{Aab}	0.91±0.02^{Bab}	0.89±0.04^{Ba}	0.88±0.04^{Ba}	0.88±0.04^{Ba}
PV(meq/kg)						
T1	0.95±0.01^{Aa}	1.61±0.02^{Bb}	1.87±0.03^{Cc}	2.04±0.02^{Bd}	2.47±0.01^{Ce}	N.P.
T2	0.98±0.01^{Aa}	1.19±0.03^{Ab}	1.28±0.03^{Bc}	1.47±0.02^{Ad}	1.62±0.03^{Ae}	1.68±0.03^{Af}
T3	0.99±0.01^{Aa}	1.21±0.02^{Ab}	1.29±0.02^{Bb}	1.46±0.05^{Ac}	1.67±0.03^{Ad}	1.70±0.03^{Ad}
T4	1.01±0.02^{Aa}	1.73±0.03^{Bb}	1.90±0.01^{Cc}	2.20±0.05^{Bd}	2.29±0.03^{Be}	3.71±0.04^{Cf}
T5	0.98±0.06^{Aa}	1.22±0.03^{Ab}	1.21±0.01^{Ab}	1.45±0.04^{Ac}	1.69±0.04^{Ad}	1.72±0.03^{Ad}
T6	1.02±0.02^{Aa}	1.21±0.03^{Ab}	1.23±0.01^{Ab}	1.46±0.04^{Ac}	1.62±0.03^{Ad}	1.81±0.03^{Be}
TBARS(mg MDA/kg)						
T1	0.35±0.01^{Aa}	0.58±0.04^{Bb}	0.82±0.04^{Cc}	0.88±0.09^{Cc}	1.14±0.12^{Bd}	N.P.
T2	0.35±0.01^{Aa}	0.46±0.03^{Ab}	0.60±0.02^{Ac}	0.63±0.045^{Ac}	0.68±0.03^{Ac}	0.86±0.03^{Bd}
T3	0.31±0.02^{Aa}	0.44±0.01^{Ab}	0.59±0.01^{Ac}	0.59±0.03^{Ac}	0.69±0.02^{Ad}	0.76±0.04^{Ae}
T4	0.33±0.03^{Aa}	0.56±0.02^{Bb}	0.74±0.03^{Bc}	0.77±0.02^{Bd}	1.17±0.02^{Be}	1.31±0.07^{Cf}
T5	0.31±0.04^{Aa}	0.43±0.01^{Ab}	0.58±0.01^{Ac}	0.64±0.02^{Ad}	0.72±0.01^{Ae}	0.79±0.02^{Af}
T6	0.31±0.02^{Aa}	0.43±0.02^{Ab}	0.55±0.04^{Ac}	0.59±0.04^{Ad}	0.69±0.03^{Ae}	0.75±0.01^{Af}
FFA(%)						
T1	0.191±0.03^{Aa}	0.31±0.02^{Bb}	0.38±0.02^{Bc}	0.60±0.03^{Bd}	0.74±0.03^{Ce}	N.P.
T2	0.23±0.03^{Aa}	0.27±0.04^{Bab}	0.30±0.03^{Abc}	0.36±0.04^{Ac}	0.49±0.03^{Bd}	0.75±0.04^{Ae}
T3	0.24±0.03^{Aa}	0.28±0.02^{Bab}	0.29±0.04^{Aab}	0.32±0.06^{Ab}	0.54±0.04^{Bc}	0.70±0.03^{Ad}
T4	0.21±0.03^{Aa}	0.29±0.03^{Bb}	0.43±0.03^{Bc}	0.59±0.05^{Bd}	0.69±0.03^{Ce}	0.88±0.03^{Bf}
T5	0.23±0.02^{Aa}	0.22±0.02^{Aa}	0.28±0.02^{Ab}	0.39±0.04^{Ac}	0.40±0.04^{Ac}	0.69±0.04^{Ad}
T6	0.24±0.01^{Aa}	0.25±0.03^{Aa}	0.22±0.05^{Aa}	0.35±0.03^{Ab}	0.41±0.03^{Ab}	0.67±0.03^{Ac}

n=-6, T1: Aerobic; T2:MAP; T3: Vacuum; T4:Wrapped Aerobic; T5:Wrapped MAP; T6:Wrapped Vacuum.

*Mean±S.E. with different superscripts row-wise (a-f) and column wise (A-C) differ significantly ($P<0.05$).

Microbiological quality

Microbiological quality of SMN during storage at refrigeration under different packaging conditions represented in Table 2. SPC was comparable on first day of storage amongst all treatments. As duration of storage increased, SPC also increased in all treatments except in T2 and T5 where it declined upto 14 day of storage and increased thereafter. It might be due to the carbon dioxide gas used in the concentration of 50% in combination with 50% nitrogen in MAP packaged product. Low SPC count was observed due to carbon dioxide that inhibited growth of bacteria by elongating lag phase of growth curve.

Coliforms and *Escherichia coli* were not seen in any treatment except in T1 that too on 28 day of storage. The

Table 2: Effect of different packaging methods on the microbiological quality of spent hen meat nuggets during storage at refrigeration temperature (4±1 ◦C) (Mean±S.E.)

Treatments/days	1	7	14	21	28	35
			SPC (cfu/g)			
T1	2.59±0.12^{Aa}	3.15 ±0.01^{Db}	3.86± 0.08^{Cc}	4.08± 0.05^{Dd}	5.76 ±0.16^{De}	7.58±0.18^{Df}
T2	2.61±0.08^{Ab}	2.07±0.02^{Aa}	2.11±0.09^{Aa}	2.99±0.04^{Bc}	3.23±0.19^{Bc}	3.68±0.20^{Cd}
T3	2.56±0.14^{Ab}	2.07±0.04^{Aa}	2.17±0.06^{Ba}	2.70±0.06^{Ac}	3.05±0.11^{Ad}	3.41±0.10^{Be}
T4	2.63±0.16^{Aa}	2.91±0.02^{Cb}	3.78±0.16^{Cc}	4.09±0.1^{Dd}	4.93±0.07^{Ce}	7.45±0.24^{Df}
T5	2.66±0.12^{Ab}	2.04±0.14^{Aa}	2.06±0.18^{Aa}	2.92±0.1^{Bc}	2.93±0.12^{Ac}	3.10±0.16^{Ad}
T6	2.66±0.15^{Aa}	2.62±0.03^{Ba}	2.86±0.09^{Bb}	3.19±0.16^{Cc}	2.98±0.11^{Ab}	3.25±0.12^{Ac}
			***Escherichia coli* (cfu/g)**			
T1	ND	ND	ND	ND	2.67±0.08^{a}	3.50±0.14^{b}
T2	ND	ND	ND	ND	ND	ND
T3	ND	ND	ND	ND	ND	ND
T4	ND	ND	ND	ND	ND	ND
T5	ND	ND	ND	ND	ND	ND
T6	ND	ND	ND	ND	ND	ND
			Coliform (cfu/g)			
T1	ND	ND	ND	ND	3.48±0.05^{a}	4.47±0.12^{b}
T2	ND	ND	ND	ND	ND	ND
T3	ND	ND	ND	ND	ND	ND
T4	ND	ND	ND	ND	ND	ND
T5	ND	ND	ND	ND	ND	ND
T6	ND	ND	ND	ND	ND	ND
			Psychrophiles (cfu/g)			
T1	ND	ND	ND	1.18±0.34^{a}	2.71±0.15^{b}	3.67±0.18^{c}
T2	ND	ND	ND	ND	ND	ND
T3	ND	ND	ND	ND	ND	ND
T4	ND	ND	ND	ND	ND	ND
T5	ND	ND	ND	ND	ND	ND
T6	ND	ND	ND	ND	ND	ND
			Yeast and molds (cfu/g)			
T1	ND	ND	ND	ND	2.53±0.08^{a}	3.56±0.12^{b}
T2	ND	ND	ND	ND	ND	ND
T3	ND	ND	ND	ND	ND	ND
T4	ND	ND	ND	ND	ND	ND
T5	ND	ND	ND	ND	ND	ND
T6	ND	ND	ND	ND	ND	ND

n=6, T1: Aerobic; T2:MAP; T3: Vacuum; T4:Wrapped Aerobic; T5:Wrapped MAP; T6:Wrapped Vacuum.

*Mean±S.E. with different superscripts row-wise (a-f) and column wise (A-D) differ significantly ($P<0.05$).

absence of coliforms and *Escherichia coli* in the present study can be attributed to the packaging environment and antibacterial activity of coliphages embedded Zein based biodegradable films. The absence of coliform in the present study represents the hygienic conditions during product preparation, high heat treatment implied during cooking, MAP and vacuum packaging conditions and application of the BBF.

Psychrophiles were detected in T1 on day 21 of storage and this could be due to post processing contamination, rise in pH, decreased a_w of product packaged aerobically. Psychrophiles were not detected in all other treatments irrespective of type of packaging conditions. Our results are in consonance with findings of Rani (2014) in chicken meat bullets. Yeasts and molds were observed in T1 on day 28 and 35 of storage. Visible growth of yeast and mold on the product prevented the workers from presenting before the sensory panelist. Therefore, Zein based BBF effectively maintained the microbiological quality of wrapped SMN.

Table 3: Effect of different packaging methods on the sensory attributes of spent hen meat nuggets during storage at refrigeration temperature (4±1° C) (Mean±S.E.)*

Treatment/Days	1	7	14	21	28	35
			Appearance And Color			
T1	7.36±0.10^{Ad}	7.26±0.13^{Ac}	6.72±0.14^{Ab}	6.03±0.13^{Aa}	N.P.	N.P.
T2	7.35±0.012^{Ae}	7.20±0.15^{Ad}	7.08±0.13^{Cc}	7.03±0.17^{Bc}	6.83±0.13^{Bb}	6.51±0.13^{Aa}
T3	7.35±0.014^{Ad}	7.16±0.15^{Ac}	7.00±0.16^{Cb}	7.06±0.15^{Bb}	7.00±0.12^{Bb}	6.67±0.14^{Aa}
T4	7.31±0.10^{Ae}	7.20±0.11^{Ad}	6.88±0.11^{Bc}	6.09±0.16^{Bb}	5.67±0.15^{Aa}	N.P.
T5	7.31±0.10^{Ad}	7.19±0.13^{Ac}	7.11±0.16^{Cc}	7.12±0.11^{Bc}	6.88±0.16^{Bb}	6.64±0.12^{Aa}
T6	7.39±0.11^{Ae}	7.17±0.11^{Ad}	7.07±0.12^{Cc}	7.09±0.13^{Bc}	6.78±0.13^{Bb}	6.68±0.11^{Aa}
			Flavor			
T1	7.31±0.12^{Ad}	7.03±0.13^{Ac}	6.77±0.16^{Ab}	6.13±0.17^{Aa}	N.P.	N.P.
T2	7.42±0.15^{Ae}	7.18±0.11^{Bd}	7.17±0.13^{Dd}	7.01±0.13^{Cc}	6.91±0.14^{Bb}	6.61±0.16^{Aa}
T3	7.33±0.10^{Ac}	7.06±0.14^{Ab}	7.07±0.11^{Cb}	7.07±0.15^{Cb}	7.00±0.11^{Bb}	6.69±0.12^{Aa}
T4	7.26±0.11^{Ad}	7.00±0.11^{Ac}	6.91±0.13^{Bc}	6.63±0.16^{Bb}	6.03±0.12^{Aa}	N.P.
T5	7.28±0.11^{Ac}	7.00±0.13^{Ab}	7.01±0.12^{Cb}	7.08±0.11^{Cb}	7.04±0.14^{Bb}	6.71±0.16^{Aa}
T6	7.28±0.10^{Ac}	7.05±0.04^{Ab}	7.07±0.16^{Cb}	6.99±0.13^{Cb}	7.03±0.16^{Bb}	6.66±0.11^{Aa}
			Texture			
T1	7.15±0.16^{Ac}	7.09±0.24^{Ac}	6.71±0.12^{Ab}	6.12±0.16^{Aa}	N.P.	N.P.
T2	7.31±0.14^{Ac}	7.20±0.18^{Ac}	6.98±0.10^{Bb}	7.02±0.14^{Bb}	6.91±0.11^{Ab}	6.61±0.015^{Aa}
T3	7.26±0.12^{Ad}	7.13±0.16^{Ac}	7.01±0.12^{Bb}	6.94±0.12^{Bb}	7.00±0.16^{Ab}	6.69±0.16^{Aa}
T4	7.16±0.14^{Ab}	7.07±0.14^{Ab}	7.02±0.14^{Bb}	6.98±0.25^{Ba}	7.02±0.24^{Ab}	N.P.
T5	7.16±0.10^{Ab}	7.08±0.18^{Ab}	7.05±0.16^{Bb}	6.94±0.12^{Bb}	7.07±0.18^{Ab}	6.80±0.16^{Ba}
T6	7.16±0.10^{Ac}	7.03±0.16^{Ab}	7.00±0.14^{Bb}	6.98±0.14^{Bb}	6.97±0.14^{Ab}	6.86±0.13^{Ba}
			Juiciness			
T1	7.34±0.13^{Ad}	7.05±0.15^{Ac}	6.63±0.13^{Ab}	6.08±0.11^{Aa}	N.P.	N.P.
T2	7.21±0.20^{Ac}	7.26±0.09^{Bc}	6.78±0.14^{Bb}	7.05±0.15^{Cb}	6.96±0.12^{Bb}	6.70±0.11^{Aa}
T3	7.19±0.18^{Ac}	7.23±0.11^{Bc}	7.00±0.21^{Bb}	7.01±0.16^{Cb}	6.97±0.15^{Bb}	6.75±0.20^{Aa}
T4	7.13±0.20^{Ad}	7.09±0.15^{Ad}	6.78±0.18^{Ac}	6.50±0.17^{Bb}	6.02±0.14^{Aa}	N.P.
T5	7.17±0.17^{Ac}	7.10±0.21^{Abc}	7.07±0.13^{Bb}	6.95±0.16^{Cb}	6.98±0.18^{Bab}	6.87±0.10^{Ba}
T6	7.20±0.14^{Ab}	7.13±0.13^{Aab}	7.06±0.20^{Ba}	6.97±0.12^{Ca}	7.00±0.24^{Ba}	6.91±0.14^{Ba}
			Overall acceptability			
T1	7.38±0.14^{Ad}	7.05±0.11^{Ac}	6.65±0.16^{Ab}	6.08±0.13^{Aa}	N.P.	N.P.
T2	7.37±0.20^{Ae}	7.15±0.14^{Ad}	7.02±0.02^{Cc}	7.04±0.14^{Cc}	6.90±0.17^{Bb}	6.64±0.24^{Aa}
T3	7.31±0.12^{Ae}	7.16±0.10^{Ad}	7.00±0.16^{Cc}	6.97±0.2^{Cb}	6.97±0.15^{Bb}	6.53±0.20^{Aa}
T4	7.24±0.13^{Ae}	7.10±0.12^{Ad}	6.79±0.14^{Bc}	6.60±0.13^{Bb}	6.02±0.17^{Aa}	N.P.
T5	7.26±0.15^{Ac}	7.05±0.1^{Ab}	7.00±0.19^{Cb}	6.95±0.15^{Cb}	6.99±0.10^{Bb}	6.88±0.14^{Ba}
T6	7.24±0.13^{Ab}	7.06±0.11^{Aa}	7.00±0.15^{Ca}	6.90±0.1^{Ca}	7.01±0.12^{Ba}	6.90±0.16^{Ba}

n=21, T1: Aerobic; T2:MAP; T3: Vacuum; T4:Wrapped Aerobic; T5:Wrapped MAP; T6:Wrapped Vacuum.

**8- point descriptive scale, where 1- extremely undesirable and 8- extremely desirable.

*Mean±S.E. with different superscripts row-wise (a-f) and column wise (A-f) differ significantly (P<0.05).

Hence, coliphages impregnated BBF along with MAP packaging conditions could be effectively used for storage of SMN for period 35 days and more, while maintaining its physico-chemical and microbiological quality.

Sensory attributes

Changes in sensory attributes of SMN packaged in different packaging conditions and refrigeration temperature are shown in Table 3. Initially Color and Appearance were comparable in all products on the 1st day of storage regardless of packaging conditions and presence or absence of BBF. Color and appearance scores decreased sharply in aerobic packaged product and this is due to moisture loss, which lead to dessication and concentration of pigments on surface of meat forming crust layer leading to poor visual scores. However in wrapped products, moisture losses were prevented due to water barrier properties of Zein based films. Added advantage of the packaging in laminates having low WVTR under MAP and vacuum conditions lead to the maintenance of appearance and color of product. Texture and juiciness was lower in aerobic packaged and unwrapped products than wrapped and packaged in laminated pouches. Sensory panelist granted high scores for juiciness and texture to T5 and T6 products than other treated products throughout storage.

Mean flavor scores also reduced and followed decreasing trend throughout storage in all treatments including different packaging conditions. Highest flavor scores were awarded to T5 and T6 throughout storage. Overall acceptability was highest for T5 and minimum for T1 throughout storage period. BBF wrapped and MAP packaged product were awarded overall acceptability scores "good to very good" on 35th day as well. Thus developed SMN wrapped in coliphage impregnated Zein based biodegradable films can be successfully stored under MAP at refrigerated conditions for 35 days without any effect on sensory quality, oxidative stability and microbial load well below acceptable limit.

CONCLUSION

It was concluded that developed spent hen meat nuggets can be successfully stored for 35 days in bioactive biodegradable wrap under MAP conditions at 4±1ºC. Incorporation of antimicrobials into biodegradable films and their subsequent wrapping on ready-to-eat products is an innovative approach for extending their shelf-life. It also serves to protect the quality during transportation and storage. Thus bioactive, biodegradable packaging is very crucial step towards improvement of food quality, extension of shelf-life and decreasing environmental burden.

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