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Effect of *Bacopa monnieri* Extract on Storage and Microbial Quality of Vacuum Packaged Chicken Nuggets

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The present study was done to assess the antioxidant potential of a herb viz. *Bacopa monnieri* L. in enhancing the shelf-life as well as adding function to chicken nuggets. Meat products are very vulnerable to spoilage due to excessive fats and protein content. Therefore, chicken nuggets fortified with 1, 2, and 3% *Bacopa monnieri* L. extracts and along with control were studied to explore the potency of *Bacopa monnieri* L. on oxidative stability and storage quality of vacuum packaged chicken nuggets on 0,15,30 and 45 days at refrigerated storage (4±1°C). Extracts of *Bacopa monnieri* L. were prepared, standardized, optimized and fortified in chicken nuggets. Chicken nuggets prepared with 2% of *Bacoppa monnieri* L. incorporation were adjudged to the best among all based on sensory attributes. Extracts of *Bacopa monnieri* fortified were safe for consumption till 45 days of refrigerated storage (4±1°C) under vacuum packaging on the basis of pH, FFA, TBARS, and microbiological profile, sensory evaluation of chicken nuggets.The total plate count, psychrotropic count, coliform count, yeast and mold counts were within the acceptable limit in vacuum packaged *Bacopa monnieri* L. fortified chicken nuggets till 45 days at refrigeration temperature(4±1°C).

Keywords: *Bacopa monnieri,* extract, storage quality, chicken nuggets.

Today, consumers demand for organic, safe, natural and high quality foods. The preference of consumer towards natural food compels the food industry to work upon and include natural antioxidant in meat products to impart oxidative stability (Camo *et al*., 2007). The herbal extract can act as a potent natural antioxidant which can be used in different meat products. This antioxidant has potency to act against lipolysis and proteolysis thereby imparting protein and lipid stability. The addition of these alcoholic aqueous extract of herbs not only improves the sensory characteristic but also enhance shelf-life of the meat products (Wojdylo *et al*., 2007).

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 (Alam *et al*., 2011).. This is excessive production of pro-oxidant at cellular level during metabolism 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. Therefore, development of value added meat product with incorporation of herbal extract as antioxidant that help us in combating oxidative stress (Tajkarimi *et al*., 2010).

The Indian herb *Bacopa monnieri* L. may serve as dietary antioxidant with various mode of

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action viz. anti lipolytic agent, anti-microbial agent. Therefore the extract of *Bacopa monnieri* L. can elongate the shelf-life of meat product when incorporated at standardized and optimized level in emulsion based meat product. The extract of *Bacopa monnieri* L. also acts as antioxidant when consumed by humans and protect them from oxidative damaged and age old cognitive decline (Simpson *et al*., 2015). The aim of present study is to develop *Bacopa monnieri* L. extract fortified value added meat product and further the product is evaluated for it various quality parameters during refrigeration storage under vacuum packaging.

MATERIALS AND METHODS

Preparation of *Bacopa monnieri* L. extract

Fresh *Bacopa monnieri* L. plant will be collected and botanically authenticated. Then it will be grounded and defatted with petroleum ether (1:10 w/v; 60-80°C) and filtered. The residue will be then soxhlet extracted with 80% ethanol (1:10 w/v) for 12 hours. The extract will be evaporated to dryness (Yield: 5.9% w/w) and dissolved in dimethyl sulphoxide (DMSO) (Venu *et al*., 2003).

Preparation of Chicken nuggets

Lean meat from spent hen was cut and minced in a Sirman mincer (MOD-TC 23 R10 U.P. INOX, Marsango, Italy). The common salt, vegetable oil, refined wheat flour (maida), nitrite, SHMP, spice mixture and condiment mixture were added as per formulation. Meat emulsion for chicken nuggets was prepared in Sirman 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. Chicken nuggets were molded in rectangular stainless steel boxes. The steel boxes of 21 cm length and 11 cm breadth were used for molding and steaming. The weighed quantity of the batter or emulsion was stuffed in mould with parchment paper and pre-smeared with refined soybean oil to avoid sticking. Mold was covered with lid and tied properly. The mixture was subjected to steam cooking for 30±2 minutes in pressure cooker. The boxes were allowed to cool at room temperature after removal from pressure cooker. The brick shaped chicken nugget so obtained were sliced and cut into pieces to get smaller nuggets. The formulation in (%) was standardized, optimized and used for preparation of chicken nuggets from spent hen meat was lean 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, 1, 2, and 3% *Bacopa monnieri* L. extracts added with replacement in control chicken nuggets (wt./wt.). The nuggets were cooled and packaged under vacuum packaging using single chamber vacuum packaging machine (Model NoDZ-500/2ES, 20m^3/h). These were stored in refrigerator (4±1°C) for evaluation of physico-chemical and sensory parameters 0th, 15th, 30th and 45th day.

Analytical Techniques

pH

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

Moisture Content

10 gm of mashed sample was transferred in pre-weight flat bottom aluminum moisture cup, which was transferred to hot air oven at 101±1p C and keep for 16-18 hrs. Dried sample was then placed in desiccators having silica gel as desiccant. After 1 hr, the cup containing dried sample was weighed. Moisture content was calculated by applying the following formula: (AOAC, 1995)

$$\text{Moisture (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

where, W_1 = Weight of empty cup, W_2= weight of cup + sample, W_3= Weight of cup+ dried sample.

Thio Barbituric Acid (TBA)

It was determined using the method of Witte *et al.* (1970). 10 g of chicken nuggets sample was blended finely with 50 ml of 20% TCA in a waring blender/ homogenizer for 2 minutes. The pestle & mortar could also have been used for homogenization of sample and was allowed to stand for 10 minutes. The extract was filtered through Whatman filter paper No.42 and in a test tube. 3 ml of this extract was mixed with equal volume of 0.1% (w/v) TBA reagent and blank

sample was prepared by mixing 3 ml 20% TCA with equal volume of 0.1% TBA reagent. The content of each test tube was thoroughly mixed and boiled for 35 minutes and further were allowed to cool down. The absorbance was measured at 532 nm by a spectrophotometer. TBA value was calculated by comparing the absorbance of test sample with a standard graph prepared by using known concentrations of malonaldehyde. For preparing standard graph, 0.3055 gm of 1,1,3,3 Tetraethoxy Propane (TEP) was dissolved in 100 ml of 95% absolute alcohol to obtain 1mg malonaldehyde/ml and was used as stock solution. To prepare working standard solution of TEP, 0.3 ml of stock solution was diluted to a volume of 100 ml by distilled water. The diluted solution contained 3 ìg/ml of malonaldehyde and from that solution a standard graph was prepared by using different concentration of malonaldehyde.

Free Fatty Acid (FFA)

For the determination of free fatty acids, the method described by Koniecko (1979) was followed. Exactly 10 gram sample blended for two minute with 50 ml of chloroform in presence of about 10g of anhydrous sodium sulphate. The mix was filtered through Whatman No.1 filter paper into a 300ml conical flask. About 2 to 3 drop of 0.2% phenolphthalein indicator were added to the chloroform extract, which was titrated against 0.1N alcoholic potassium hydroxide was recorded. Percent free fatty acid was calculated as

$$\text{FFA (\% Oleic acid)} = \frac{(0.1 \times \text{ml } 0.1\text{N alcoholic KOH} \times 0.282 \times 100)}{\text{Wt. of sample}}$$

Microbiological Profile

Total plate count, Psychrotrophic count, Coliform count and Yeast and Mould count in the sample were determined by method described by APHA (1984). Readymade media (Hi-Media) were used for the analysis.

Sensory Evaluation

The sensory evaluation of fresh and stored samples was carried for various attributes viz.color and appearance, flavor, juiciness, texture and overall acceptability by a panel of trained members composed of scientists based on a eight-point Hedonic scale, wherein 8 denoted “extremely desirable” and 1 denoted “extremely undesirable” (Keeton, *et al*., 1983). Seven members of the panel replicated the experiment thrice ($n = 21$). Panelists were seated in a room free of noise and odors and suitably illuminated. Coded samples for sensory evaluation were prepared.

Statistical Analysis

The result will be analyzed statistically for analysis of variance and least significant difference tests as per Snedecor and Cochran (1997). 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 *Bacopa monnieri* L.extract was prepared. The extracts of *Bacopa monnieri* L.was added in standardized formulation of chicken nuggets substituting proportionately (wt./wt.) at the level of 1%, 2% and 3%. The products were vacuum packaged and evaluated based on physico-chemical, sensory and microbiological profile on 0^{th}, 15^{th}, 30^{th} and 45^{th} day kept under refrigeration storage at (4±1°C).

RESULTS AND DISCUSSION

Physico-chemical parameters

The changes in physico-chemical profile of *Bacopa monnieri* L. extract fortified vacuum packaged chicken nuggets at refrigeration temperature (4 ± 1^0C) presented in Table 1.

pH

The pH of *Bacopa monnieri* L.extract fortified vacuum packaged chicken nuggets was recorded to be significantly ($p<0.05$) lower as compared to vacuum packaged control. The pH was increased significantly on successive storage days irrespective of levels of fortification of *Bacopa monnieri* L.extract in vacuum packaged chicken nuggets including vacuum packaged control chicken nuggets. However, the inclination in pH level was significantly ($p<0.05$) lower in treated product as compared to vacuum packaged control. It may be due to the fact that *Bacopa monnieri* L. extract contains bacoside and other phenols and flavonoids which are proton donors and acidic in nature. This was supported by the finding of Velasco and Williams, (2011) and their work was also suggestive of decline of pH in meat product with increase in successive increase level of *Bacopa monnieri* L. extract incorporation in vacuum packaged chicken nuggets.

Table 1. Changes in physico-chemical profile of *Bacopa monnieri* extract fortified v acuum packaged chicken nuggets at refrigeration temperature (4±1^{0}C). (Mean ±SE)*

Treatments	0-day	15th day	30thday	45thday
pH				
(Control)	6.24±0.017^{Ad}	6.40±0.006^{Ac}	6.73±0.028^{Ab}	6.98±0.031^{Aa}
Bacopa monnieri (1%)	5.95±0.008^{Bd}	6.11±0.005^{Bc}	6.40±0.027^{Bb}	6.73±0.058^{Ba}
Bacopa monnieri (2%)	5.57±0.034^{Cd}	5.74±0.028^{Cc}	6.08±0.026^{Cb}	6.36±0.020^{Ca}
Bacopa monnieri (3%)	5.50±0.040^{Cd}	5.70±0.019^{Cc}	6.03±0.093^{Cb}	6.33±0.030^{Ca}
Moisture				
(Control)	61.37±0.665^{Aa}	60.23±0.663^{Aab}	59.47±0.698^{Aab}	58.45±0.698^{Ab}
Bacopa monnieri (1%)	61.51±0.343^{Aa}	60.30±0.342^{Aab}	59.55±0.344^{Abc}	58.55±0.349^{Ac}
Bacopa monnieri (2%)	61.49±0.366^{Aa}	60.34±0.385^{Aab}	59.58±0.382^{Abc}	58.63±0.339^{Ac}
Bacopa monnieri (3%)	61.96±0.529^{Aa}	60.85±0.529^{Aab}	59.99±0.536^{Abc}	58.99±0.515^{Ac}
TBA(mg malonaldehyde/kg)				
(Control)	0.314±0.017^{Ad}	0.443±0.009^{Ac}	0.681±0.014^{Ab}	1.036±0.252^{Aa}
Bacopa monnieri (1%)	0.252±0.015^{Bd}	0.343±0.008^{Bc}	0.572±0.010^{Bb}	0.891±0.013^{Ba}
Bacopa monnieri (2%)	0.168±0.013^{Cd}	0.244±0.015^{Cc}	0.493±0.012^{Cb}	0.772±0.008^{Ca}
Bacopa monnieri (3%)	0.153±0.086^{Cd}	0.235±0.019^{Cc}	0.474±0.016^{Cb}	0.763±0.007^{Ca}
FFA(% oleic acid)				
(Control)	0.117±0.0019^{Ad}	0.133±0.0012^{Ac}	0.217±0.0013^{Ab}	0.309±0.0010^{Aa}
Bacopa monnieri (1%)	0.105±0.0014^{Bd}	0.120±0.0015^{Bc}	0.189±0.0015^{Bb}	0.235±0.0013^{Ba}
Bacopa monnieri (2%)	0.078±0.0016^{Cd}	0.086±0.0014^{Cc}	0.126±0.0017^{Cb}	0.153±0.0012^{Ca}
Bacopa monnieri (3%)	0.075±0.0017^{Cd}	0.083±0.0018^{Cc}	0.123±0.0012^{Cb}	0.150±0.0013^{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.

Table 2. Changes in microbiological profile of *Bacopa monnieri* extract fortified vacuum packaged chicken nuggets at refrigeration temperature (4±1^{0}C). (Mean ±SE)*

Treatments	0-day	15th day	30thday	45thday
Total Plate Count (log_{10}cfu/g)				
(Control)	2.52±0.014^{Ad}	3.41±0.011^{Ac}	3.67±0.012^{Ab}	4.31±0.018^{Aa}
Bacopa monnieri (1%)	2.33±0.015^{Bd}	3.16±0.016^{Bc}	3.34±0.010^{Bb}	3.83±0.011^{Ba}
Bacopa monnieri (2%)	1.82±0.014^{Cd}	2.59±0.011^{Cc}	2.83±0.013^{Cb}	3.17±0.017^{Ca}
Bacopa monnieri (3%)	1.78±0.011^{Cd}	2.57±0.012^{Cc}	2.78±0.012^{Cb}	3.12±0.011^{Ca}
Psychrotrophic Count (log_{10}cfu/g)				
(Control)	ND	ND	1.65±0.017^{Ab}	2.62±0.017^{Aa}
Bacopa monnieri (1%)	ND	ND	1.05±0.016^{Bb}	2.19±0.015^{Ba}
Bacopa monnieri (2%)	ND	ND	0.65±0.012^{Cb}	1.05±0.012^{Ca}
Bacopa monnieri (3%)	ND	ND	0.57±0.011^{Cb}	0.97±0.011^{Ca}
Coliform Count (log_{10}cfu/g)				
(Control)	ND	ND	ND	ND
Bacopa monnieri (1%)	ND	ND	ND	ND
Bacopa monnieri (2%)	ND	ND	ND	ND
Bacopa monnieri (3%)	ND	ND	ND	ND
Yeast and Mould Count (log_{10}cfu/g)				
(Control)	ND	ND	2.74±0.011^{Ab}	3.46±0.013^{Aa}
Bacopa monnieri (1%)	ND	ND	2.25±0.011^{Bb}	3.12±0.011^{Ba}
Bacopa monnieri (2%)	ND	ND	1.49±0.010^{Cb}	2.32±0.012^{Ca}
Bacopa monnieri (3%)	ND	ND	1.37±0.008^{Cb}	2.24±0.017^{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.

Table 3. Changes in the sensory attributes of aerobically packaged *Bacopa monnieri* extract fortified vacuum packaged chicken nuggets during refrigerated storage at 4±1°C (Mean ±SE)*

Treatments	Storage period(days)			
	0-day	15th day	30thday	45thday
Colour and appearance				
Bacopa monnieri (0%)	6.70±0.103^{Ca}	6.05±0.099^{Cb}	5.62±0.117^{Dc}	4.99±0.128^{Ad}
Bacopa monnieri (1%)	7.03±0.117^{Ba}	6.50±0.106^{Bb}	6.00±0.090^{Bc}	5.43±0.132^{Bd}
Bacopa monnieri (2%)	7.46±0.118^{Aa}	6.90±0.083^{Ab}	6.37±0.096^{Ac}	5.98±0.088^{Ad}
Bacopa monnieri (3%)	6.40±0.096^{Da}	5.70±0.106^{Db}	5.18±0.102^{Cc}	4.59±0.139^{Dd}
Flavour				
Bacopa monnieri (0%)	6.99±0.116^{Ba}	6.31±0.109^{Bb}	5.79±0.120^{Bc}	5.28±0.126^{Bd}
Bacopa monnieri (1%)	7.23±0.073^{ABa}	6.51±0.093 ^{ABb}	5.99±0.099^{ABc}	5.49±0.089^{ABd}
Bacopa monnieri (2%)	7.38±0.092^{Aa}	6.68±0.101^{Ab}	6.18±0.106^{Ac}	5.74±0.101^{Ad}
Bacopa monnieri (3%)	6.24±0.092^{Ca}	5.82±0.102^{Cb}	5.18±0.137^{Cc}	4.47±0.096^{Cd}
Texture				
Bacopa monnieri (0%)	7.38±0.068^{Aa}	6.95±0.113^{Ab}	5.59±0.095^{Bc}	4.69±0.123^{Ad}
Bacopa monnieri (1%)	7.50±0.076^{Aa}	6.97±0.089^{Ab}	5.62±0.079^{Bc}	4.74±0.111^{Ad}
Bacopa monnieri (2%)	7.62±0.078^{Aa}	7.09±0.088^{Ab}	5.79±0.107^{Ac}	4.73±0.122^{Ad}
Bacopa monnieri (3%)	7.39±0.075^{Aa}	6.80±0.084^{Ab}	5.49±0.099^{ABc}	4.57±0.112^{Ad}
Juiciness				
Bacopa monnieri (0%)	6.66±0.103^{Ba}	6.12±0.106^{Bb}	5.39±0.135^{Bc}	4.76±0.093^{Bd}
Bacopa monnieri (1%)	6.87±0.106^{ABa}	6.34±0.128^{ABb}	5.68±0.125^{ABc}	4.96±0.102^{ABd}
Bacopa monnieri (2%)	6.98±0.107^{Aa}	6.46±0.095^{Ab}	5.79±0.128^{Ac}	5.09±0.087^{Ad}
Bacopa monnieri (3%)	7.17±0.089^{Aa}	6.62±0.121^{Ab}	6.12±0.097^{Ac}	5.32±0.068^{Ad}
Overall acceptability				
Bacopa monnieri (0%)	6.79±0.168^{BCa}	5.89±0.116^{BCb}	5.66±0.130^{BCb}	5.18±0.105 ^{BCc}
Bacopa monnieri (1%)	6.98±0.100^{Ba}	6.06±0.098^{Bb}	5.88±0.121^{Bb}	5.47±0.087^{Bc}
Bacopa monnieri (2%)	7.29±0.089^{Aa}	6.42±0.117^{Ab}	6.29±0.104^{Ab}	5.75±0.103^{Ac}
Bacopa monnieri (3%)	6.56±0.113^{Ca}	5.66±0.119^{Cb}	5.48±0.141^{Cb}	5.07±0.104^{Cc}

*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.

Moisture

The moisture level recorded in *Bacopa monnieri* L. extract fortified vacuum packaged chicken nuggets and control was comparable. This may be due to the fact that the extract prepared contains similar moisture levels as present in meat emulsion. However, the moisture level observed in *Bacopa monnieri* L. extract fortified vacuum packaged chicken nuggets and vacuum packaged control products decreased significantly ($p<0.05$) on successive refrigeration storage days. This fact was supported by the finding of Shan *et al*., (2005) and Wojdyolo *et al*., (2007) who worked on oregano (*Organum vulgare* L.), rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) respectively in various emulsion based meat products.

TBA value

The TBA value is quantitative indication of lipid peroxidation in meat products. In order to know the rate of lipid peroxidation, the malondialdehyde content was evaluated by assaying meat product during storage.TBA value was significantly ($p<0.05$) decreased with increased in the level of incorporation of *Bacopa monnieri* L. extract while it was found to be significantly ($p<0.05$) increased on successive storage days in *Bacopa monnieri* L. extract incorporated vacuum packaged chicken nuggets including control. The TBA value indicated that control vacuum packaged chicken nugget was not suitable for consumption on 45th day of refrigeration storage whereas all levels of *Bacopa monnieri* L. extract incorporated vacuum packaged chicken nugget was found to

be suitable for consumption even on 45th day. This was an indicative of the fact that *Bacopa monnieri* L. extract had preventive effect on lipid peroxidation and hence enhance its shelf-life. This was due to the fact that mainly bacoside and other phenols and flavonoid present in *Bacopa monnieri* L. acted as anti-lipolytic factor due to which the shelf life of meat product was enhanced. The plant extract had positive effect on lipid oxidation by reducing the production of 2-TBA and malondialdehyde formation in herbal extract incorporated vacuum packaged meat product during refrigeration storage. Tanabe *et al*., (2002) also supported our present finding who reported the incorporation of phenols and flavonoids in pork product reduced production of malondialdehyde and lowered TBA value. Fasseas *et al*., (2007) reported that extract of various herbal plants and found out reduction in TBA value and lipid oxidation. This finding was in congruence with the finding of Phrompttayarat *et al*., (2007) who worked on *Bacopa monnieri* L. essential oil in different emulsion based meat and poultry products.

FFA

The FFA value is quantitative indication of lipolysis in meat products. The FFA value of *Bacopa monnieri* L. extract fortified vacuum packaged chicken nuggets recorded significantly lower ($p>0.05$) in comparison to vacuum packaged control product. This was an indicative of the fact that *Bacopa monnieri* L. extract had preventive effect on lipolysis and hence enhance its shelf-life. The FFA value has increased significantly ($p<0.05$) on successive refrigeration storage days in *Bacopa monnieri* L. extract fortified vacuum packaged chicken nuggets including control. This also suggested that *Bacopa monnieri* L. extract incorporated vacuum packaged chicken nuggets had better shelf life than control. It was due to the fact that bacoside present in extract of *Bacopa monnieri* L. has antilipolytic activity due to and which free fatty acid production was less. The present finding was also in parallel with finding of Simitzis *et al*., (2008) who reported dietary natural antioxidants obtained from different herbs had positive effect oxidative stability by producing fewer amounts of free fatty acids during refrigeration storage. It was supported by the work of Phrompttayarat *et al*., (2007) who worked on *Bacopa monnieri* L. essential oil in different meat and poultry product. Our present finding was also supported by the finding of Djenane *et al*., (2002) who concluded that surface application of extract of various herbs has positive effect on oxidative stability of beef steaks packaged in vacuum and modified atmospheric packaging.

Microbiological Characters

The changes in microbiological profile of *Bacopa monnieri* extract fortified vacuum packaged chicken nuggets at refrigeration temperature (4 ± 1^0C) depicted inTable-2.

Total Plate Count

The total plate count was lowered in *Bacopa monnieri* extract incorporated vacuum packaged chicken nuggets than control. This was indicative of anti-microbial nature of *Bacopa monnieri* extract. The total plate count was increased on successive refrigeration storage days in *Bacopa monnieri* extract incorporate vacuum packaged chicken nuggets including control. The TPC of control vacuum packaged chicken nuggets was indicative of fact that the product was not suitable for consumption on 45th day of storage. The TPC value of *Bacopa monnieri* extract incorporated vacuum packaged chicken nuggets were found to be in the range of 3 $\log_{10}$ cfu/gm which was indicative of fact that *Bacopa monnieri* extract incorporated vacuum packaged chicken nuggets were suitable for consumption even on 45th day of refrigeration storage. The herbal extract had affected microbial cell by various antimicrobial mechanisms. It may disrupt enzyme system, disrupt genetic material of bacteria attacking on phospholipidbilayer cellular membrane and forming fatty acid hydroperoxidase (Arques *et al*., 2008; Burt *et al*., 2007). The herbal extract had antimicrobial activity and when incorporated in meat product could elongate its shelf life during refrigeration storage. The bacoside has antimicrobial effect against both Gram positive and Gram negative bacteria (Proestos *et al*., 2008). The present finding was also supported by (Ceylan and Fung, 2004) who reported significantly decline in microbial load with incorporation of herbal extract in various meat product.

Psychrotrophic Count

Psychrotropic were not detected till 15h day of refrigeration storage. The psychrotropic were found to be lower on 30th and 45th day of refrigeration storage in *Bacopa monnieri* extract

incorporated vacuum packaged chicken nuggets as compare to control. It was also observed that control chi vacuum packaged cken nugget was not found to be suitable for consumption on 45th day. But, *Bacopa monnieri* extract incorporated vacuum packaged chicken nugget was found to be suitable for human consumption even on 45th day of refrigeration storage. It may be due the fact that the principle component bacoside present in *Bacopa monnieri* extract had significant antimicrobial effect at refrigeration temperature. The bacoside interact with phospholipid bilayer of microbial cell wall and cell membrane and disrupt it. The present result was also supported by finding Rota, *et al*., (2008) who suggested Psychrotropic antimicrobial affect of essential oils and extracts of herbal plants. It also defunct electron transport system, ion gradient and other enzyme dependent cellular mechanism of psychrotropic bacteria (Burt, 2004).

Coliform Count

The coliform was not detected at any day of refrigeration storage in any of the vacuum packaged product. It may be due to the fact that strict hygienic condition was followed during meat product processing. It may also be due to antimicrobial effect against coliform by bacoside present in *Bacopa monnieri* extract. Our present finding was supported by reports of Ben Sassi, *et al*., (2008); Graumann and Holley (2008); Ibrahim, *et al*., (2008); Kuete *et al*., (2008) and Winward, *et al*., (2008) who concluded that the extract and essential oil obtained from various herbs had significant antimicrobial effect against almost all coliforms.

Yeast and Moulds

The yeast and moulds count was not detected till 15th day of storage but it was appeared in all product from 30th day onward. The yeast and mould count of *Bacopa monnieri* extract incorporated vacuum packaged chicken nuggets was significantly lower than control on 30th and 45th day of storage. It was also indicative of the fact that vacuum packaged control chicken nugget was not suitable but *Bacopa monnieri* extract incorporated vacuum packaged chicken nuggets was found to be suitable for human consumption even on 45th day of refrigeration storage. The appearance of yeast and moulds on 30th day may be due to the fact that yeast and moulds requires incubation period of approximately 10 days. Our finding was supported by Razzaghi-Abyaneh *et al*., (2008) and El. Seedi *et al*., (2008) who reported the ethanolic extract of Bacopa monnieri and other herbs significantly reduces yeast and moulds count in various meat product. Moreover these extract possess natural fungicidal effect against food borne fungi (Fisher and Phillips, 2008). It was also effective against mycotoxin (Friedman, 2007; Musyimi *et al*., 2008; Kong *et al*., 2007; and Lopez *et al*., 2007).

Sensory Parameters

The changes in the sensory attributes of vacuum packaged *Bacopa monnieri* extract fortified chicken nuggets during refrigerated storage at 4±1°C projected in Table-3.All the sensory attributes viz. color and appearance, flavour, texture, juiciness and overall acceptability was found to be lower on successive refrigeration storage in *Bacopa monnieri* extract incorporated vacuum packaged chicken nuggets including control. The color and appearance, flavour and juiciness of 2% *Bacopa monnieri* extract incorporated vacuum packaged chicken nuggets was found to be significantly higher than 1% and 3% *Bacopa monnieri* extract incorporated and vacuum packaged control chicken nuggets. The texture value of *Bacopa monnieri* extract incorporated and control chicken nuggets were comparable with each other. The Overall acceptability of 2% incorporated *Bacopa monnieri* extract in vacuum packaged chicken nugget was higher than 1% and 3% *Bacopa monnieri* extract incorporated and vacuum packaged control chicken nuggets.

The control chicken nuggets have been quickly spoiled on all parameters of sensory attributes as compared to *Bacopa monnieri* extract fortified chicken nuggets. The *Bacopa monnieri* extract fortified vacuum packaged chicken nuggets was found to be acceptable on the basis of sensory attributes even on 45th day of refrigeration storage but the control chicken nuggets was rejected on 45th day of refrigeration storage on the basis of sensory attributes.

Natural antioxidant can positively affect color and appearance parameter and maintained the original color of product for longer duration during refrigeration storage (Djenane *et al*., 2003; Carpenter *et al*., 2007; Chouliars *et al*., 2007; Nerin

et al., 2006 and Simitzis *et al*., 2008). The herbal extract has positive effect by inhibiting discoloration and off- odor formation in different meat product during refrigeration as well as deep freeze storage (Djenane *et al*., 2003; Nerin *et al*., 2006 and Camo *et al*., 2008). The herbal extract can act as a very good flavoring agent too. It can also act as a good binding agent. The overall sensory attributes can also be enhanced with herbal extract fortification in various meat products (Chaves *et al*., 2008).

CONCLUSIONS

The ethanolic: aqueous (80:20) extract of *Bacopa monnieri* L. were standardized and optimized to be used in preparation of value added vacuum packaged chicken nuggets. The developed product depicted significant ($p>0.05$) anti microbial, anti lipolytic and anti oxidant activity. The incorporation of *Bacopa monnieri* L. extract (2%) in value added vacuum packaged chicken nuggets has enhanced not only sensory scores but also its shelf life. The result revealed the possible application of *Bacopa monnieri* L. extract (2%) as a natural source of anti oxidant in development of value added vacuum packaged chicken meat product with potential health benefits.

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