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In vitro evaluation of *Hydrilla verticillata* extract as a natural preservative for chicken meat

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ABSTRACT

The intent of this research is to study the preservative effect of ethanolic extract of *Hydrilla verticillata* enriched with chitosan coating on the meat sample to increase the shelf life of the palatable product. The chemical, microbiological, sensory, and nutritional analysis such as pH, the amount of lipid peroxidation, total bacterial count, protein, and fat content of the fresh chicken meat coated with ethanolic extract of *H. verticillata* and chitosan were performed for 21 days. The results of the experiment show that *H. verticillata* extract enriched with chitosan coating met the need to act as a preservative by degrading the growth of microorganisms, maintaining the pH, and also by increasing the nutritional values.

1. Introduction

Natural preservatives are compounds found in natural sources which is used for long term preservation of organoleptic qualities (color, flavour, taste, smell, freshness), prevention of rapid degradation and to prolong the shelf life of food (*Bondi et al.,* 2017). The natural preservatives are witnessing higher demand owing to their acceptance in processing by relevant regulatory agencies coupled with rising health consciousness among customers. Chicken meat is a predominant dietary protein source and the treatment to preserve its quality plays a vital role. Treatment procedures may follow physical, chemical and biological processes. The physical process includes dehydration, freeze-drying methods etc., and chemical process where synthetics are added such as benzoates, nitrites, etc. The biopreservation process includes the addition of enzymes and plant extracts which have natural antioxidants that promote health benefits. Moreover, consumer's preference for natural food preservatives and concern regarding the safety of synthetic preservatives urged the food industry to look for natural alternatives (*Esmaeili et al.,* 2021).

Hydrilla verticillata is a submerged herbal medicinal aquatic plant that contains more of beneficial compounds which possess both antimicrobial and antioxidant properties (*Pal & Nimse,* 2006). The presence of the phytochemical in *H. verticillata* namely phytol may act as a natural preservative as it has both the antimicrobial and antioxidant activities that play an integral role in acting as a natural preservative. The phenol and flavonoids content in it also contributes to the high antioxidant capacity. Due to its essential properties and presence of vital bioactive compounds, the ethanolic extract of *H. verticillata* can be used as a preservative (*Byju et al.,* 2013).

Chitosan is a natural, biodegradable, biorenewable and non-toxic substance that has been considered for applications in the food industry (*Sinha et al.,* 2022). This is due to its physicochemical properties, film forming and barrier properties against pathogenic microbes, anti-microbial and anti-fungal activities *(Xing et al., 2016)*. Application of chitosan

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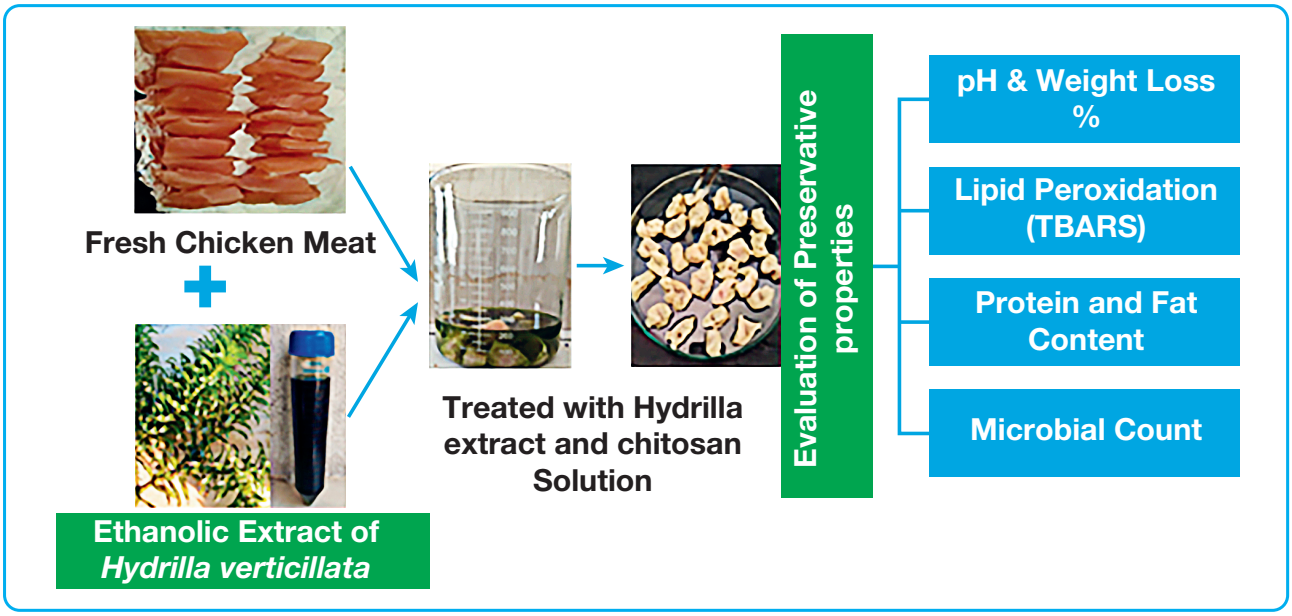

Figure 1. Comprehensive Overview of the Synergistic Preservative Effects of Hydrilla verticillata and Chitosan on Chicken Meat

to meat will be a barrier to water vapour that will reduce the moisture content and weight loss, maintain the color of the meat, retard the lipid peroxidation and increase the shelf life and storage quality. When chitosan is combined with the plant extract, it additionally enriches the preservative action. The 1% chitosan is most commonly preferred as it effectively inhibits the growth of bacteria, putrefaction, reduces the thiobarbituric acid (TBA) value and increases anti-oxidation *(Shafiei & Mostaghim, 2022)*. Hence, the experiment involved the application of extract derived from *H. verticillata* along with chitosan to analyze the preservative effect on the chicken meat.

2. Materials and Methods

Preparation of ethanolic extract of Hydrilla verticillata

The preparation of ethanolic extract of *H. verticillata* was done as described in our previous article (*Prabha et al.,* 2019).

Preparation of 1% chitosan solution

4 g of chitosan was dissolved in 4 mL of glacial acetic acid and it was stirred in the magnetic stirrer until it is completely dissolved for about 10 min at 50 °C and then 150 mL of distilled water was added to the mixture and stirred again until it is mixed properly. Then the solution was made up to 300 mL with distilled water.

Preparation of chicken samples

Fresh chicken breast meat was brought from the market and cut into small pieces each weighing approximately 15 g and divided into three groups namely:

- Group I – Control
- Group II – Treated with hydrilla extract
- Group III – Treated with hydrilla extract and chitosan

The group I chicken pieces were dipped in distilled water for 1.5 min, the group II chicken pieces were dipped in extract solution of *H. verticillata* for 10 min and the group III chicken pieces were first treated with extract solution for 10 min and then with 1% chitosan solution for 1.5 min. Finally, all the groups were stored at refrigerated condition at 4 °C.

Evaluation of preservative properties

Chemical, microbiological, nutritional and sensory analysis are important in evaluation of preservation properties. The data is presented as the mean ± standard deviation of the mean (SDM) calculated over a period of 21 days.

Measurement of pH

The pH for all the groups of the sample was done by homogenizing 0.5 g of sample with 5 mL of distilled water for 1 min. The homogenized samples were kept at room temperature for 10 min and then pH was determined using the pH meter and the values were recorded *(Karthik et al., 2021)*.

2.1 Analysis of lipid oxidation

Determination of thiobarbituric acid reactive substances (TBARS)

4 g of sample was blended with 8 mL of trichloroacetic acid (5 mg/100 mL) and 8 mL of 0.5% butylated hydroxy toluene (BHT) with the help of mortar and pestle and then the solution was filtered through Whatmann 4 filter paper. 5 mL of filtrate was added with 5 mL of thiobarbituric acid and they were heated in the boiling water bath for 30 min and measured at 532 nm *(Pandi et al., 2022)*. They are expressed as mg malonaldihyde/kg meat.

Analysis of weight loss %

Weight loss was calculated by initially weighing the weight of the sample meat of all groups and then the final weight for the consecutive days *(Adu et al., 2019)*.

The weight loss in percentage was calculated using the formula given below:

$$\text{Weight loss (\%)} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Final Weight}} \times 100$$

2.2 Nutritional analysis

Determination of protein content

The protein content of all the groups of sample was determined by Lowry's method. The concentration of protein can be determined using colorimeter at 670 nm (*Karthik et al.,* 2021).

Determination of fat content

The fat content of different batches of sample was determined by Liebermann-Burchard method *(Adu et al., 2019)*.

2.3 Microbiological analysis

Pour plating technique for counting the total bacterial co unt

1.1 g of sample from each group was homogenized with 10 mL of 0.1% sterile peptone water. From this homogenate, 1 mL was added to 9 mL of 0.1% sterile peptone water and the appropriate serial dilutions were carried out. Two dilutions of 10^{-2} and 10^{-3} were taken and 1 mL from each dilution was first placed into the plate and then the melted plate count agar was poured over it and then the plate was incubated at 37 °C and the numbers of colonies were calculated every day for a period of 21 days (*Mehdizadeh & Mojaddar Langroodi,* 2019).

2.4 Sensory analysis

It was performed by evaluation of the acceptability (total sensory evaluation score) as a composite of odor, color and appearance using a nine-point hedonic scale. The scale points were: excellent, 9; very good, 8; good, 7; acceptable, 6; poor (first off odor, off-taste development) < 6; a score of 6 would be taken as the lower limit of acceptability. The sample would be defined as unacceptable after development of first off-odor or off-taste.

2.5 Statistical analysis

All data expression is done using mean ± standard deviation. Significant results were reported by one-way analysis of variance follow-up test by Tukey's HSD post hoc multiple comparisons with the help of GraphPad Prism Scientific Software. $p<0.05$ is considered as statistically significant.

3. Results and Discussion

One of the main purposes of food industry is to optimize the preservation technologies of perishable foods to reach a final product with optimal quality.

As pH is the measurement of acidity, it will affect the water holding capacity and the color of the meat, which in turn will influence the overall quality of the meat. So maintaining the pH value of the meat is very important. The pH values of three groups of samples were determined by using a pH meter. The comparative results of three groups are depicted in Figure 2. The untreated control meat was spoiled after one week, but the hydrilla and hydrilla with chitosan treated meat samples retained pH 6.11 and 5.56 respectively after three weeks. It was observed that the only 10–13% changes in pH on the coated meat during the storage period. In this study, the pH values of the treated samples were significantly ($p<0.05$) lower than the control group during the storage period. This is mainly due to the phytochemicals present in the *H. verticillata* extract and acidic properties of chitosan solution and it prevents the microbial growth on the surface of the samples, which is due to the antimicrobial property (*Eldaly et al.,* 2018). During storage, there is a gradual increase in pH values in both Experimental groups II and III, primarily due to the activity of endogenous enzymes, bacterial metabolites, and

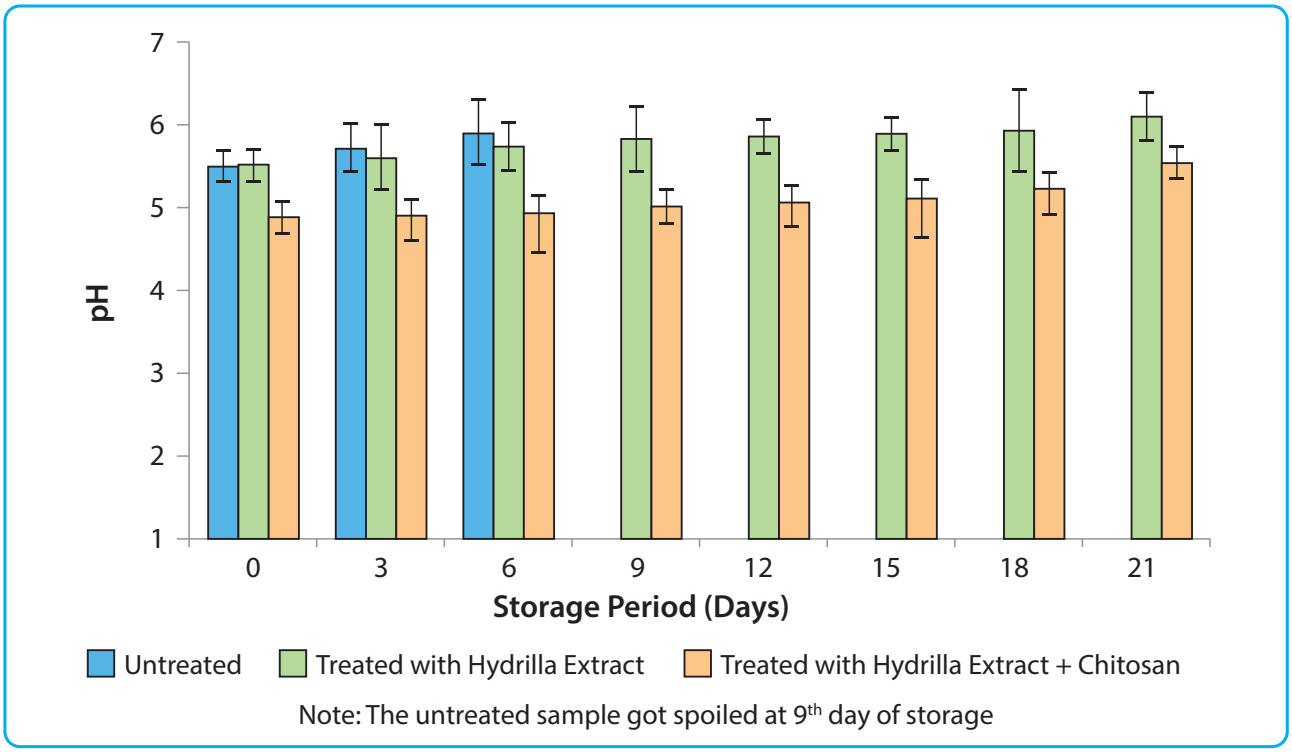

Figure 2. Effect of *Hydrilla verticillata* extract and chitosan coating on pH

the production of volatile organic compounds such as ammonia (*Alam et al.,* 2018). Notably, a significant difference was observed between these two groups, attributed to the presence of chitosan in Experimental group III. Chitosan plays a crucial role in mitigating pH fluctuations in meat samples by buffering the pH, inhibiting microbial growth, forming a protective film, and chelating metal ions. These mechanisms collectively contribute to the preservation of meat quality (*Thambiliyagodage et al.,* 2023).

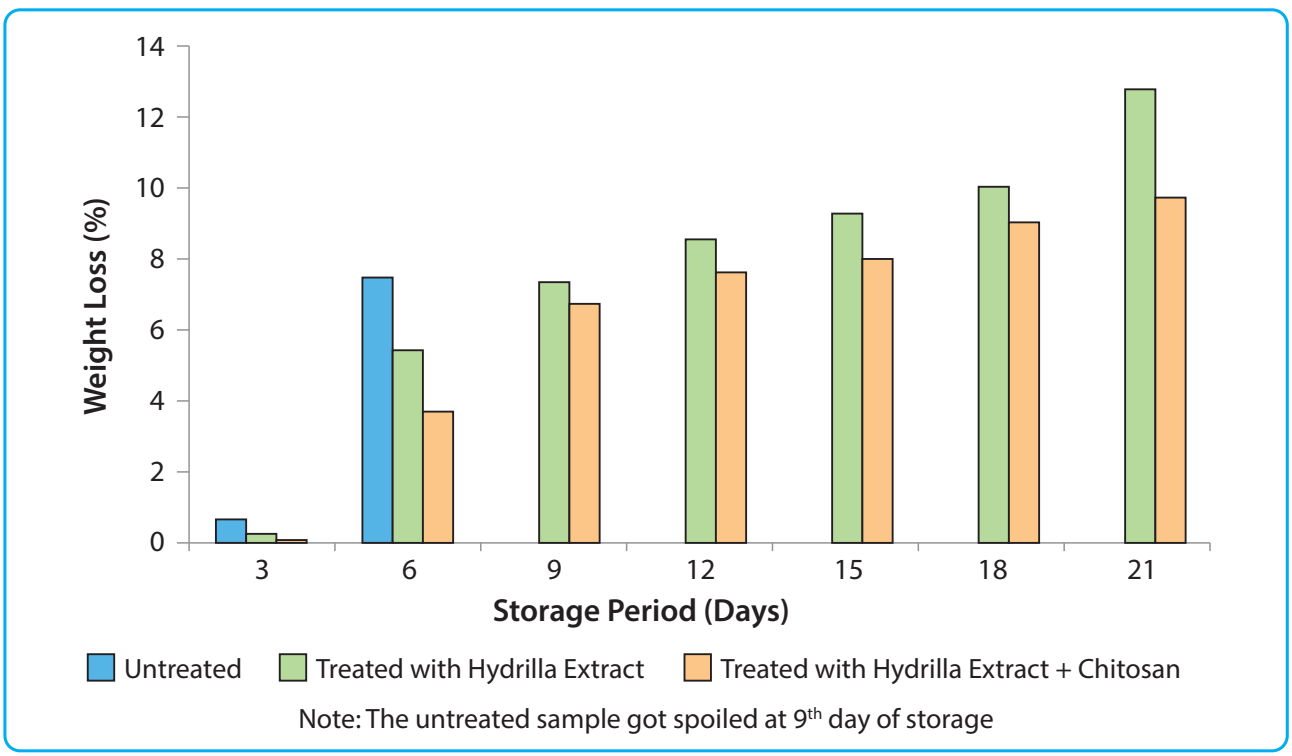

Figure 3. Effect of *Hydrilla verticillata* extract and chitosan coating on Weight loss %

Weight loss in meat occurs due to dehydration of meat during storage under refrigerated conditions. The main reason for dehydration is due to the fact that surface of the meat is exposed to mass transfer exchange (evaporation) with the environment. The weight loss percentages for three groups of sample were represented in the Figure 3. The study revealed that around 10% weight loss is observed on 9th day for untreated meat, however the same percentage of weight loss is observed on 18th and 21st day for hydrilla and hydrilla with chitosan coated meat. The coated samples showed a significantly lower weight loss than the uncoated sample meats. This is because the *H. verticillata* extract and the chitosan coating retained the moisture content by maintaining the weight which is mainly required to manage the quality of meat (*Mehdizadeh & Mojaddar Langroodi*, 2019).

Lipid peroxidation is a main factor that limits the shelf life of muscle foods and it is measured in terms of TBARS. The concentrations of TBARS formed due to lipid peroxidation in the chicken samples were found out using the standard calibration curve and expressed as mg/kg. As seen in this experiment, initially there was no significant difference in the TBARS values, but upon storage, there was rapid increase in TBARS in the uncoated sample as compared to the coated samples (Figure 4). Lipid peroxidation generally involves the degradation of polyunsaturated fatty acids and the production of secondary decomposition products, including carbonyls and hydrocarbon compounds. The oxidative stability of meat depends on the balance of anti-oxidants and the composition of oxidizable substrates, including PUFAs, cholesterol, proteins, and pigments (*Pereira & Vicente,* 2013). The antioxidants present in the hydrilla plays effective role in the inhibition of lipid peroxidation by donating hydrogen and helps to form the hydroxyl groups (*Jonaidi Jafari et al.,* 2018). Moreover, the scavenging activity of chitosan enhance the antioxidant activity of the formulated preservative (Ngo & Kim, 2014). The observed results revealed that *H. verticillata* extract and chitosan coating inhibited the lipid oxidation in all the meat samples during the storage.

The concentrations of protein in the three groups of sample were found out using the standard calibration curve and the concentration is expressed as mg/ml. The results of the experiment are represented in Figure 5. It was observed that the minor change in protein content 17% and 13% on the hydrilla and hydrilla with chitosan coated meat. The untreated meat samples could be stored under refrigeration for 6 days but the hydrilla extract treated samples could be stored for 21 days by retaining near normal protein content of the meat. The antimicrobial activity can help prevent protein degradation

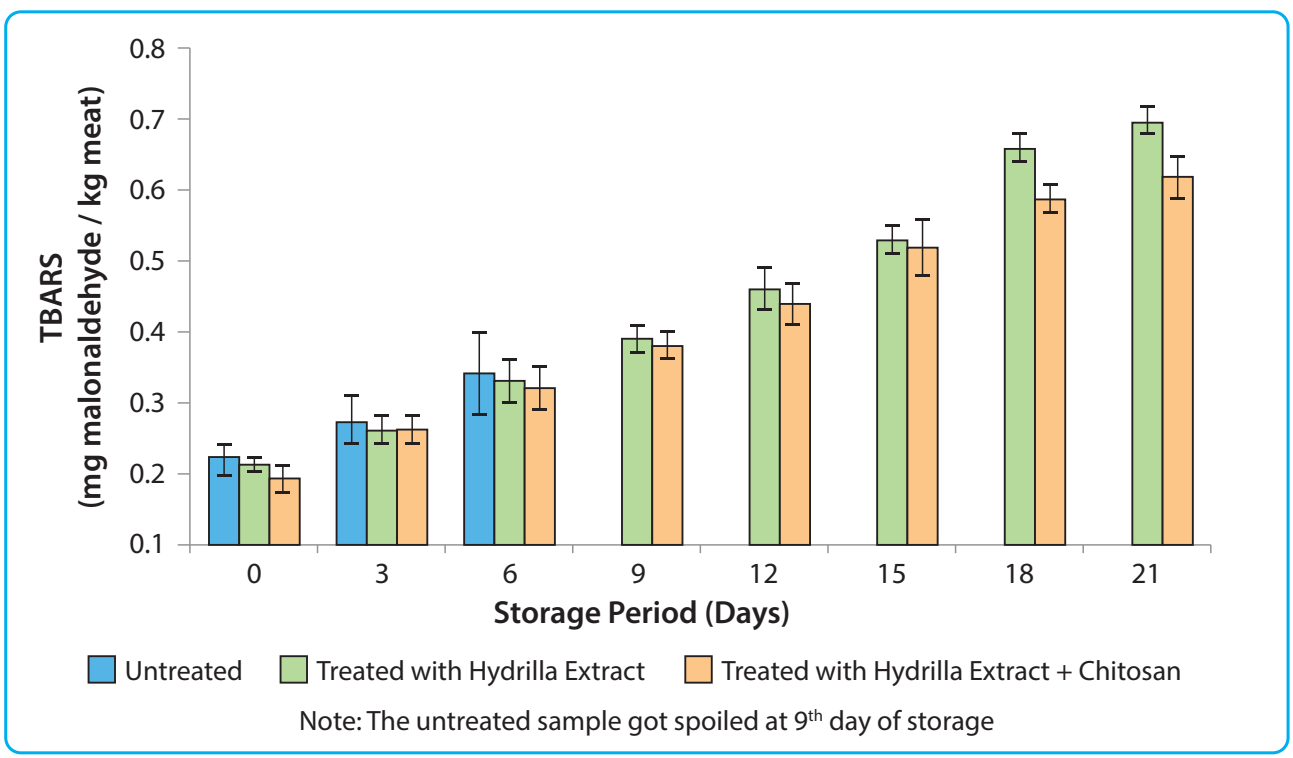

Figure 4. Effect of *Hydrilla verticillata* extract and chitosan coating on TBARS

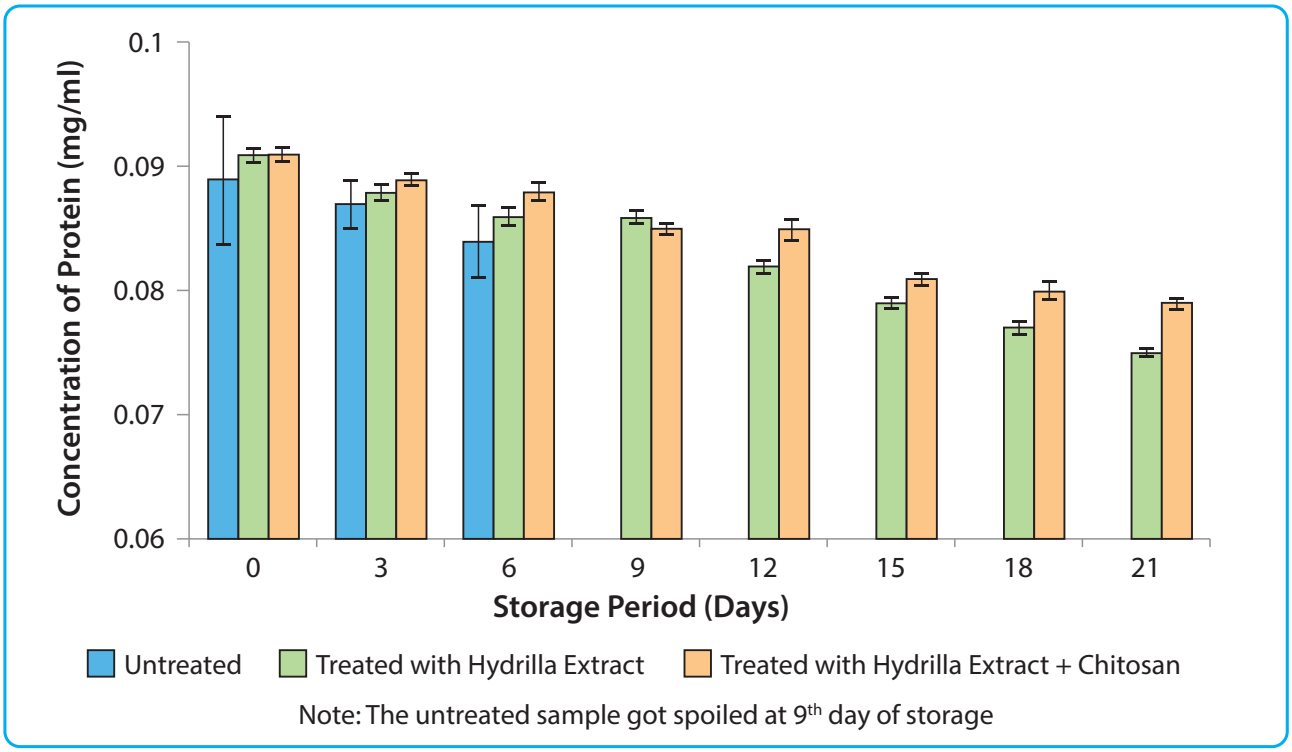

Figure 5. Effect of *Hydrilla verticillata* extract and chitosan coating on Protein content

caused by microbial spoilage, which can lead to nutrient loss. The protease inhibitors present in the hydrilla extract can help preserve the protein content of meat products by inhibiting proteolytic enzymes (*Olvera-Aguirre et al.,* 2023).

The concentrations of fat in the three groups of sample were found out using the standard calibration curve and the concentration is expressed as mg/mL. The results are represented in Figure 6. There is no change in the fat content upto 6 days for treated and

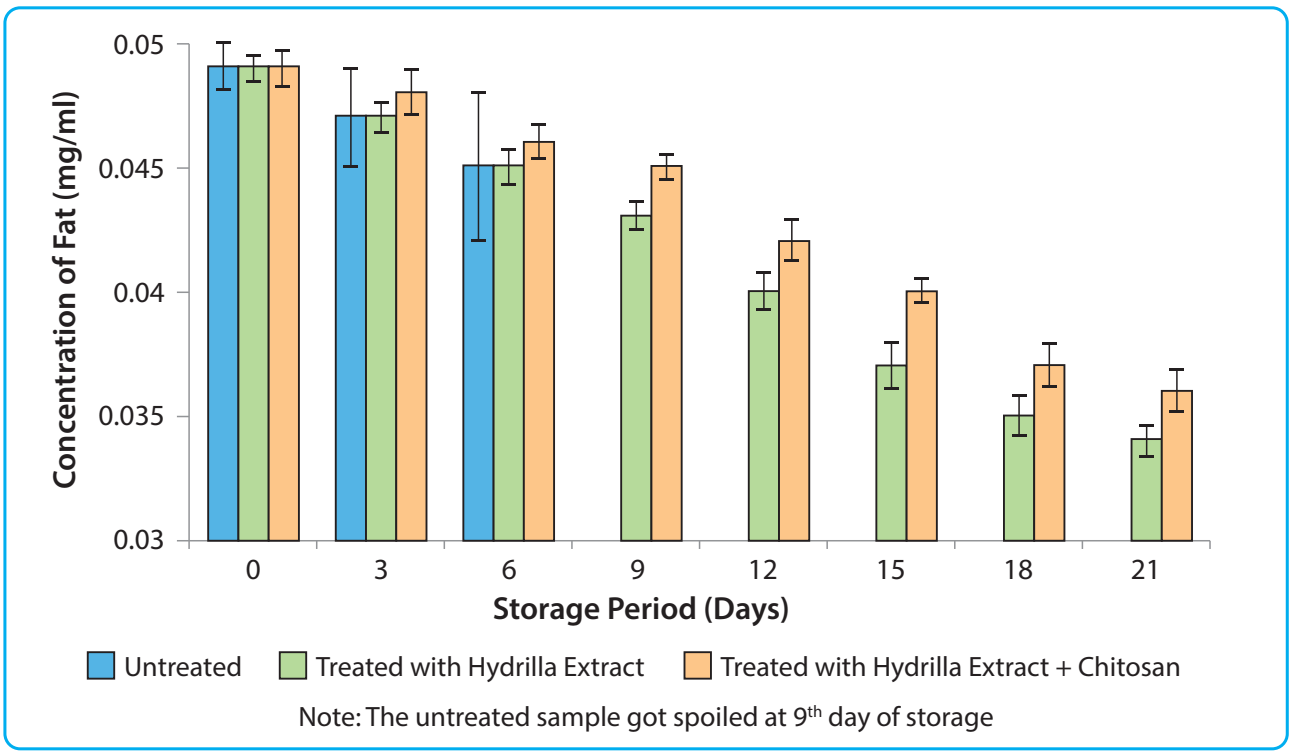

Figure 6. Effect of *Hydrilla verticillata* extract and chitosan coating on Fat content

untreated meat samples, but afterwards slight change in in fat content is observed on the coated samples whereas the uncoated samples are spoiled. The results revealed that 75% of the fat is retained during the storage period 21 days on the hydrilla and hydrilla with chitosan coated meat. A variety of things happen during the processing and storage of preserved meat that can affect its nutritional content. The nutrient loss of meat is a major concern during the refrigerated storage. Meat is a valuable source of protein, iron, vitamin B_{12} as well as other B complex vitamins, zinc, selenium and the fat content (*Bustabad,* 1999). The major source of nutrient in chicken meat is protein and fat content. It is observed that the application of *H. verticillata* extract and chitosan coating maintained the protein and fat content at a constant rate without major loss. The hydrilla extract is rich in antioxidants, such as polyphenols, flavonoids, and vitamins. The antioxidants help combat oxidative reactions that can lead to nutrient degradation in meat products. By scavenging free radicals and inhibiting lipid and protein oxidation, plant extracts can help preserve the nutritional content of vitamins, minerals, and amino acids in meat (*Petcu et al.,* 2023).

The ethanolic extract of *H. verticillata* contains phytol as one of its chemical constituents, along with other compounds like chlorophyll, carotenoids, polyphenols, and more. Some studies have shown that phytol can scavenge free radicals and protect cells from oxidative damage. Phytol has also shown antimicrobial activity against a range of microorganisms, including bacteria, fungi, and some parasites. Its antimicrobial effects are attributed to its ability to disrupt microbial cell membranes and interfere with their growth and replication. This makes phytol a potential candidate for the development of antimicrobial agents or as a natural preservative in food products. Ethyl palmitate and ethyl linolenate, the chemical compounds that can be used as a preservative in various food products and found naturally in the hydrilla extract. These esters can function as an antioxidant and helps prevent the oxidation of fats and oils in food and products. Oxidation of fats can lead to off-flavors, rancidity, and a decrease in product quality. By inhibiting lipid oxidation, ethyl palmitate can extend the shelf life of meat and meat products (*Lin & Long,* 2023). Hence, the hydrilla extract may be used as natural antioxidants, antimicrobial agents, or ingredients in dietary supplements.

The total bacterial count was determined using the pour plate technique and it is expressed as logarithm of colony forming units (log CFU/g). The results of the comparative study are displayed in Figure 7. Even though the slight increase in bacterial count is observed on the coated meat, it could be eliminated during cooking process. *H. verticillata* has been documented for its broad antimicrobial activity

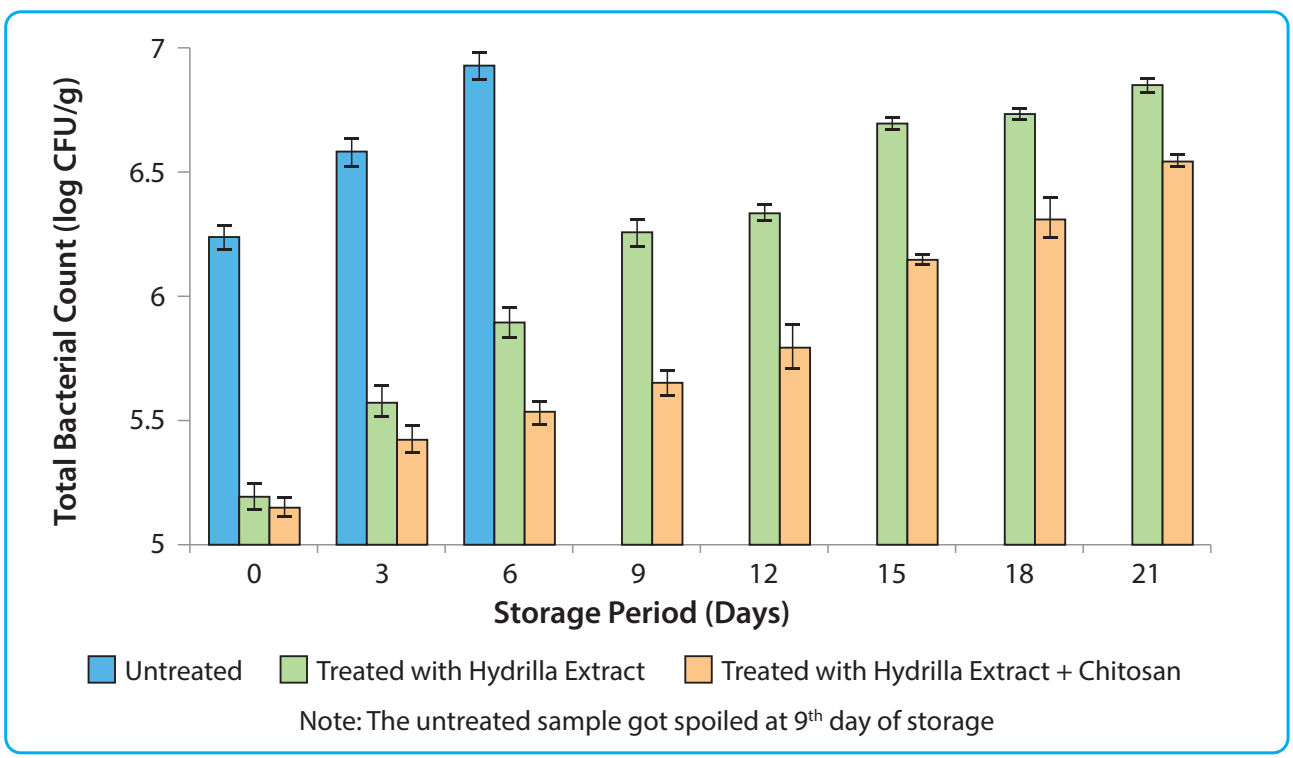

Figure 7. Effect of *Hydrilla verticillata* extract and chitosan coating on total bacterial count

against bacteria and fungi. The antibacterial action of plant extract is related to prevent cell division, causing the changes to membrane phospholipid and fatty acid value and prohibits RNA and DNA synthesis. Furthermore, chitosan as a coating solution acts as an oxygen barrier around the bacterial cell and thus prevents the growth of aerobic bacteria and the antimicrobial property of chitosan is associated with its unique polycationic property, which interrupts the microbial cell membrane (*Pereira & Vicente,* 2013). The results indicated that plant extract along with chitosan coating decreased the bacterial population in the treatment. If the plant extract is applied alone it showed a low antibacterial impacts, but when the plant extract and the chitosan is applied together, it leads to stability of the antibacterial properties for a significant period. This is due to the phenomenon that allowing plant extract to hydrolyze the peptidoglycan layer surrounding the cytoplasmic membrane of bacteria, increasing the antibacterial effect of chitosan (*Darmadji & Izumimoto,* 1994). The data revealed that the coated sample meats led to a significant reduction in total bacterial count over the time of storage period. This reduction in microbial count is due to the antimicrobial effect of *H. verticillata* extract and chitosan (1%) on the spoilage bacteria . The distinction between Experimental groups II and III is primarily due to the presence of chitosan in Experimental group III. Chitosan effectively inhibits bacterial growth through several mechanisms, including disrupting the bacterial cell membrane, blocking nutrient absorption, inducing osmotic imbalance, chelating essential metal ions, interfering with DNA and RNA synthesis, and generating oxidative stress. These combined actions render chitosan a potent antimicrobial agent (*Ardean et al.,* 2021).

The results (Table 1) indicated that the hydrilla and hydrilla with chitosan coated meat have excellent sensory characteristics for three days, followed by good for another 3–4 days, and then reached the acceptable limit. However, the untreated sample reached the poor sensory characteristics at the 6th day storage period. The role of sensory evaluation is to provide valid and reliable information for consumer acceptability. The sensory scores of the samples were not affected by *H. verticillata* extract and chitosan as it does not produce any off-flavors, color and the appearance of the sample were not objectionable and either of which could potentially lead to rejection of products by the consumer. Whereas, the uncoated sample meats produced off-flavor during the 9th day of storage which indicates the effects of *H. verticillata* and chitosan on preserving the sensory characteristics of chicken meat. According to sensory evaluation, the shelf life of the meat samples is determined to be 6 days for the untreated samples and 21 days for those treated with hydrilla extract. Additionally, the group treated with chitosan was able to maintain good quality for 15 days, while the hydrilla extract group maintained good quality for 12 days.

4. Conclusion

The *H. verticillata* being a weed plant with profound uses has also resulted to act as a potent natural preservative. Since chicken meat is largely consumed by many the nature of the chicken meat is difficult to maintain as days pass by. In this case, the extract from *H. verticillata* is used in retaining the raw nature, taste, intrinsic factors, color, and the texture of the chicken meat for many days. The re-

Table 1. Sensory characteristics of meat sample during chilled storage

Storage period (Days)	Untreated	Treated with Hydrilla Extract	Treated with Hydrilla Extract + Chitosan
0	9	9	9
3	7	9	9
6	5	8	9
9	NA	8	8
12	NA	8	8
15	NA	7	8
18	NA	7	7
21	NA	7	7

Legend: NA-Not applicable (The sample got spoiled at 9th day of storage)

sults of the comparative study represented that the preservative effect of *H. verticillata* extract enriched with chitosan coating sustained the quality of chicken meat under 4 °C by maintaining the pH, protein, fat, TBARS, microbial growth and also the sensory characteristics such as color, odor and appearance during the storage period of 21 days. The antioxidant present in the extract prevents the free radicals and the antimicrobial activity against the microbes ensure the preservative properties of the hydrilla extract. These results suggest that *H. verticillata* extract along with the chitosan (1%) coating can be applied as natural preservative to the meat products in the food industry to preserve quality and extend the shelf life .

In vitro evaluacija ekstrakta Hydrilla verticillata kao prirodnog konzervansa za pileće meso

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INFORMACIJE O RADU

Ključne reči:
Antioksidativno dejstvo
Antimikrobno dejstvo
Konzervirajuće dejstvo
Vodena biljka
Rok trajanja

APSTRAKT

Cilj istraživanja je da se ispita efekat konzervacije etanolnog ekstrakta Hydrilla verticillata obogaćenog hitozanom na uzorku mesa kako bi se produžio rok trajanja proizvoda. Hemijske, mikrobiološke, senzorne i nutritivne analize kao što su pH, količina peroksidacije lipida, ukupan broj bakterija, sadržaj proteina i masti u svežem pilećem mesu koje je obloženo etanolnim ekstraktom H. verticillata i hitozanom, su rađene u periodu od 21 dan. Rezultati eksperimenta pokazuju da je ekstrakt H. verticillata obogaćen hitozanskom prevlakom delovao kao konzervans na degradaciju rasta mikroorganizama, održavanje pH, kao i povećanje nutritivnih vrednosti.

Disclosure statement: No potential conflict of interest was reported by authors.

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