

*Original Research***Effect of Carrageenan Edible Film with Oleoresin of *Mentha piperita* (Mint) on Quality of Buffalo Meat Steaks****N. Manjunath^{1*}, Renuka Nayar², Shelcy S Akkara¹, Magna Thomas³, Kavitha Rajagopal² and C. Sunanda⁴**

¹MVSc Scholar, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Pookode, Kerala Veterinary and Animal Sciences University, Pookode, Wayanad- 673 576, Kerala, INDIA

²Assistant Professor, Department of Livestock Products Technology, College of Veterinary and Animal Sciences, Pookode, Wayanad- 673 576, Kerala, INDIA

³Assistant Professor, Department of Dairy Science, College of Veterinary and Animal Sciences, Pookode, Wayanad- 673 576, Kerala, INDIA

⁴Assistant Professor, Department of Statistics, College of Veterinary and Animal Sciences, Pookode, Wayanad-673 576, Kerala, INDIA

*Corresponding author: manjunathgowdakvc@gmail.com

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Abstract

A study was conducted to evaluate the effect of carrageenan edible film with *Mentha piperita* (mint) oleoresin on quality of buffalo longissimus dorsi steaks. C -Control steaks without edible film, T1- Steaks with carrageenan film, T2 -Steaks with carrageenan film incorporated with 1.5% mint oleoresin were the samples. The samples were aerobically packed and stored in refrigerator ($4\pm 1^{\circ}\text{C}$) and evaluated on days 0, 3, 6 and 9 and 12th day. Cooking and drip losses and pH were significantly ($p < 0.01$) higher for C. Water holding capacity and colour values significantly reduced for all on storage. TBARS numbers and tyrosine values were significantly lower and total phenolic content and DPPH activity were significantly higher for T2. C and T1 had a shelf life of 6 days and T2 had 9 days. Incorporation of 1.5% mint oleoresin in carrageenan edible film enhanced the shelf life of buffalo meat steaks with acceptable sensory attributes.

Key words: Buffalo Meat Steaks, Carrageenan Film, Mint Oleoresin, Refrigerated Storage, Shelf Life

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Introduction

Edible films are thin sheets processed from edible biopolymers such as protein, lipids and polysaccharides and often contain food grade supplements such as antibacterial and antioxidant agents (Gennadios *et al.*, 1997). Plasticizers and other supplements are generally incorporated along with biopolymers to alter the functional properties of films. These films enhance the quality and shelf life of food materials, preventing

them from microbial deterioration (Kester and Fennema, 1986), decreasing the clustering of food granular particles, enhancing the visual and palpable features on surfaces of the food product and improving the physical outlook (Cuq *et al.*, 1995). They also function as hurdles to gases such as oxygen and carbon dioxide, and they act as carriers of effective substances, such as antibacterial agents, antioxidants, Carrageenan is extracted from red seaweeds (Rhodophyta) and is used as a stabilizing, and gelling agent. Addition of natural antioxidants and antimicrobials into the biodegradable edible film may extend the shelf life of meat packed in them by delaying deterioration. Mint is a popular herb used widely in Indian cooking and also for healing several common food borne diseases (Choudhury *et al.*, 2006). Mint oleoresin is manufactured by solvent extraction from garden mint leaves and is considered as a more potent antioxidant than other spice extracts. The major effective volatile component is menthol and it has antioxidant and reducing activities (Zheng and Wang, 2001). A study was carried out to evaluate the effect of carrageenan edible film incorporated with garden mint on quality of aerobically packed buffalo *Longissimus dorsi* steaks stored under chiller (4 ± 1 °C) condition.

Materials and Methods

Longissimus dorsi muscles from buffalo carcasses aged 8-9 years were procured from Malabar meat, Brahmagiri Development Society, Sulthan bathery and brought to the department of Livestock Products Technology, College of Veterinary and Animal Sciences, Pookode under refrigerated conditions and cut into steaks.

Preparation of Edible Film

Edible film was prepared with refined carrageenan and glycerol as plasticizer, the levels of which were standardised after preliminary trials as, one per cent each. Refined Carrageenan (Marine hydrocolloids, Kochi, Kerala) was mixed with the filtered water and was heated to 90 °C with constant stirring till it was completely dissolved. After cooling to 70°C, glycerol was added, mixed and then cooled to ambient temperature and the solution was divided into two, one without oleoresin and the remaining incorporated with 1.5% *Mentha piperita* (mint) oleoresin (Plant lipids, Kolenchery, Kochi), the concentration of which was standardised after preliminary sensory evaluation. Carrageenan solutions were formed into films after pouring on glass trays and drying in an incubator at 45°C (45-50 % relative humidity) for 48 h. The formed films were peeled and stored under dry and hygienic conditions. *Longissimus dorsi* steaks were divided in to three groups and subjected to various treatments as: C- Control steaks without edible film, T1 - Steaks with edible film that does not contain oleoresin. T2- Steaks with edible film incorporated with oleoresin of 1.5% *Mentha piperita* (mint).

Control as well as treatment steaks were aerobically packed in high density polyethylene (HDPE) pouches (above 65µm) and sealed by continuous sealing machine (Sevanasepack- CS3H, Kochi) and kept in chiller

(4 ± 1^0 C) for storage. The samples were subjected to physico-chemical, microbiological and sensory evaluation on days 0, 3, 6, 9 and 12.

Physico-chemical Characteristics

Drip loss of the samples was calculated on days 3, 6 and 9 and it was the loss of weight of raw steaks on chiller storage. Cooking loss was calculated as per Boccard *et al.* (1981). 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). Thiobarbituric acid reactive substances (TBARS) number was determined as per Witte *et al.* (1970) with modifications. Tyrosine values of control and treatment samples were estimated as per the method described by Pearson (1968). The concentration of total phenolics of film forming solution as well as mint oleoresin was determined by the Folin-Ciocalteu (F-C) assay (Escarpa and Gonzalez, 2001) with slight modification. 2,2-diphenyl-1-picryl hydrazyl (DPPH) assay was done to evaluate the antioxidant activity of the film forming solution and oleoresin as per the modified method of Singh *et al.* (2002). Colour values of the samples were determined objectively as per Page *et al.* (2001) 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) using Universal Testing Machine Shimadzu Texture Analyzer Model EZ-SX (Shimadzu Corporation, Kyoto, Japan).

Microbiological Parameters

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).

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.

Sensory Attributes

The sensory evaluation of raw as well as cooked steaks was conducted by a semi trained panel using a three-point Hedonic scale score card for raw steaks and a nine-point Hedonic scale score card for cooked steaks.

Statistical Analysis

The data obtained were statistically analysed 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).

Results and Discussions

Drip loss of C was significantly ($p < 0.01$) higher than the film wrapped samples, T1 and T2 on all days except day 3. In contrast to this, Rodriguez-Turienzo *et al.* (2012) observed an increased drip loss in salmon fish coated with whey protein concentrate when compared with uncoated fish.

Table 1: Physico-chemical parameters of control and treatment steaks on different chiller storage days.

Storage period (4±1 °C)							
Parameters	Treatments	Day 0	Day 3	Day 6	Day 9	Day 12	F-value (p-value)
Drip loss %	C	-	9.35±0.167 ^C	17.51±0.441 ^{aB}	21.29±0.294 ^{aA}	22.10±0.447 ^{aA}	514.025 ^{**} (<0.001)
	T1	-	7.37±0.495 ^C	9.87±0.296 ^{bB}	9.48±1.724 ^{cBC}	15.44±1.042 ^{cA}	21.679 ^{**} (<0.001)
	T2	-	10.61±2.547 ^{BC}	9.18±0.73 ^{bC}	12.79±0.554 ^{bcB}	18.51±1.099 ^{bA}	7.624 ^{**} (0.008)
Cooking loss %	C	26.77±0.597 ^a	29.74±0.229 ^a	30.53±1.151 ^a	32.22±0.556 ^a	22.58±3.979	4.40 ^{ns} (0.121)
	T1	16.62±2.995 ^b	13.70±1.986 ^b	17.50±0.796 ^b	21.49±2.550 ^b	20.92±4.961	1.069 ^{ns} (0.393)
	T2	15.56±1.309 ^b	13.62±1.828 ^b	18.38±3.359 ^b	19.90±2.063 ^{bc}	20.35±2.691	1.888 ^{ns} (0.177)
pH	C	5.91±0.062 ^C	6.19±0.081 ^{BC}	6.38±0.051 ^{AB}	6.48±0.195 ^{AB}	6.61±0.076 ^{bcA}	6.657 ^{**} (<0.001)
	T1	6.27±0.160 ^{B^C}	6.48±0.050 ^{AB}	6.28±0.049 ^{BC}	6.19±0.041 ^C	6.87±0.133 ^{abA}	7.375 ^{**} (<0.001)
	T2	6.24±0.154	6.24±0.126	6.49±0.099	6.29±0.092	6.44±0.126 ^c	0.771 ^{ns} (0.227)
WHC	C	15.08±0.201 ^{aBC}	14.16±0.105 ^{bD}	14.66±0.105 ^C	15.66±0.105 ^{abA}	15.16±0.105 ^{aB}	19.407 ^{**} (<0.001)
	T1	14.16±0.105 ^{bC}	13.25±0.112 ^{cD}	14.41±0.154 ^C	15.83±0.167 ^{aA}	15.08±0.083 ^{aB}	49.745 ^{**} (<0.001)
	T2	15.25±0.112 ^{aA}	15.08±0.154 ^{aAB}	14.33±0.105 ^D	14.83±0.105 ^{cBC}	14.25±0.171 ^{bC_D}	12.179 ^{**} (<0.001)

**significant at 1 % level, *significant at 5% level, ns- not significant; Means with same lower case of alphabets as superscripts are not significantly different between treatments; Means with same upper case of alphabets as superscripts are not significantly different between storage period

During storage the drip loss significantly ($p < 0.01$) increased for all samples from day 6 onwards. Control had significantly ($p < 0.01$) greater cooking loss when compared with the treatments with edible film on all days. Noori *et al.* (2018) who observed significantly ($p < 0.05$) higher cooking loss for uncoated chicken

breast fillet and fillets coated with sodium caseinate alone when compared to fillet coated with sodium caseinate containing ginger essential oil when stored under chiller (4 °C) condition. No significant difference was noticed during the chiller storage period except in T2 which had a significantly higher cooking loss on day 9 than day 6. There was no significant difference in pH of the samples on any of the days of evaluation except on day 12, where T2 showed significantly ($p < 0.05$) lower values than C and T1. pH significantly ($p < 0.05$) increased for C and T1 on storage, however, it remained same for T2. Vital *et al.* (2016) also reported an increase in pH of *Longissimus dorsi* steaks stored in chiller condition.

On chiller storage there was a significant ($p < 0.01$) decrease in water holding capacity in all three samples. However, Chamanara *et al.* (2012) observed that there was an increase in water holding capacity of Rainbow trout treated with chitosan incorporated with one percent *Thymus vulgaris* essential oil when compared to control trout during the 15-day chiller storage period. Control samples showed significantly ($p < 0.01$) higher TBARS values than T1 and T2 on all days except on day 12. The lower values of treatment steaks might be due to the effect of carrageenan films and oleoresins incorporated that might have retarded lipid oxidation. The values increased for C and T2 on storage, but remained constant for T1. Chatli *et al.* (2014) observed significant ($p < 0.05$) increase in TBARS values across the storage in chiller stored goat meat chunks with chitosan edible films incorporated with cinnamaldehyde oil.

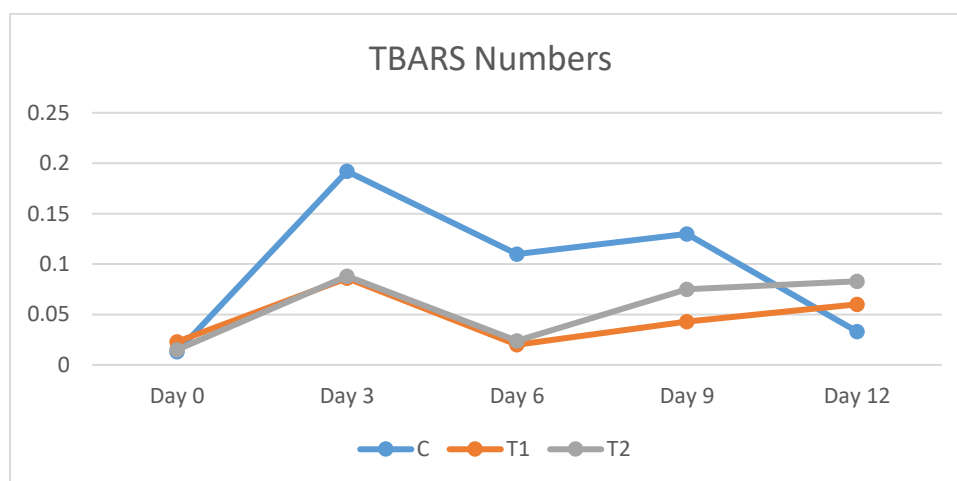


Fig. 1: TBARS numbers of control and treatment steaks at different storage period

Tyrosine values of C and T1 were significantly higher on all days of storage and lower values were observed for T2. Lower values in T2 might be due to the action of mint oleoresin which might have retarded proteolytic changes. Khare *et al.* (2016) observed that tyrosine values of control chicken breast fillets were higher than the chitosan coated samples when stored under chiller (4 ± 1 °C) condition. All samples showed a significant ($p < 0.01$) increase in the values on storage. Lijin *et al.* (2018) also observed an increase in tyrosine values of chiller (4 ± 1 °C) stored chicken breast fillets with storage, irrespective of sodium alginate coating or incorporation of clove bud oleoresin.

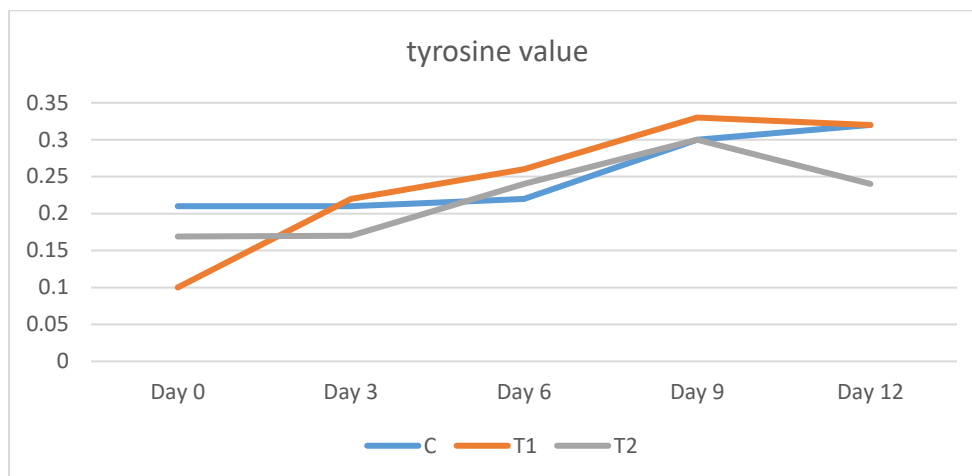


Fig. 2: Tyrosine values of control and treatment steaks at different storage intervals

Values for lightness of control samples were significantly ($p < 0.01$) higher than the treatments on almost all days. This might be due to the effects of carrageenan film and oleoresins which reduced the light reflection from the treatment steaks. Across storage there was significant ($p < 0.05$) decrease in L values in all samples. Contradictory results were reported by Vital *et al.* (2016) who observed increase in the lightness values across chiller storage in *Longissimus dorsi* steaks. Redness values were significantly ($p < 0.01$) lower in T2 when compared to C and T1 and might be due to the slightly greenish tinge of oleoresin containing films. Redness values significantly ($p < 0.01$) decreased across storage in all samples. On almost all days of storage C showed significantly ($p < 0.01$) lower b values and b value decreased across storage in all the samples. A decrease in yellowness values of frozen vacuum-packed beef on storage had also been reported by Rojas *et al.* (2008).

The shear force value was significantly ($p < 0.01$) higher for T2 on days 0 and 3. The values significantly ($p < 0.05$) lowered on day 12 in T1 and in C and T2, the values did not vary significantly across storage. Total phenolics of film forming solution was assessed and expressed as μg tannic acid equivalent (TAE) /ml and it was observed to be 0.38 ± 0.02 for plain carrageenan film forming solution and 17.85 ± 0.213 μg TAE /ml for mint incorporated film forming solution. The film forming solution containing mint oleoresin with a phenolic content of $50 \mu\text{g}$ tannic acid equivalents had a DPPH activity of $41 \pm 0.32\%$ whereas that of plain carrageenan solution was only $0.98 \pm 0.021\%$. Mint oleoresin (1 in 100 ml dilution, $50 \mu\text{g}$ of phenolics as tannic acid equivalents) showed a DPPH activity of $61 \pm 0.03\%$. The high phenolic content in mint oleoresin with high DPPH activity might have contributed to the significantly lower TBARS numbers and increased shelf life of T2.

Table 2: Hunter colour values of control and treatment steaks on different storage days

Storage period (4±1 °C)							
Hunter Colour values	Treatments	Day 0	Day 3	Day 6	Day 9	Day 12	F-value (p-value)
L	C	25.57±0.478 ^{ba}	22.52±0.406 ^{abc}	23.39±0.124 ^{ab}	26.33±0.383 ^{aA}	22.12±0.054 ^{aC}	46.123 ^{**} (<0.001)
	T1	28.67±0.360 ^{aA}	22.48±0.094 ^{aC}	21.70±0.616 ^{bDC}	24.34±0.173 ^{aB}	20.81±0.543 ^{bd}	57.893 ^{**} (<0.001)
	T2	26.11±0.246 ^{ba}	22.58±0.169 ^{aB}	22.315±0.108 ^{bb}	17.45±0.322 ^{bcD}	18.91±0.645 ^{dC}	116.28 ^{**} (<0.001)
a	C	13.35±0.588 ^{aBA}	10.43±0.093 ^{bcC}	13.81±0.280 ^{aA}	8.94±0.196 ^{cD}	11.78±0.283 ^{aB}	38.514 ^{**} (<0.001)
	T1	11.24±0.240 ^{bb}	12.43±0.131 ^{aA}	9.38±0.074 ^{cC}	12.40±1.050 ^{cBA}	8.46±0.0967 ^{bcD}	14.127 ^{**} (0.009)
	T2	9.42±0.367 ^{cC}	10.99±0.409 ^{bb}	10.50±0.106 ^{bb}	14.48±0.327 ^{aA}	8.07±0.124 ^{cD}	80.134 ^{**} (<0.001)
b	C	6.43±0.115 ^{bb}	6.88±0.420 ^{AB}	6.10±0.145 ^{dB}	7.72±0.154 ^A	5.37±0.112 ^{dC}	16.602 ^{**} (<0.001)
	T1	9.14±0.101 ^{aA}	7.74±0.290 ^C	7.47±0.204 ^{cC}	7.11±0.634 ^{BC}	8.42±0.192 ^{aB}	8.596 ^{**} (<0.001)
	T2	9.94±0.161 ^{aA}	7.40±0.184 ^{BC}	6.25±0.137 ^{dC}	7.09±0.218 ^C	7.84±0.032 ^{bb}	64.001 ^{**} (<0.001)

**significant at 1 % level, *significant at 5% level, ns- not significant; Means with same lower case of alphabets as superscripts are not significantly different between treatments; Means with same upper case of alphabets as superscripts are not significantly different between storage periods

T2 had significantly (p<0.01) lower aerobic plate counts when compared to C and T1 on days 9 and 12 and as the counts of C and T1 increased on storage, that of T2 remained similar. Seol *et al.* (2009) also recorded lower aerobic plate counts in chiller stored chicken breast wrapped with 2% carrageenan film incorporated with ovo-transferrin and EDTA when compared to the control samples wrapped with carrageenan (2%) alone. Psychrotrophic counts of C were significantly (p<0.05) higher than T1 and T2 on almost all days and the counts increased on storage in all samples.

Proximate Principles

Proximate principles like protein, fat, ash, energy and carbohydrate levels were similar for all samples. There was significant (p<0.05) difference in moisture between samples on all days except day 9 and T2 showed lowest values on all days of analysis.

Sensory Attributes

Sensory evaluation of control and treatments was done by semi trained panellists for various traits such as colour/appearance, odour and overall acceptability for raw meat and colour/appearance, juiciness, tenderness, flavour and overall acceptability for cooked meat. On 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 9 days under chiller condition, showing that mint added films resulted in 1.33 times more shelf life than C and T1. No significant difference in appearance, odour or overall acceptability scores was noticed for raw steaks neither in the control nor the treatments till spoilage changes became noticeable in the samples. For cooked steaks colour scores did not show any variation. But, Kristam *et al.* (2016) observed that chicken nuggets coated with edible polysaccharide film of sodium alginate had a higher colour score compared to uncoated cooked chicken nugget samples stored in chiller ($4 \pm 1^{\circ}\text{C}$) condition. Flavour scores did not vary significantly between samples except on day 0 where oleoresin added T2 scored higher. Across storage, samples did not show significant difference in flavour till spoilage changes became noticeable in the samples. There was no significant difference in scores of juiciness between samples on any storage day and across storage. On days 0 and 3, T2 scored higher for tenderness than C and T1. There was no significant difference in the overall acceptability scores between samples and across storage, till till spoilage changes became noticeable in the samples. Keokammerd *et al.* (2008) observed that cooked chicken treated with rosemary oleoresin recorded higher acceptability scores compared to chicken without rosemary oleoresin stored in chiller ($3 \pm 1^{\circ}\text{C}$) condition.

Conclusion

Thus, it can be concluded that incorporation of 1.5% *Mentha piperita* (mint) oleoresin in carrageenan edible film enhanced the shelf life of buffalo steaks from 6 days to 9 days. It might be because of reduced proteolysis and lipid peroxidation 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 shows the anti-microbial action of oleoresins. Carrageenan film alone did not increase the shelf life, however, reduced the drip and cooking losses of buffalo steaks.

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