



Oxidative stability and storage quality of *Mentha longifolia* L. extract fortified fish nuggets at refrigeration temperature

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Abstract: The present study was experimented to explore antioxidant potential of herb viz. *Mentha longifolia* L. in elongating the shelf life of emulsion based fish product. Since the fish meat product contain excessive amount of protein and fat which are vulnerable to spoilage due to proteolysis and lipolysis. Therefore fish nuggets incorporated with 0% (Control), 1%, 2%, and 3% of *M. longifolia* extract along with control was studied to explore the potential of *Artemisia nilagirica* L. on oxidative stability and storage of aerobically packaged fish nuggets at refrigeration temp on 0, 7, 14 and 21st day. Aqueous ethanolic extract of *M. longifolia* L. was prepared and incorporated in fish nuggets. Fish nuggets prepared with *M. longifolia* L. (1%) extract were recorded to be the best among all based on various sensory attributes with overall acceptability of 7.76±0.19. Extract of *M. longifolia* L. fortified fish nuggets were safe for human consumption till 21st day of refrigeration (4±1)°C storage under aerobically packaging on the basis of TBA value, FFA value, microbiological profile and sensory evaluation. The pH, TBA, FFA, total plate count, yeast and mould count were recorded to 6.49±0.008, 0.825±0.0012, 0.320±0.0005, 4.35±0.010, 1.26±0.013 and 2.20±0.008 respectively, and were found within acceptable range on 21st day of refrigeration storage. Thus, it can be interpreted that 1% aqueous ethanolic extracts of *M. longifolia* can be utilized in enhancing the shelf-life and improving the sensory scores of fish nuggets.

Keywords: Fish nuggets, *Mentha longifolia* L., Oxidative stability, Storage quality

INTRODUCTION

Most of the disease that we encounter today is lifestyle disease which also includes degenerative disease. The occurrence of these diseases is due to excessive formation of pro-oxidant (free radical and reactive oxygen species) metabolism in the body. To counter these pro-oxidant, the antioxidant are also secreted at cellular level but due to several reason like genetic factor, dietary habit, physiological status, work load and environment pollution etc. This is excessive production of pro-oxidant at cellular level which leads to oxidative damage of the cell resulting in various lifestyle diseases (diseases, cancer, cardiovascular disease, atherosclerosis, Alzheimer and Parkinson). Therefore in today's prospective natural antioxidants are essential dietary requirement thus development of value added meat product with incorporation of herbal extract as antioxidant of herbal extract as antioxidant help us in combating oxidative stress. (Tajkarimi *et al.*, 2010).

Lipid oxidation in meat is concern to the consumer because it causes physical and chemical changes of food quality and produces undesirable taste, texture, appearance and nutritional value. (Conde *et al.*, 2011). Consumer demand for safe, natural, wholesome and high quality food. Consumer prefer organic food which

forces food industry to include natural antioxidant in meat products to impart oxidative stability. (Camo *et al.*, 2008). The herbal extract contain various antioxidant, antimicrobial and anti-lipolytic character. (Wojodylo *et al.*, 2007).

M. longifolia belongs to family Lamiaceae, commonly called as wild mint is a fast growing, perennial herb, growing widely all over India. The species possesses anti microbial and anti oxidant properties (Daferera *et al.*, 2003; Gulluce *et al.*, 2007). The anti-oxidant and anti microbial properties of *M. longifolia* L. due to piperitenone oxide as active principle present in it. The other active principal present in it is mono terpenoids, menthone, menthol, limonine and hesperidine (Mounira *et al.*, 2009 and Ahmad *et al.*, 2013). The addition of herbal extract to fish products not only enhances shelf-life of the products but also adds function to it. Keeping in view of the above facts, the present study was conducted with the objective of evaluating *M. longifolia* L. extracts on oxidative stability and storage quality of fish nuggets.

MATERIALS AND METHODS

Raw material: Boneless fish meat of *Pangasius pangasius* fish belonging to Pangasidae family was

procured from local market of Jammu. *M. longifolia* were collected from nursery. It was further identified and authenticated by Department of Botany, Jammu University.

Preparation of *M. longifolia* L. extract: Fresh *M. longifolia* will be collected and botanically authenticated. The leaves were separated and dried at 40°C for 6 hours in hot air oven. The dried leaves were grounded into fine powder form. The powder was dissolved in 90% ethanol aqueous solution for four days under dark condition. The extract were filtered and evaporated to 10% dryness v/v using rotatory evaporator. The extract was kept in dark at refrigeration temperature until use. (Mounira, *et al.*, 2009)

Preparation of fish nuggets: Fish meat was cut and minced in a meat mincer (MOD-TC 23 R10 U.P. INOX, Marsango, Italy). Meat emulsion for fish nuggets was prepared in bowl chopper [MOD 25 2.8G 4.0, Marsango, Italy]. Crushed ice was added and blending continued for 1.5 minutes. Addition of refined vegetable oil, spice mixture, condiments and other ingredients and again mixed for 1.5 to 2 minutes to get the desired emulsion. The weighted quantity of the meat batter or emulsion was stuffed in rectangular stainless steel mould (17.5cm ×11.5cm ×5 cm) with parchment paper re-smearred with oil to avoid sticking. Mould was covered with lid and tied with thread. The mixture was subjected to steam cooking for 50±5 minutes in pressure cooker. The boxes were allowed to cool at room temperature after removal from pressure cooker. The brick shaped fish nugget so obtained were sliced and cut into pieces to get smaller nuggets.

The formulation in (%) was standardized, optimized and used for preparation of fish nuggets from fish meat - 68.6, added water- 9.1, vegetable oil- 8.9, condiment mixture-4.9, refined wheat flour - 4.1, spice mixture-1.9, table salt-1.6, monosodium glutamate- 0.4, sodium tripolyphosphate - 0.4, sodium nitrite -100 ppm. The fortification was done in the ratio of 1, 2, and 3% *M. longifolia* proportionately (wt./wt.) in control. These were stored in refrigerator (4±1°C) for evaluation of physico-chemical and sensory parameters 0th, 7th, 14th and 21st day. The nuggets were cooled and then stored in low density polyethylene pouches (200 gauge). These developed designer fish nuggets were stored in refrigerator at (4±1°C) for evaluation of physico-chemical and sensory parameters.

Analytical techniques:

pH: The pH of cooked nuggets was measured soon after its preparation by the method of Keller *et al.* (1974). The pH was recorded by immersing combined glass electrode of digital pH meter (Systronics Digital pH meter 802, Serial No. 603).

Proximate composition: Moisture, Crude protein, Crude fats, emulsion stability and cooking yield in both control and treated samples were done as per standard procedures. (AOAC, 2000).

Thio barbituric acid (TBA): It was determined using the method of Witte *et al.* (1970).

Free fatty acid (FFA): It was determined by method described by Koniecko (1979).

Microbiological profile: Total plate count, Psychrotrophic count, Coliform count and Yeast and Mould count in the sample were determined by method described by APHA (2015). Readymade media (Hi-Media) were used for the analysis.

Sensory evaluation: A semi-trained experienced sensory panelist consisting of scientists of the Faculty of Veterinary Sciences and Animal Husbandry evaluated the sensory attributes viz: appearance, flavour, juiciness, texture and the overall acceptability of fresh and stored samples using 8 point descriptive scale (Keeton *et al.*, 1983).

Statistical analysis: The result will be analyzed statistically for analysis of variance and least significant difference tests as per Snedecor and Cochran (1989). In significant effects, least significant differences were calculated at appropriate level of significance for a pair wise comparison of treatment means.

Experimental design: The ethanolic-aqueous *M. longifolia* extract was prepared. The extracts of *M. longifolia* was added in standardized formulation of chicken sausages substituting proportionately (wt./wt.) at the level of 1%, 2% and 3%. The products were evaluated based on physico-chemical, sensory and microbiological profile on 0th, 7th, 14th and 21st day during refrigeration storage at (4±1°C).

RESULTS AND DISCUSSION

Physico-chemical parameters: Table 1 depicted the effect of *M. longifolia* extract on physico-chemical properties of fish nuggets. The incorporation of *M. longifolia* extract in fish nuggets significantly ($p < 0.05$) lowered pH value of fish nuggets. It may be attributed to ethanol, menthol and methanone content of *M. longifolia* extract. This finding was similar with finding of (Al-Ankari *et al.*, 2004), who studied use of *M. longifolia* in broiler chicken diet and reported acidic pH (~5.9) of broiler chicken meat. It was further supported by (Gulluce *et al.*, 2007), who also reported acidic nature (pH~6.0) of *M. longifolia* extract. Other physico chemical and proximate parameters viz. moisture, protein, fat, emulsion stability was all comparable to control. Similarly, Gulluce, *et al.* (2007) reported no alteration in meat emulsion and product characteristic on inclusion of essential oil and menthol extract from *M. longifolia*.

Table 2 depicted the effect of *M. longifolia* extract on sensory attributes of fish nuggets. All the sensory scores viz. colour, appearance, flavour, juiciness, texture and over all acceptability of 1% *M. longifolia* extract incorporated fish nuggets was significantly ($p < 0.05$) higher than other types of fish nuggets. The different herbal extract including *M. longifolia* has

Table 1. Effect of *Mentha longifolia* on physico-chemical properties of fish nuggets (Mean \pm SE)*

Parameters	0%	1%	2%	3%
pH	6.48 \pm 0.31 ^c	6.37 \pm 0.14 ^b	6.34 \pm 0.38 ^b	6.24 \pm 0.23 ^a
Moisture	58.96 \pm 1.27	58.16 \pm 1.40	58.06 \pm 1.43	57.66 \pm 0.24
Protein	16.33 \pm 0.50	16.35 \pm 0.36	16.26 \pm 0.31	16.16 \pm 0.31
Fat	10.93 \pm 0.46	10.51 \pm 0.47	10.71 \pm 0.38	10.46 \pm 0.57
Emulsion stability	90.90 \pm 1.40	91.15 \pm 1.44	91.71 \pm 1.31	91.91 \pm 1.24
Cooking yield	87.86 \pm 1.19	88.65 \pm 1.34	89.95 \pm 1.21	89.99 \pm 1.25

*Mean \pm SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05).n=6 for each treatment.(n=6)

Table 2. Effect of *Mentha longifolia* on sensory attributes of fish nuggets. (Mean \pm SE)*

Attributes	0%	1%	2%	3%
Colour and Appearance	6.18 \pm 0.17 ^b	6.50 \pm 0.12 ^c	7.43 \pm 0.13 ^d	4.01 \pm 0.12 ^a
Flavour	6.28 \pm 0.17 ^b	6.65 \pm 0.18 ^c	7.12 \pm 0.11 ^d	4.42 \pm 0.13 ^a
Juiciness	6.22 \pm 0.18 ^b	6.42 \pm 0.16 ^c	7.34 \pm 0.13 ^d	4.23 \pm 0.11 ^a
Texture	7.38 \pm 0.14 ^c	7.43 \pm 0.16 ^d	6.87 \pm 0.11 ^b	4.39 \pm 0.17 ^a
Overall Acceptability	7.26 \pm 0.13 ^b	7.41 \pm 0.11 ^c	7.76 \pm 0.19 ^d	4.52 \pm 0.19 ^a

*Mean \pm SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05).n=21 for each treatment.

positive effect on color, appearance and flavor of meat produce which was supported Mancini and Hunt (2005), who extensively done research on various aspect of changes in color and meat product. Our finding was strongly supported by Cornforth and Jay singh (2004). The control fish nuggets have been quickly spoiled on all parameter of sensory attributes as compare to *M. longifolia* extract treated fish nuggets. The treated fish nuggets were found suitable for human consumption even on 21st day of refrigeration storage on the basis of sensory attributes evaluation. The herbal extract had positive effect by inhibiting discoloration and off odour formation in different meat product under vacuum packaging during refrigeration storage (Nerin *et al.*, 2006 and Camo, 2008). Natural antioxidant can positively affect color and appearance parameter and maintain original color of product for longer period. (Carpenter, 2007 and Simitzis *et al.*, 2008). The herbal extent can also act as very good flavor and binding agent. The sensory character can also be enhanced with herbal extract incorporation in various meat products. (Chaves *et al.*, 2008).

Table 3 showed storage quality of *M. longifolia* treated fish nugget in terms of physico-chemical properties during refrigeration storage. The TBA value in case of control fish nuggets was recorded to be significantly (p<0.05) greater than treated fish nuggets on all days of refrigeration storage. However, the TBA value in *M.*

longifolia extract treated fish nuggets were found to be suitable for human consumption even on 21st day of refrigeration storage, as the value of TBA were found to be less than 1. This may be attributed to anti-lipolytic effect and anti-oxidant effect because of mono terpenoids, menthnone, menthol, limonine and hesperidine as active principal present in *M. longifolia* extract (Mounira, 2009 and Ahmad *et al.*, 2003).

The pH value of all types of fish nuggets were reported significantly (p<0.05) higher value during refrigeration period. However the pH value of ground *M. longifolia* extract treated fish nuggets recorded comparatively lesser inclination in pH value as compared to control fish nuggets. The acidic nature was attributed to methanol, ethanol, menthanone and piperatone oxide content of *M. longifolia* extract. The less inclination of pH was reported with incorporation of essential oil of poppy seed in Turkish Sucuk (Gok *et al.*, 2011).

The FFA contents of all types of fish nuggets was significantly (p<0.05) higher during their refrigeration storage. However the rate of inclination of FFA values was significantly (p<0.05) lower in *M. longifolia* extract incorporated fish nuggets as compared to control fish nuggets. Our present finding was also supported by (Djenane *et al.*, 2002), who concluded that topical application of various herbal extract had positive effect on oxidative stability of beef steak

Table 3. Storage quality of *Mentha longifolia* L. treated fish nugget in terms of physico-chemical properties during refrigeration storage (4±1°C). (Mean ±SE)*

Treatments	Storage days			
	0 th day	7 th day	14 th day	21 st day
TBA(mg malonaldehyde/kg)				
Control	0.302±0.0134 ^{Ad}	0.429±0.0005 ^{Ac}	1.051±0.0011 ^{Ab}	1.305±0.0005 ^{Aa}
<i>M. longifolia</i> (1%)	0.298±0.0182 ^{ABd}	0.394±0.0009 ^{Bc}	0.674±0.0006 ^{Bb}	0.825±0.0012 ^{Ba}
<i>M. longifolia</i> (2%)	0.293±0.0280 ^{BCd}	0.362±0.0005 ^{Cc}	0.625±0.0007 ^{Cb}	0.781±0.0005 ^{Ca}
<i>M. longifolia</i> (3%)	0.289±0.0145 ^{Ca}	0.325±0.0005 ^{Dc}	0.561±0.0007 ^{Db}	0.761±0.0005 ^{Dd}
pH				
Control	5.88±0.007 ^{Ad}	5.97±0.009 ^{Ac}	6.26±0.011 ^{Ab}	6.73±0.005 ^{Aa}
<i>M. longifolia</i> (1%)	5.85±0.166 ^{ABd}	5.87±0.009 ^{Bc}	6.11±0.011 ^{Bb}	6.49±0.008 ^{Ba}
<i>M. longifolia</i> (2%)	5.82±0.133 ^{BCd}	5.73±0.010 ^{Cc}	6.02±0.007 ^{Cb}	6.36±0.008 ^{Ca}
<i>M. longifolia</i> (3%)	5.79±0.170 ^{Cd}	5.49±0.009 ^{Dc}	5.59±0.005 ^{Db}	6.30±0.008 ^{Da}
FFA				
Control	0.093±0.0005 ^{Ad}	0.135±0.0008 ^{Ac}	0.225±0.0005 ^{Ab}	0.362±0.0006 ^{Aa}
<i>M. longifolia</i> (1%)	0.091±0.0063 ^{Ad}	0.115±0.0009 ^{Bc}	0.205±0.0009 ^{Bb}	0.320±0.0005 ^{Ba}
<i>M. longifolia</i> (2%)	0.085±0.0140 ^{Bd}	0.095±0.0008 ^{Cc}	0.180±0.0008 ^{Cb}	0.234±0.0009 ^{Ca}
<i>M. longifolia</i> (3%)	0.080±0.0217 ^{Cd}	0.076±0.0008 ^{Cc}	0.131±0.0007 ^{Cb}	0.215±0.0047 ^{Ca}

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05).n=6 for each treatment.(n=6)

Table 4. Changes in microbiological profile of *Mentha longifolia* L. extract treated fish nugget at refrigeration temperature (4±1°C). (Mean ±SE)*

Treatments	0 th day	7 th day	14 th day	21 st day
Total Plate Count (log₁₀cfu/g)				
Control	2.70±0.0076 ^{Ad}	3.74±0.0088 ^{Ac}	4.15±0.0114 ^{Ab}	5.10±0.0076 ^{Aa}
<i>M. longifolia</i> (1%)	2.35±0.0073 ^{Bd}	3.29±0.0111 ^{Bc}	3.85±0.0076 ^{Bb}	4.35±0.0096 ^{Ba}
<i>M. longifolia</i> (2%)	1.80±0.0066 ^{Cd}	3.10±0.0076 ^{Cc}	3.50±0.0101 ^{Cb}	3.72±0.0076 ^{Ca}
<i>M. longifolia</i> (3%)	1.79±0.0057 ^{Dd}	2.88±0.0080 ^{Dc}	3.15±0.0060 ^{Db}	3.49±0.0060 ^{Da}
Psychrotrophic Count (log₁₀cfu/g)				
Control	ND	ND	1.59±0.011 ^{Ab}	2.74±0.0152 ^{Aa}
<i>M. longifolia</i> (1%)	ND	ND	ND	1.26±0.0125 ^{Ba}
<i>M. longifolia</i> (2%)	ND	ND	ND	0.84±0.0116 ^{Ca}
<i>M. longifolia</i> (3%)	ND	ND	ND	0.21±0.0116 ^{Da}
Coliform Count (log₁₀cfu/g)				
Control	ND	ND	ND	ND
<i>M. longifolia</i> (1%)	ND	ND	ND	ND
<i>M. longifolia</i> (2%)	ND	ND	ND	ND
<i>M. longifolia</i> (3%)	ND	ND	ND	ND
Yeast and Mold Count (log₁₀cfu/g)				
Control	ND	ND	2.73±0.0101 ^{Ab}	3.60±0.0110 ^{Aa}
<i>M. longifolia</i> (1%)	ND	ND	ND	2.20±0.0076 ^{Ba}
<i>M. longifolia</i> (2%)	ND	ND	ND	1.79±0.0107 ^{Ca}
<i>M. longifolia</i> (3%)	ND	ND	ND	1.34±0.0125 ^{Da}

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05).n=6 for each treatment.

Table 5. Changes in the sensory attributes of aerobically packaged *Mentha longifolia* L. extract treated fish nugget during refrigerated storage at (4±1°C). (Mean ±SE)*

Treatments	Storage Period (Days)			
	0 th day	7 th day	14 th day	21 st day
Colour and appearance				
Control	6.59±0.100 ^{Ca}	6.07±0.109 ^{Cb}	5.59±0.097 ^{Dc}	4.95±0.141 ^{Ad}
<i>M. longifolia</i> (1%)	7.08±0.096 ^{Ba}	6.42±0.096 ^{Bb}	6.04±0.090 ^{Bc}	5.40±0.121 ^{Bd}
<i>M. longifolia</i> (2%)	7.47±0.118 ^{Aa}	6.85±0.093 ^{Ab}	6.42±0.096 ^{Ac}	5.97±0.090 ^{Ad}
<i>M. longifolia</i> (3%)	6.39±0.086 ^{Da}	5.88±0.116 ^{Db}	5.38±0.102 ^{Cc}	4.54±0.127 ^{Dd}
Flavour				
Control	6.53±0.094 ^{Ba}	5.93±0.130 ^{Bb}	5.58±0.074 ^{Bc}	3.96±0.108 ^{Bd}
<i>M. longifolia</i> (1%)	6.74±0.131 ^{Ba}	6.12±0.119 ^{Bb}	5.77±0.112 ^{Bc}	4.09±0.105 ^{Bd}
<i>M. longifolia</i> (2%)	6.77±0.081 ^{Ba}	6.22±0.073 ^{Bb}	5.89±0.113 ^{Bc}	4.16±0.070 ^{Bd}
<i>M. longifolia</i> (3%)	7.15±0.098 ^{Aa}	6.74±0.061 ^{Ab}	6.35±0.061 ^{Ac}	4.85±0.076 ^{Ad}
Texture				
Control	7.41±0.088 ^{Aa}	6.82±0.105 ^{Ab}	5.57±0.086 ^{Ac}	4.62±0.112 ^{Ad}
<i>M. longifolia</i> (1%)	7.43±0.110 ^{Aa}	6.87±0.137 ^{Ab}	5.64±0.092 ^{Ac}	4.65±0.140 ^{Ad}
<i>M. longifolia</i> (2%)	7.51±0.912 ^{Aa}	6.96±0.126 ^{Ab}	5.71±0.127 ^{Ac}	4.77±0.146 ^{Ac}
<i>M. longifolia</i> (3%)	7.35±0.076 ^{Aa}	6.75±0.101 ^{Ab}	5.52±0.087 ^{Ac}	4.60±0.052 ^{Ac}
Juiciness				
Control	6.80±0.100 ^{Ca}	6.33±0.105 ^{Cb}	5.59±0.127 ^{Cc}	4.88±0.090 ^{Cd}
<i>M. longifolia</i> (1%)	7.04±0.083 ^{BCa}	6.54±0.108 ^{BCb}	5.85±0.115 ^{BCc}	5.11±0.074 ^{BCd}
<i>M. longifolia</i> (2%)	7.16±0.110 ^{Ba}	6.66±0.099 ^{Bb}	5.97±0.094 ^{Bc}	5.21±0.100 ^{Bd}
<i>M. longifolia</i> (3%)	7.47±0.080 ^{Aa}	6.97±0.094 ^{Ab}	6.40±0.101 ^{Ac}	5.52±0.050 ^{Ad}
Overall acceptability				
Control	6.75±0.094 ^{Ca}	6.21±0.083 ^{Cb}	5.41±0.134 ^{Cc}	3.96±0.116 ^{Cd}
<i>M. longifolia</i> (1%)	6.87±0.096 ^{Ca}	6.33±0.089 ^{Cb}	5.55±0.123 ^{Cc}	4.17±0.062 ^{Cd}
<i>M. longifolia</i> (2%)	7.16±0.076 ^{Ba}	6.73±0.107 ^{Bb}	5.94±0.096 ^{Bc}	4.58±0.115 ^{Bd}
<i>M. longifolia</i> (3%)	7.44±0.096 ^{Aa}	7.16±0.081 ^{Ab}	6.34±0.083 ^{Ac}	5.01±0.091 ^{Ad}

*Mean± SE with different superscripts in a row wise (lower case alphabet) and column wise (upper case alphabet) differ significantly (P<0.05). Mean values are scores on 8 point descriptive scale where 1- extremely poor and 8- extremely desirable. n = 21 for each treatment.

packaged in vacuum packaging. The present finding is also in agreement with Simitzis, *et al.* (2008), who reported dietary natural antioxidant obtained from different plant has positive effect on oxidative stability by producing lesser amount of free fatty acid under vacuum packaging during refrigeration storage.

Table 4 depicted the changes in microbiological profile of aerobically packaged *M. longifolia* extract incorporated fish nuggets during refrigeration storage. The total plate count in *M. longifolia* extract incorporated in fish nuggets were recorded significantly (p<0.05) lower in comparison to controlled fish nuggets. It clearly indicated that *M. longifolia* extract was having antibacterial effect. The extract and essential oil of *M.*

longifolia showed strong antimicrobial activity against 30 aerobic microorganisms (Gulluce, *et al.*, 2007). The extract might act upon microbes in various mechanisms. It might disrupt enzyme system; disrupt genetic material of bacteria attacking on phospholipid bilayer of cellular membrane (Arques *et al.*, 2008; Burt *et al.*, 2007). The present finding was also supported by Ceylan and Fung (2004) antimicrobial activity of herbal extract responsible of significant decline in microbial load of extracted treated meat product. The herbal extract had antimicrobial activity and thus when incorporated in meat product could elongate its shelf life under vacuum packaging or aerobic packaging during refrigeration storage. The antioxidant and

antimicrobial nature of extract was due to piperitenone oxide, terpenoids, menthone, menthol, limonine and hesperidine as active principle present in it (Mounira, 2009 and Ahmad *et al.*, 2013).

The psychrophilic count were found to be significantly ($p < 0.05$) lower in comparison in treated fish nuggets as compared to controlled fish nuggets. However, psychrophiles were not detected till 7th day of refrigeration storage. These findings will be attributed to anti-psychrophilic potential of *M. longifolia* extract. The psychrophiles did not appear till 7th day of refrigeration storage due to more than 7th day of incubation period of most of psychrophilic bacteria. The coliforms were not detected on any day of storage period in any of the fish nuggets. It may be due to strict hygienic condition and correct method followed during preparing and processing of various types of fish nuggets. The active principle interacted with phospholipid bilayer of microbial cell wall and cell membrane and finally disrupted it. It defunct electron transport system, ion gradient and other enzyme dependent cellular mechanism of psychrotropic bacteria (Burt, 2007). The present result was also supported by finding of (Rota *et al.*, 2008), who suggested psychotropic antimicrobial effect of herbal extract during vacuum packaged and refrigeration storage. The yeast and mould count were not detected till 7th day which may be attributed to longer incubation period of most yeast and mould. The yeast and mould were recorded significantly ($p < 0.05$) lower in treated fish nuggets as compared to control. It may be attributed to antifungal effect of *M. longifolia* extract in fish nuggets. More over these extract possess natural fungicidal effects against food born fungi (Fisher and Phillip, 2008; Daferera *et al.*, 2000).

Table 5 presented the changes in sensory attributes of *M. longifolia* extract fortified aerobically packed fish nuggets during refrigeration storage. All the sensory scores irrespective of control or treated fish nuggets were recorded significantly lower values during all days of refrigeration storage however 1% incorporation of *M. longifolia* extracts incorporated fish nuggets were recorded highest sensory values. The rate of decline of sensory values in treated product shows significantly ($p < 0.05$) lower as compared to control fish nuggets. This clearly exhibited antimicrobial, anti-lipolytic, anti-mycotic and antioxidant property of *M. longifolia* Fortification of herbal extracts including *M. longifolia* in meat product was recorded higher colour, texture, juiciness, and flavour scores (Kim, 2013; Pinero *et al.*, 2008). The herbal extent could also act as very good flavor and binding agent. The sensory character could also be enhanced with herbal extract incorporation in various meat products. (Cornforth and Jay, 2004 and Chaves *et al.*, 2008)

Conclusion

The leaves extract of (1%) *M. longifolia* - was used in preparation of aerobically packaged value added fish nuggets. The developed product exhibited antimicrobial (3.49 ± 0.006), anti-mycotic (1.34 ± 0.01), anti-lipolytic (0.215 ± 0.004) and antioxidant activity. 1% *M. longifolia* extracts treated value added fish nuggets has enhanced sensory scores (5.01 ± 0.091) as well as extended shelf life of 21 days at refrigeration temperature under aerobic packaging. The result revealed the possible application of (1%) *M. longifolia* extract as a natural source of antioxidant in development of value added fish nuggets with potential health benefits.

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