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Effect of carrageenan edible film with oleoresins of *Piper nigrum* (black pepper) on quality of buffalo meat steaks

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Abstract

This study was aimed to develop carrageenan edible film with oleoresin of *Piper nigrum* (black pepper) and to study its effect on the quality and shelf life of buffalo *Longissimus dorsi* steaks during chiller storage at 4 ± 1 °C. Steaks wrapped with carrageenan edible film with 0.5 percent pepper oleoresin (T1) were compared with those wrapped with edible film without oleoresin (T1) and unwrapped (C) steaks for physico-chemical, microbiological and sensory characteristics. Drip loss and cooking loss of C were higher on all days compared to others. C and T1 showed significant increase in pH on storage. Thiobarbituric acid reacting substances numbers of C were significantly higher. Tyrosine values significantly increased on storage for all. C had higher 'L' values when compared to treatments and T2 had lower 'a' values compared to C and T1. C and T1 showed significant decrease in 'b' values across the storage days. Total phenolic content of pepper oleoresin was found to be 15.51 ± 0.79 µg TAE /ml, and DPPH radical scavenging activity of oleoresin containing carrageenan solution was $37 \pm 0.18\%$. C and T1 had higher total viable and psychrotropic counts on day 12th compared to T2. Flavour, juiciness and overall acceptability sensory scores of cooked steaks did not vary across storage till the respective day of spoilage C and T1 had shelf life of 6 days and T2 had a shelf life of 12 days. Carrageenan edible film with pepper oleoresin doubled the shelf life of meat steaks without affecting the sensory attributes under chiller storage.

Keywords: Carrageenan, edible films, black pepper, oleoresin, *Longissimus dorsi*, chiller storage

1. Introduction

Meat is a nutrient rich food and is highly susceptible to microbial deterioration; therefore, its preservation is very important to maintain the quality characteristics. Currently petro-chemical based stuffs are used for packaging of food and other items due to their versatility. However, these materials pose a threat to the environment because of their non-biodegradability and this has instigated the researchers to develop packaging materials with biodegradable properties. Carrageenan is a water soluble biopolymer, extracted from red seaweed and has gel forming ability and has the ability to form thin edible films due to polysaccharide cross linking (Karbowski *et al.* 2006) ^[1]. Further the functional characteristics of these edible films can be improved by adding oleoresins of spices or herbs and this may enhance the shelf life and improve the quality characteristics of food wrapped in these films. Spices are generally used to increase the flavour of any meat product and black pepper is one among the commonly used spices in Indian cuisine. This research work has been aimed to develop carrageenan edible film with oleoresin of *Piper nigrum* (black pepper) and to study its effect on the quality and shelf life of buffalo *Longissimus dorsi* steaks during chiller storage at 4 ± 1 °C.

2. Materials and methods

Longissimus dorsi muscles from buffalo carcasses aged 8-9 years were procured from Malabar Meat, Brahmagiri Development Society, Sulthanbathery and brought to the department of Livestock Products Technology, College of Veterinary and Animal Sciences, Pookode under refrigerated conditions. The preparation of edible films and treatments are as follows.

2.1 Preparation of edible film

An edible film was prepared with the refined carrageenan and glycerol as plasticizer, the levels of which were standardised after preliminary trials as,

- a) Carrageenan (one percent)
- b) Glycerol (one percent)

Refined Carrageenan (Marine hydrocolloids, cochine, Kerala) was mixed with the filter water and was heated to 90 °C with constantly stirring till it was completely dissolved. After cooling to 70 °C Glycerol was added and, mixed and then cooled to ambient temperature and the solution was divided into two, one without oleoresin and the remaining incorporated with *Pepper nigrum* (black pepper) (0.5%) oleoresin (Plant lipids, Kolenchery, Cochine) the concentration were standardized after preliminary sensory evaluation and poured on the OHP sheet kept on glass tray then incubated at temperature 45 °C Humidity ranging from 35-40 percent for two days then it is peeled from the OHP sheet and stored under dry condition.

Fillets were divided into three groups and subjected to various treatments such as: C- Control steaks without edible film, T1 - Steaks with edible film that does not contain oleoresin. (1% carrageenan), T2- Steaks with edible film incorporated with oleoresins of *Pepper nigrum* (black pepper) (0.5%).

2.2 Physico-chemical attributes

Drip loss of breast fillets was calculated using the following formula:

$$\text{Drip loss (per cent)} \times 100 = \frac{\text{Initial weight (g)} - \text{Final weight (g)}}{\text{Initial weight (g)}}$$

Cooking loss was calculated as per Boccard *et al.* (1981) [2]. pH of the samples was measured using a digital pH meter as described by AOAC (2012). Water holding capacity was estimated as per Wardlaw *et al.* (1973) [3]. Thiobarbituric acid reactive substances (TBARS) number were determined as per Witte *et al.* (1970) [4] with modifications. Tyrosine values of control and treatment samples were estimated as per the method described by Pearson (1968) [5]. The concentration of total phenolics of coating solution was determined by the Folin-Ciocalteu (F-C) assay (Escarpa and Gonzalez, 2001) [6] with slight modification. 2, 2-diphenyl-1-picryl hydrazyl (DPPH) assay was done to evaluate the antioxidant activity of the coating solution. This was done by the modified method of Singh *et al.* (2002) [7]. The DPPH radical scavenging activity was expressed as percentage maximum of coating solution. The DPPH activity of oleoresin was also calculated by the above method. The aliquot containing 50µg of phenolics was obtained from 1 in 100 ml dilution of pepper oleoresin. Colour values of the samples were determined objectively as per Page *et al.* (2001) [8] using Hunter Lab Mini Scan XE Plus Spectrophotometer (Hunter Lab, Virginia, USA) with diffuse illumination. Warner-Bratzler Shear Force (WBSF) of each sample was determined by the method outlined by Wheeler *et al.* (1997) [9] using Universal Testing Machine Shimadzu Texture Analyzer Model EZ-SX (Shimadzu Corporation, Kyoto, Japan).

2.3 Microbiological parameters

All the microbiological parameters were determined by following standard methods of American Public Health Association. Readymade media (Hi-Media and Sisco Research Laboratories, India) were used for all the microbiological examinations after serial dilution of the samples.

Aerobic plate count (APC) was evaluated as per the procedure of Morton (2001). Psychrotrophic count was expressed as per the procedure of Beuchat and Cousin (2001) [10].

2.4 Proximate principles

Samples were analysed for proximate principles like moisture on days 0, 3, 6, 9 and 12 and fat, protein and ash on day 0 as per AOAC (2012). The proximate principles were expressed as percentage of sample on wet matter basis. Carbohydrate and energy values of the samples were also calculated.

2.5 Sensory evaluation

The sensory evaluation of poultry meat was conducted by a semi trained panel consisting of faculty and post graduate students from the Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Pookode using a three point Hedonic scale score card for raw fillets and a nine point Hedonic scale score card for cooked steaks.

2.6 Statistical analysis

The data obtained were statistically analyzed by one-way ANOVA, repeated measures ANOVA, Kruskal-Wallis test, Wilcoxon signed rank test, Friedman test, Mann Whitney test using SPSS software (VERSION 21) as per Snedecor and Cochran (1994) [11].

3. Results and Discussion

There was a major ($p < 0.01$) difference in drip loss between the samples on all days except on day three and also across the storage days, with C having the highest loss. The values increased from day 6 onwards for C and T1 and from day nine only for T2. Significant ($p < 0.01$) variation in cooking loss between control and treatments were observed with C having significantly ($p < 0.01$) higher cooking loss in comparison to the treatments on all days. Across storage there was no significant difference in any one of the sample. James *et al.* (2018) [12] opined that coating of chicken breast fillets with sodium alginate incorporated with clove bud natural resin effectively decreased the cooking loss under chiller condition. There was no significant variation in pH of T2 on storage, however in C and T1 there was a significant ($p < 0.05$) increase on storage. Yingyuad (2006) [13] reported a increase in pH of control vacuum pre-packed grilled pork samples when compared to chitosan coated samples stored under chiller condition. In C and T1, water holding capacity increased on day 3 followed by a decrease up to day 12. In T2 the value increased on day 6 followed by a decrease up to day 12. Thiobarbituric acid reacting substances numbers of C were considerably ($p < 0.01$) higher on all days increased across storage, however, in T2 there was no significant variation on storage. Wu *et al.* (2000) [14] reported that carrageenan treated chiller stored beef patties had less TBA numbers when compared to gluten treated beef patties. Tyrosine values of C and T1 showed a significant ($p < 0.01$) increase on storage, whereas in T2 there was no change.

Colour values of *Longissimus dorsi* steaks were assessed and it was observed that C had higher L values in comparison to treatments. During storage L values reduced significantly ($p < 0.01$) from day 3 onwards. T2 had significantly ($p < 0.01$) lower 'a' values than C and T1 and except in T2 the values decreased on storage. Martinez (2006) [15] recorded a major decrease in lightness and yellowness values of chiller stored pork sausages containing 0.5% black pepper packed in 20%

CO₂ and 80% O₂ when compared to control sausages without pepper.

Shear force values for *Longissimus dorsi* steaks values were considerably ($p < 0.05$) reduced on day twelve in T1 compared to control and T2. Total phenolic values (μg tannic acid equivalents (TAE)/ml) were assessed to be 0.38 ± 0.02 , 15.51 ± 0.79 and 1.52 ± 0.18 for plain carrageenan film forming solution, for solution containing black pepper and pepper oleoresin, respectively. DPPH activity of film forming solutions as well as oleoresin (1 in 100 dilution) with a phenolic content of $50 \mu\text{g}$ tannic acid equivalents was assessed. DPPH activity was $0.98 \pm 0.02\%$, $37 \pm 0.18\%$ and $57 \pm 0.51\%$ for for plain carrageenan film forming solution, for solution containing black pepper and pepper oleoresin, respectively.

T2 had significantly ($p < 0.01$) lower aerobic plate counts when compared to C and T1 on day 9 and on day 12. There was a significant ($p < 0.05$) increase in the counts in C and T1 during storage and C showed a significant increase from day 3 onwards. However, in T2 the counts remained similar during storage.

There was significant ($p < 0.05$) variation in psychrotrophic counts of samples on all days of storage with C showing higher counts. There was significant ($p < 0.01$) increase in the counts on storage in all the samples and the increase occurred from day three onwards. Kapoor *et al.* (2014) [16] noted that on addition of black pepper oil there was a reduction in the total aerobic microbial population in orange juice stored under chiller (4 ± 1 °C) condition for twenty eight days.

On proximate evaluation, it was observed that moisture content considerably ($p < 0.05$) reduced for C compared to the T1 and T2. Wu *et al.* (2000) [14] observed that chiller stored carrageenan film wrapped beef patties had a less moisture loss compared to unwrapped beef patties. There was no significant difference in protein, ash, fat carbohydrate and energy values between samples.

Sensory evaluation was done up to day 6 for C and T1 and up to day 12 for T2. Appearance and overall acceptability scores of raw steaks did not vary on storage until the day of spoilage but raw odour scores of T2 considerably lowered on storage. Divakar *et al.* (2018) [17] observed that all the organoleptic characteristics like colour, odour and overall acceptability of raw buffalo *Longissimus dorsi* samples decreased on day ninth of chiller (4 ± 1 °C) storage.

For cooked steaks colour scores significantly ($p < 0.05$) reduced only for C. Across storage, samples did not show significant difference in flavour and juiciness till their respective days of spoilage. Significant ($p < 0.05$) difference in tenderness scores was observed only on days 0 and 3 with C showing lowest scores followed by T1 and then T2. There was no significant difference in the overall acceptability scores between samples and across storage, till the respective days of spoilage of each sample. James *et al.* (2018) [12] noted increased tenderness, juiciness and flavour scores in chicken breast fillets coated with sodium alginate incorporated with clove bud natural resin when compared to control when kept in chiller.

By sensory evaluation, spoilage of samples was assessed and it was observed that C and T1 had a shelf life of 6 days and T2 had a shelf life of 12 days. It might be because of reduced proteolysis and lipid peroxidation in T2 due to the anti-proteolytic and antioxidant properties of the oleoresin. Also,

comparatively reduced aerobic and psychrotrophic counts were observed in steaks wrapped with oleoresin incorporated film which showed the anti-microbial action of oleoresin. Carrageenan film alone in T1 did not increase the shelf life, however, reduced the drip and cooking losses of buffalo steaks. Houicher (2013) observed that 1% of mint on *Sardina pilchardus* fish fillets resulted in a longer chiller (3 ± 1 °C) shelf life when compared with control.

The cost of control *Longissimus* steaks was Rs 280 per kg and costs of production of T1 and T2 were Rs. 285.75 and Rs. 289 per kg, respectively.

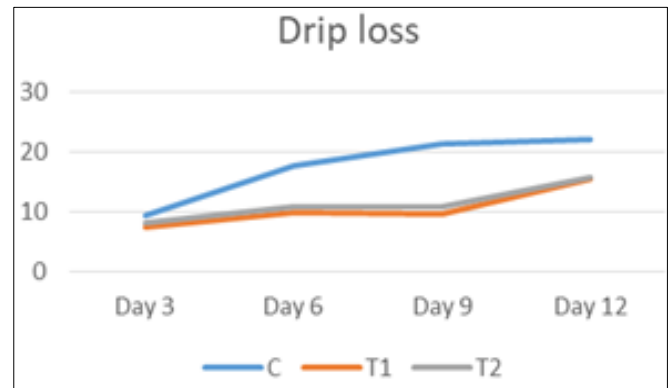


Fig 1: Drip loss values of control and treatment steaks at different storage intervals

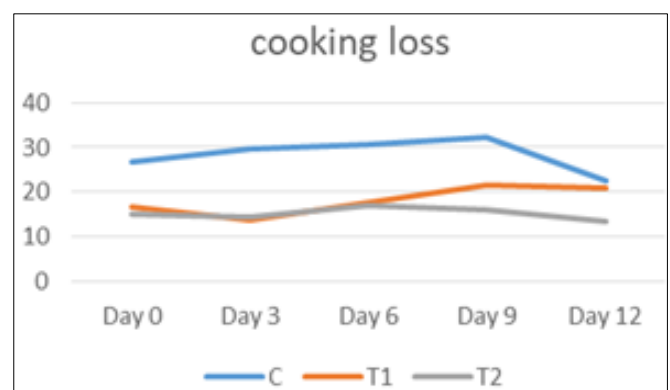


Fig 2: Cooking loss values of control and treatment steaks at different storage intervals

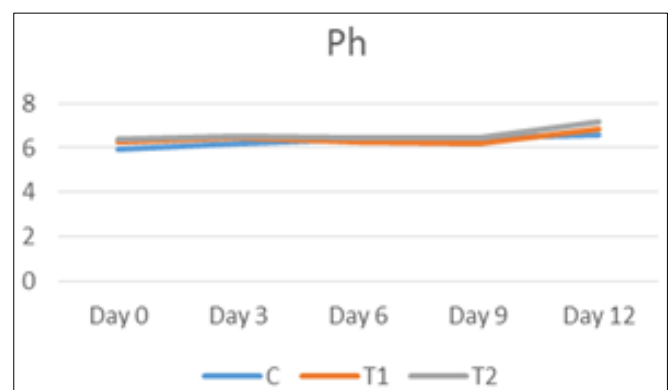


Fig 3: Ph values of control and treatment steaks at different storage intervals

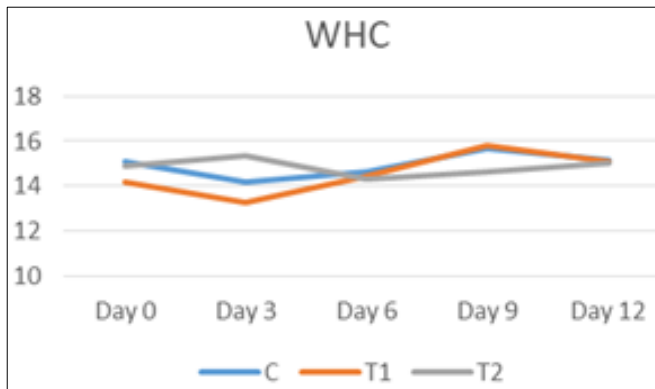


Fig 4: water holding capacity values of control and treatment steaks at different storage intervals

4. Conclusions

Steaks wrapped with carrageenan edible film incorporated with black pepper oleoresin had double the shelf life of unwrapped steaks and steaks wrapped with plain carrageenan solution. This shows that pepper oleoresin at 0.5% level in carrageenan edible film could be used to enhance the keeping quality of chiller stored buffalo meat without affecting the sensory attributes.

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