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RESEARCH ARTICLE

EFFECT OF COATING CHEMICALLY DERIVED CHITOSAN FROM SHRIMP SHELL WASTES ON PHYSICAL, MICROBIOLOGICAL AND SENSORY CHARACTERISTICS OF CHICKEN SAUSAGES

Mithushan P[1*], Pagthinathan M[1], Ravikumar S[2], and Vahaful Nisath MF[1]

[1]Department of Animal Science, Faculty of Agriculture, Eastern University, Sri Lanka

[2]Coastal Aquaculture Extension and Monitoring Unit, Batticaloa, Sri Lanka

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ABSTRACT

Sausages have high moisture and protein content, making them extremely vulnerable to microbial deterioration. The antimicrobial qualities of chitosan coating prolong the shelf-life of sausages. The aim of this present study was to evaluate the effect of 0, 0.25, 0.5 and 1% (w/v) of chitosan coatings on microbiological, physical and sensory attributes of chicken sausages in cold storage (-10 °C). Chitosan was extracted from shrimp shell and dissolved in acetic acid to prepare 0.25, 0.5 and 1% of edible chitosan solution and control (0%). Sausages were coated by dipping in chitosan solutions. To find the best percentage of chitosan coating, physical, microbiological and sensory attributes were examined during the storage period. A number of TAB colonies was shown to have increased during the storage; nevertheless, the values were within the acceptable level (5.00 Log (CFU/g) in 0.25, 0.5 and 1% chitosan treated samples in two weeks of storage. The number of TAB colonies was not significantly (P>0.05) reduced between the samples treated with 0.25, 0.5 and 1% of chitosan. Further, hardness values were increased with the chitosan coating, while 1% chitosan-coated sausages had the highest value for hardness (27.93 N). Similarly, the WHC also increased with the chitosan coating. There was no significant difference observed (P>0.05) among 0.25, 0.5, and 1.0% of chitosan coating in WHC. pH values improved with the chitosan coating. Moisture and colour were not significantly (p>0.05) affected by chitosan coating. During storage, deteriorative changes occurred slowly in coated sausages. 1% chitosan coated sausages had the highest overall acceptability and maximum scores for odour, colour, taste, appearance and texture on the day 1 and 4th week of storage. Chitosan coating can be utilized as an edible coating material for the preservation of meat products.

Keywords: Antimicrobial, Demineralization, Deproteinization, Film coating, Microbial spoilage, Shelf-life

INTRODUCTION

Shrimp aquaculture is one of the biggest seafood production industries around the globe, which provides high protein-rich food. Due to their deliciousness and nutritional value, worldwide consumption of shrimp and shrimp-derived products is increasing, with demand rising annually. The world shrimp processing industries produce enormous amounts of shrimp waste annually which has dramatically increased recently (Suresh, 2012). This by-product is being wasted in enormous quantities, increasing environmental pollution. The most prevalent amino polysaccharide, chitin is found in the cell walls of shrimp exoskeletons (Bostan and Isin Mahan, 2011 ; Kandra *et al*., 2012). Chitin has a limited range of applications due to its insolubility. The structure must be

Corresponding author: prabamithushan@gmail.com

altered chemically to increase its application. Chitosan is a deacetylated derivative of chitin that can dissolve in mild acids (Robertson, 2004). Chitosan's antioxidant and antimicrobial properties have attracted the attention of many researchers (Lekjing, 2016 ; Arslan and Soyer, 2018). The food industry has extensively used chitosan's non-toxic and non-allergenic qualities as a preservative, food additive, packing material, edible film, etc. (Dong *et al*., 2020 ; Umaraw *et al*., 2020).

Sausage is one of the ready-to-cook meat products which can be easily spoiled by microbial contamination. It is believed that a major portion of the microbial population in vacuum-packed sausages consists of lactic acid bacteria. (Bostan and Isin Mahan, 2011). Many researchers conducted experiments on chitosan application as an edible coating material for raw meat. Using chitosan as a coating material for meat products, particularly chicken sausage, is rarely discussed (Pabast *et al*., 2018). In addition to that, the effectiveness of chitosan, which is extracted from the chemical method as an edible coating material, is still not discussed much before. Therefore, the study was designed to produce chitosan film-coated sausages in order to enhance their shelf-life in terms of their microbiological, physical, and sensory qualities.

MATERIALS AND METHODS

Raw material preparation for chitosan extraction

The chemical method of chitosan extraction was carried out according to Arachchi *et al*. (2018) with minor alterations as indicated in the figure 1. Shrimp shells were obtained from a local market in Chenkalady, Batticaloa. It was washed with tap water to remove meat leftovers and other impurities; then, it was dried at 105 °C in an oven until its weight remained constant. The powdered shrimp shell underwent deacetylation, demineralization, deproteinization, and purification.

In brief, finely ground Shrimp shell powder (particle size of 250mm) was treated with 0.25 M HCl at room temperature with a solution is to solid ratio of 40 mL/g for demineralization. This reaction proceeded under agitation at 250 rpm in the ORBIT Shaker for 2 hours. Then 1.0 M NaOH was used to deproteinize the demineralized shrimp shell powder at 70°C with a solution that was a solid ratio of 20 mL/g in the sample. This reaction was conducted under agitation for 3 hours in a VELP SCIENTIFICA Magnetic stirrer. The residue from the deproteinization process was purified with hot ethanol and boiled in acetone (10 mL/g sample) to remove other

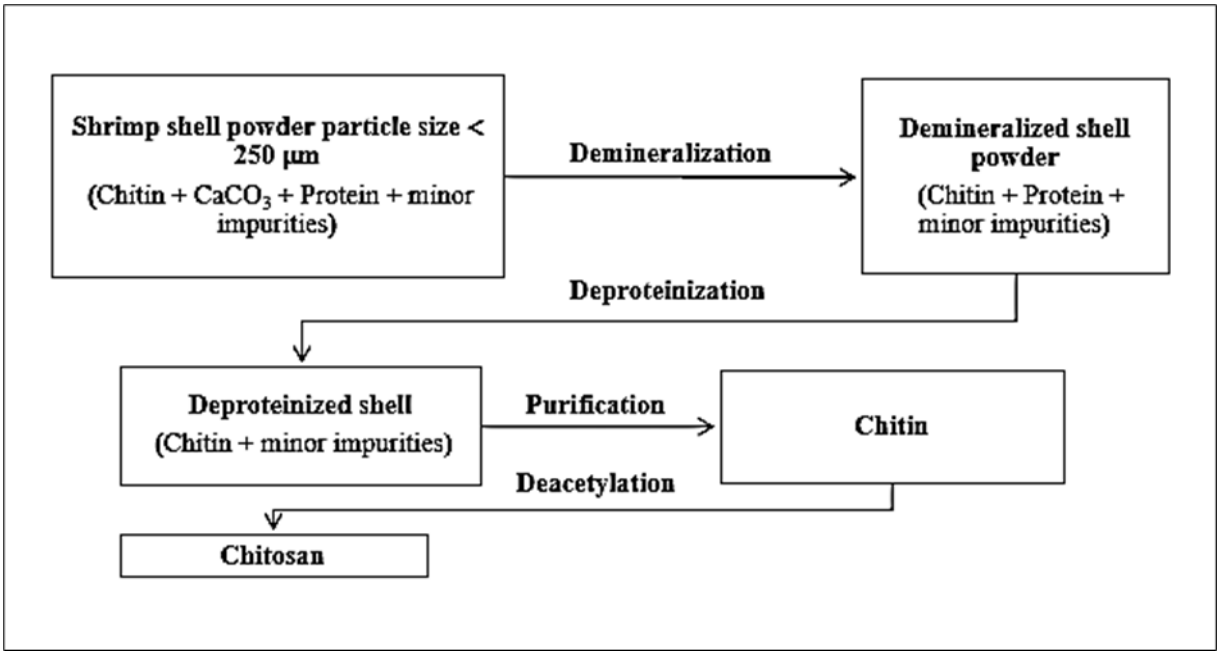

Figure 1: Chemical extraction procedure for chitosan

minor impurities. Purified chitin was treated with 45% NaOH for 7 hours at 100 °C to deacetylate into chitosan. Finally, it was washed several times with distilled water and oven-dried to a constant weight at 105 °C. Extracted chitosan was packed in a polyethene bag and stored in the refrigerator at 4 °C.

Preparation of edible chitosan coating

Edible chitosan coating solution was made based on the method described by Dong *et al*. (2020) with little adjustments. Chitosan solutions; 0, 0.25, 0.5 and 1.0% (w/v) were made by dissolving 0, 0.25, 0.5, and 1 g of chitosan powder, respectively, in 100 mL of 1% (v/v) acetic acid and the resulting solution was then heated in a water bath at 45 °C for a period of 10 minutes. Once the chitosan had completely dissolved in the acetic acid, 1 mL of glycerol was added as a plasticizer. Coating solutions were stored in the refrigerator in empty beakers at 4 °C for further analysis.

Chicken sausage preparation and coating

The preparation of chicken sausages was performed according to the formulation used by Mohan, (2014). The sausage mixture includes Potato starch, Table salt and Spices such as Chilli powder, Pepper powder, Garlic, Onion, Curry leaves and Ginger. The sausage mixture was inserted into a 2.5 cm-diameter natural goat intestine casing and tied in desired lengths (Shehata, 1989). Stuffed raw sausages were allowed to be smoked for 30 minutes using coconut husk. Then, it was poached at 75 ºC until the internal temperature reached 72 ºC. Finally, it was allowed to cool for 5 minutes. Sausages were coated with chitosan coating solution according to the method described by Bostan and Isin Mahan, (2011). Sausages were randomly selected and equally divided into four groups. Three groups of sausages were submerged for 15 minutes in chitosan solution at 0.25, 0.5, and 1.0%, respectively. Similarly, another group of sausages was submerged in tap water as a control for the study. Samples were air dried and kept for 4 weeks in cold storage (-10 °C) for further analysis.

Determination of pH

The technique described by Dong *et al*. (2020) was used to measure the pH value. Five grams of crushed sausage samples were mixed with 45 mL of distilled water, and the mixture was allowed to chill for 30 minutes at 4 °C. The pH was measured at room temperature (about 30 °C) by using a digital pH meter (OHAUS®, model – ST3100). Standard buffer solutions were used to calibrate the pH meter.

Determination of hardness

The hardness was measured using a Food Rheology Tester (IMADA, model – FTR series). The sausage samples were equilibrated at room temperature for 30 minutes and cut into 2.5 cm in diameter and 2 cm thick pieces (Schubring, 2002). A test speed of 1 mm/s was utilized to cut the sample pieces using a wedge probe (FR – K60 – 2030J).

Water holding capacity (WHC)

The press method was used to measure the wa ter-holding capacity. One gram of sample was placed on humid filter paper (Whatman No.1,11 cm in diameter) that was placed between two glass plates and subjected to a specified pressure by 1 kg constant weight for 1 minute (Joo, 2018). The filter paper's weight was recorded before and after the sample was placed. The weight difference after one minute of compression was calculated.

Determination of Moisture

Moisture was determined using the oven-dry method, based on the procedure described by AOAC (1995). A 5 g crushed sausage sample was used to obtain the moisture contents, and an air-drying oven at 105 °C was used to obtain a constant weight.

Colour measurement

According to the method of Ly *et al*. (2020), the colour parameters were measured using a Colorimeter (KONIKA MINOLTA, model CR20). For every sample, three distinct places were chosen randomly to measure colour and analyze the L*, a*, and b* values.

Determination of spoilage

The sausages were inspected for symptoms of degradation. The initial point of contamination was determined by an unpleasant smell, strange flavour, or strange appearance according the description given by Bostan and Isin Mahan, (2011).

Microbial analysis

The microbiological evaluation of sausage samples was conducted on the day 1, 2nd and 4th weeks of the storage. Total Aerobic Bacterial count (TAB) was taken during this period. One gram of minced samples representing each treatment was diluted up to 10^{-3} dilution factor. Nutrient Agar (NA) media was used to culture TAB. Diluted samples were inoculated into a sterile Petri dish, which contains growth media by the streak plate technique described in Sue Katz (2012) in an aseptic condition in the Bio Safety Cabinet (Heal ForceÒ, model – HFSafe 1200CC). Finally, the plates were incubated at 37 °C for 24 hours in the incubator (POL-EKO APARATURAÒ). The number of bacteria colonies were counted under the colony counter (GALLENKAMP colony counter).

Sensory evaluation

Sensory evaluation was performed on the day 1 and 4th weeks of storage. Briefly, 30 untrained panelists were selected and used for the evaluation. The sensory samples were fried at 50 °C temperature for 5 minutes. It was cut into 2 cm long pieces and labelled with appropriate treatment numbers. The panelists were allowed to sit on private seats under appropriate lighting areas and served with the samples in a random manner. The organoleptic characteristics, including odour, texture, appearance, taste and colour, were evaluated using a nine-point hedonic scale (Tolga and Sukran, 2010).

Statistical analysis

The experiment was conducted using a Completely Randomized Design (CRD) model. Data were subjected to statistical analysis using version 9.1.3 SAS software. Data were analyzed by the MANOVA at a 5% significance level, while Duncan's Multiple Range Test was used for mean separation. The Sensory analysis was carried out by Minitab 17.1.0 software using the Non-parametric Friedman test.

RESULTS AND DISCUSSION

Physical evaluation of extracted chitosan

The chitosan yield of the present study was 28.76% (w/w). However, the study conducted by Puvvada *et al*. (2012) was able to extract the chitosan yield of 35.49% (w/w). The variation in the chitosan yield might cause a difference in reaction time during the extraction. The moisture and ash contents of the chitosan samples were 7.3% and 1.6% on a dry basis, respectively, while the pH value of the chitosan samples was 8.5. The above-mentioned values are similar to those of Puvvada *et al*. (2012) and Divya *et al*. (2014). Further, the permitted level of moisture content in chitosan-coated chicken sausage should be below 10% on a dry basis (Gandhi *et al*., 2014), and this study fulfils the required level.

Physical attributes of chitosan-coated chicken sausages

Moisture

As indicated in the Table 1, the moisture level of sausages gradually dropped with the storage time at (-10 °C). According to El-Nashi *et al*. (2015), the primary source of moisture reduction was water vapour evacuation from the sausage surface due to the water vapour pressure difference with the surrounding cold air. The moisture content of chicken sausage is not significantly ($P>0.05$) influenced by the concentration of chitosan, which indicates that the addition of chitosan of less than 1% cannot prevent moisture loss in chicken sausages to a certain degree. Dong *et al*. (2020) found that chitosan coating concentrations of 2% or 3% are needed to prevent moisture loss. However, the higher concentrations, 2% or 3%, cause excessive viscosity and reduction in aroma (Dong *et al*., 2020), which affects consumer preference.

pH

The pH value gradually dropped, with the storage period increased as in the Figure 2.

Table 1: Changes in the moisture content during the time period of storage

Treatments	Moisture % Week 1	Week 2	Week 3	Week 4
T_0	70.53 ± 0.62^{ab}	69.65 ±1.05^{abc}	68.20 ± 1.27^{bc}	67.75 ±1.00^{bc}
T_1	71.38 ±0.79^{a}	69.13 ± 1.41^{abc}	68.76 ± 0.30^{abc}	67.38 ±0.27^{c}
T_2	70.32 ±0.49^{ab}	69.81 ± 0.50^{abc}	68.41 ± 0.76^{bc}	68.16 ±0.74^{bc}
T_3	70.36 ±1.54^{ab}	67.66 ± 4.07^{bc}	68.19 ± 2.00^{bc}	67.34 ±1.85^{c}

T_0 : 0% Chitosan coated Sausages, T_1 : 0.25% Chitosan coated Sausages, T_2 : 0.5% Chitosan coated Sausages, T_3 : 1% Chitosan coated Sausages. The numerical values indicates means ± standard deviations of replicates, Means in the column that have the same letters do not vary significantly ($p > 0.05$).

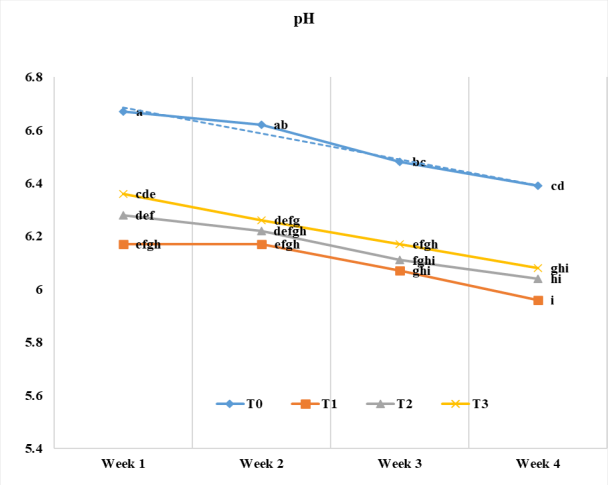

Figure 2: The changes in pH of chitosan coated chicken sausage during storage period

All values represent mean values of three replicates (mean ± SD). Means with different superscripts significantly differ (P<0.05).

T0: 0% Chitosan coated Sausages, T_1 : 0.25% Chitosan coated Sausages, T_2 : 0.5% Chitosan coated Sausages, T_3 : 1% Chitosan coated Sausages.

The reason for the pH decline during the storage period might be lactic acid production by lactic acid fermenting bacteria (Wenjiao *et al*., 2013). Dong *et al*. (2020) also reported in their study that the acid is produced by the microbial breakdown of organic materials, which lowers the pH level. In addition, the uncoated sausages have the highest pH value than the other treatments in all four weeks of storage. It could result from the accumulation of basic substances like ammonia, which is produced by microbial activity (Soultos *et al*., 2008). The pH values between 0.25, 0.5 and 1% chitosan-coated sausages were significantly increased with the concentration of chitosan coating (P<0.05) during storage.

Hardness

The comparison between hardness values of the chitosan-coated chicken sausage and without chitosan-coated sausages was studied. According to the results in the table 2, the coating with chitosan significantly (P<0.05) increases the hardness value of the chicken sausages. A higher value for hardness was detected in 1% chitosan-coated sausages, while a lower hardness value was recorded for the uncoated sausages. However, there were no significant differences (P>0.05) among 0.25 and 0.5% in hardness. Hajidoun, (2013) stated that the addition of different concentrations of chitosan significantly (P<0.05) improved the hardness. Moisture loss during storage significantly influences sausage hardness (Chen *et al.,* 2019).

Table 2: Hardness and WHC changes during storage period

Treatments	Hardness (N)	WHC (%) 1st week	2nd week
T_0	18.90 ± 2.31^{c}	14.33 ± 1.52^{bc}	13.00 ± 1.73^{c}
T_1	24.4 ± 1.51^{b}	21.33 ± 2.52^{a}	18.33 ± 4.51^{ab}
T_2	26.6 ± 0.95^{ab}	20.33 ± 0.57^{a}	18.67 ± 4.16^{ab}
T_3	27.93 ± 1.45^{a}	20.00 ± 1.73^{a}	18.00 ± 2.00^{ab}

T0: 0% Chitosan coated Sausages, T1: 0.25% Chitosan coated Sausages, T2: 0.5% Chitosan coated Sausages, T3 – 1% Chitosan coated Sausages. The numerical values indicate means ± standard deviations of replicates,

Means in the column that have the same letters do not vary significantly ($p > 0.05$).

Water Holding Capacity (WHC)

The mean WHC percentages of chitosan-coated and uncoated sausages during the 1st and 2nd week of storage were measured (Table 2). The chitosan-coated sausages have a significantly higher (P>0.05) WHC percentage than the uncoated sausage group. It might be due to the moisture retention potential of chitosan. A similar finding was recorded by Chattopadhyay *et al.* (2019) They showed that the inclusion of chitosan improves WHC in pre-emulsified fish sausages. There was no noticeable variation (P>0.05) in the WHC percentage among 0.25, 0.5, and 1.0% of chitosan coating, and no significant difference was observed between the first and second weeks of storage.

Colour

The L*, a*, and b* values are used to assess objective colour and compute colour differences. L* denotes lightness from black to white on a scale of 0-100, whereas a* and b* denote without numerical bounds. It is known that negative a* is associated with green, positive a* with red, negative b* with blue, and positive b* with yellow. According to the study, the lightness(L*), redness(a*), and yellowness(b*) values of the samples were not significantly affected (P>0.05) by coating with chitosan during the whole period of storage. L* value ranged from 45.00 to 54.13. a* value ranged from 6.43 to 7.86. b* value ranged from 14.00 to 19.08.

Determination of Spoilage

The sausage sample underwent daily assessment for deteriorative changes in room temperature and cold storage from day one. At room temperature, deteriorative changes such as unpleasant odour, off-flavour or appearance were detected in chitosan-coated sausages after four days from the day of preparation. The deteriorative changes occurred in chitosan-coated sausages in cold storage after the 4th week of storage. In uncoated sausages, these deteriorative changes occurred faster than chitosan-coated sausages. Gita *et al*. (2022) reported similar results.

Microbiological Analysis

The effects of different concentrations of chitosan coating on a number of total aerobic bacteria (TAB) during storage were assessed in the study as per the table 3. Initially, on day one the TAB counts were roughly 2.76 Log (CFU/g) and did not significantly differ between the samples treated with chitosan and control. The number of bacterial colonies was increased with the extension of the storage period. The total aerobic count of the smoked and cooked sausages should not exceed 1×10^5 CFU/g (5.00 Log (CFU/g) according to the Food Act No.26 of 1980 of Ministry of Health, Sri Lanka (Food Control Administration Unit of Ministry of Health, 2020). The TAB in the control sample had almost reached the permissible limit in two weeks. In contrast, the TAB count of samples treated with chitosan (0.25, 0.5 and 1%) was lower than the acceptable level that could be tolerated for a period of 14 days of storage. It could be due to the antibacterial activity of chitosan (Ganan, 2009 ; Yilmaz, 2020). The number of TAB colonies was not significantly (P>0.05) reduced between the samples treated with 0.25, 0.5 and 1% of chitosan. This observation indicates that the chitosan coating of 0.25% is sufficient to inhibit bacterial growth in sausage. This was supported by the study conducted by Bostan and Isin Mahan, (2011), which showed that a chitosan concentration of 0.25% was sufficient with respect to slowing down the development of aerobic bacteria. However, all the samples surpassed the acceptable limit in the 4th week of storage.

Table 3: TAB count in sausage during cold storage

Treatments	TAB count (Log CFU/g)	
	Week 2	Week 4
T_0	4.97 ± 0.13^{c}	5.38 ± 0.01^{a}
T_1	4.56 ± 0.09^{d}	5.18 ± 0.03^{b}
T_2	4.50 ± 0.09^{d}	5.12 ± 0.00^{b}
T_3	4.44 ± 0.13^{d}	5.19 ± 0.01^{b}

T0: 0% Chitosan coated Sausages, T1 : 0.25% Chitosan coated Sausages, T2: 0.5% Chitosan coated Sausages, T3: 1% Chitosan coated Sausages.

The numerical values indicates means ± standard deviations of replicates, Means in the column that have the same letters do not vary significantly (p>0.05)

Sensory evaluation

According to consumer acceptability of sensory panel results, higher consumer preference for odour, colour, appearance, and texture was observed in 1% chitosan-coated sausage (Figures 3 and 4). The panelists assigned a higher value to the Overall acceptability for the 1% chitosan-coated sausage group. At the same time, it had lower overall acceptability for uncoated sausages.

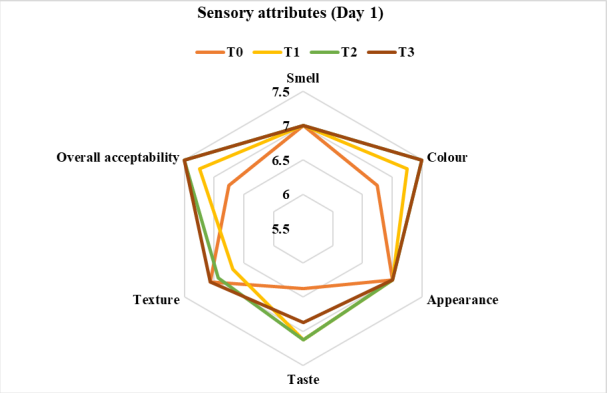

Figure 3: Sensory attributes on Day 1

T0: 0% Chitosan coated Sausages, T1: 0.25% Chitosan coated Sausages, T2: 0.5% Chitosan coated Sausages, T3: 1% Chitosan coated Sausages.

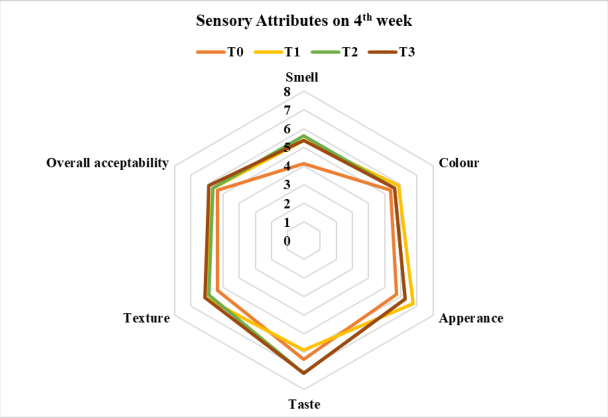

Figure 4: Sensory attributes on 4th week

T0: 0% Chitosan coated Sausages, T1: 0.25% Chitosan coated Sausages, T2: 0.5% Chitosan coated Sausages, T3: 1% Chitosan coated Sausages.

CONCLUSION

The findings of this study indicate that 1% (w/v) chitosan coating sausage leads to the retention of acceptable quality attributes, an enhancement in microbiological security, and an extension of the shelf life of chicken sausage at cold storage (-10 °C). These quality attributes were achieved by limiting pH decline and water movement and suppressing the development of aerobic bacteria. According to these findings, chitosan coating can be used to coat sausages as an edible coating to prevent microbial contamination and ensure safety from microbes.

AUTHOR CONTRIBUTION

PM, MP, and SR conceptualized and designed the study. PM and MFVN performed the experiments, analyzed the data and drafted the manuscript. MP and SR supervised the study and PM critically revised the manuscript.

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