



Oxidative stability and storage quality analysis of *Ocimum sanctum* L. extracts incorporated chicken nuggets

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Abstract: The present study was done to explore the antioxidant potential of locally available herb holy basil viz. *Ocimum sanctum* L. in enhancing the shelf-life of emulsion based chicken nuggets. Chicken nuggets are widely cherished meat cuisine but it is vulnerable to spoilage due to excessive fats and protein content. Thus, chicken nugget fortified with 1, 2, and 3% of itsethanolic-aqueous extracts of *O. sanctum* and along with control was studied to explore the potency of holy basil on oxidative stability and storage quality of chicken nuggets on 0,7,14 and 21 days at refrigeration temperature. 80% ethanolic aqueous extracts of *O. sanctum* were prepared, standardized, optimized and incorporated in chicken nuggets. Chicken nuggets prepared with fortification of 3% *O. sanctum* extract were adjudged best among all with overall acceptability of 7.16 ± 0.071 value in sensory analysis. The *O. sanctum* extract treated chicken nugget's pH, FFA, TBA, Total plate count, Psychrophilic count, Yeast and Mold count were found to be in acceptable range of 4.49 ± 0.008 , 0.320 ± 0.0005 , 0.979 ± 0.0012 , $<4, <2, <2 \log_{10} \text{cfu/g}$ respectively on 21 days of refrigeration storage. Extracts of *O. sanctum* fortified chicken nuggets were safe for human consumption even 21 days of refrigerated storage ($4 \pm 1^\circ\text{C}$) on the basis of pH, FFA, TBA value, microbiological profile and sensory evaluation. The results proved the antioxidant potential of holy basil *O. sanctum* and chicken nuggets fortified with (3%) *Ocimum sanctum* extract could be safe for a period of 21 days in refrigerated ($4 \pm 1^\circ\text{C}$) storage without any marked loss of physico-chemical, microbial and sensory quality.

Keywords: Antioxidant, Chicken nuggets, *Ocimum sanctum*, Oxidative stability, Storage quality

INTRODUCTION

Consumer demands for safe, natural and high quality foods. The preference of consumer towards natural or organic food compels the food industry to include natural antioxidant in meat products to impart oxidative stability (Camo *et al.*, 2008). The herbal extract can act as a potent natural antioxidant which can be used in different meat products. The addition of these extract not only improves the sensory characteristic but also enhance self-life (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. 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)

Ocimum sanctum Linn. (Family: Labiatae), locally known as 'Tulsi' in Hindi and 'Holy Basil' in English, is herb found throughout India. The Indian herb *O. sanctum* - may serve as dietary antioxidant with various mode of action viz. anti-microbial agent, anti lipolytic agent. Therefore the extract of *O. sanctum* can elongate the shelf-life of meat product when incorporated at standardized and optimized level in emulsion based meat product. The extract of *O. sanctum* also act 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 *O. sanctum*. extract incorporated value added meat product and further the product is evaluated for it quality parameters during refrigeration storage.

MATERIALS AND METHODS

Raw material: Spent hen meat of over 72 weeks age was selected. The spent hen was humanely slaughtered

in the meat science lab of Division of Livestock Products Technology, SKUAST-Jammu. *Ocimum sanctum* L. were collected locally. It was further identified and authenticated by Department of Botany, Jammu University.

Preparation of *O. sanctum* extract: Fresh *O. sanctum* plant will be collected and botanically authenticated. The leaves will be separated and dried at 40°C for 6 hours in hot air oven. The dried leaves will be ground into fine powder form. The powder will be dissolved in 80% ethanol aqueous solution for four days by changing solution daily. The extract will be filtered and evaporated to 10% dryness v/v using rotatory evaporator (Hannan *et al.*, 2006).

Preparation of chicken nuggets: Lean meat from spent hen was cut and minced in a meat mincer (MOD-TC 23 R10 U.P. INOX, Marsango, Italy). 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 in length and 11 cm in breadth were used for molding and further steaming was done. The weighed quantity of the batter or emulsion was stuffed in mould with parchment paper and pre-smear with 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, *O. sanctum* 1, 2, and 3% extracts added with replacement in control chicken nuggets (wt./wt.). The nuggets were cooled and then stored in low density polyethylene pouches (200 gauge). These were stored in refrigerator (4±1°C) for evaluation of physico-chemical and sensory parameters 0th, 7th, 14th and 21st day.

Experimental design: The ethanolic-aqueous *Ocimum sanctum* L. extract was prepared and was added in standardized formulation of chicken nuggets 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).

Analytical techniques

pH: The pH of cooked nuggets was measured soon after its preparation by the method of (Keller *et al.*, 1974).

Moisture content: It was calculated as per method.

(AOAC, 1995).

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

Free fatty acid (FFA): The method described by (Koniecko, 1979) was followed.

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: It 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 comfortably seated in a room free of noise and odors and suitably illuminated. Coded samples for sensory evaluation were prepared.

Statistical analysis: The results were analyzed statistically for analysis of variance in one way as well as two way and least significant difference tests as per (Snedecor and Cochran, 1989). In significant effects, least significant differences were calculated for a pair wise comparison of treatment means.

RESULTS AND DISCUSSION

Physico-chemical parameters: The changes in physico-chemical profile of *O. sanctum* sp extract fortified chicken nuggets at refrigeration temperature (4±1°C) presented in Table 1.

pH: The pH of *O. sanctum* extract incorporated chicken nuggets was recorded to be significantly lower as compared to control. The pH was increased significantly on successive storage days irrespective of levels of incorporation of *O. sanctum* extract in chicken nuggets including control. However the inclination in pH level was significantly lower in treated product as compared to control. It may be due to the fact that *O. sanctum* extract contains urolic acid, apigenin, and luteolin which are proton donors and acidic in nature. The poultry meat recorded lower pH when fed on diet incorporated with *O. sanctum* powder Lanjewar *et al.* (2009). The acidic pH of *O. sanctum* extract was helpful in treatment of wilt disease of tomato plant (Murthy *et al.*, 2014).

Moisture: The moisture level recorded in *O. sanctum* extract incorporated 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 *O. sanctum* extract incorporated chicken nuggets and control decreased significantly ($p < 0.05$) on successive refrigeration storage days. This fact was supported by the finding of (Shan *et al.*, 2005; Wojdy-

Table 1. Changes in physico-chemical profile of *Ocimum sanctum* extract fortified chicken nuggets at refrigeration temperature (4±1°C). (Mean ±SE)*.

| TREATMENTS | 0-day | 7 th day | 14 th -day | 21 st -day |
|----------------------------------|-----------------------------|----------------------------|----------------------------|----------------------------|
| pH | | | | |
| <i>O. sanctum</i> (Control) | 5.88±0.007 ^{Ad} | 5.97±0.009 ^{Ac} | 6.26±0.011 ^{Ab} | 6.73±0.005 ^{Aa} |
| <i>O. sanctum</i> (1%) | 5.85±0.166 ^{ABd} | 5.87±0.009 ^{Bc} | 6.11±0.011 ^{Bb} | 6.49±0.008 ^{Ba} |
| <i>O. sanctum</i> (2%) | 5.82±0.133 ^{BCd} | 5.73±0.010 ^{Cc} | 6.02±0.007 ^{Cb} | 6.36±0.008 ^{Ca} |
| <i>O. sanctum</i> (3%) | 5.79±0.170 ^{Cd} | 5.49±0.009 ^{Dc} | 5.59±0.005 ^{Db} | 6.30±0.008 ^{Da} |
| Moisture | | | | |
| <i>O. sanctum</i> (Control) | 61.77±0.679 ^{Aa} | 60.51±0.575 ^{Aab} | 59.94±0.334 ^{Aab} | 58.82±0.688 ^{Ab} |
| <i>O. sanctum</i> (1%) | 61.92±0.333 ^{Aa} | 60.76±0.329 ^{Aab} | 60.02±0.692 ^{Abc} | 58.90±0.329 ^{Ac} |
| <i>O. sanctum</i> (2%) | 61.02±0.494 ^{Aa} | 60.81±0.375 ^{Aab} | 60.05±0.372 ^{Abc} | 58.14±0.359 ^{Ac} |
| <i>O. sanctum</i> (3%) | 61.34±0.519 ^{Aa} | 60.41±0.494 ^{Aab} | 60.52±0.514 ^{Abc} | 58.52±0.505 ^{Ac} |
| TBA (mg malonaldehyde/kg) | | | | |
| <i>O. sanctum</i> (Control) | 0.302±0.0134 ^{Ad} | 0.429±0.0005 ^{Ac} | 1.051±0.0011 ^{Ab} | 1.305±0.0005 ^{Aa} |
| <i>O. sanctum</i> (1%) | 0.298±0.0182 ^{ABd} | 0.394±0.0009 ^{Bc} | 0.674±0.0006 ^{Bb} | 1.250±0.0012 ^{Ba} |
| <i>O. sanctum</i> (2%) | 0.293±0.0280 ^{BCd} | 0.362±0.0005 ^{Cc} | 0.625±0.0007 ^{Cb} | 1.221±0.0005 ^{Ca} |
| <i>O. sanctum</i> (3%) | 0.289±0.0145 ^{Ca} | 0.325±0.0005 ^{Dc} | 0.561±0.0007 ^{Db} | 1.161±0.005 ^{Dd} |
| FFA (% oleic acid) | | | | |
| <i>O. sanctum</i> (Control) | 0.093±0.0005 ^{Ad} | 0.135±0.0008 ^{Ac} | 0.225±0.0005 ^{Ab} | 0.362±0.0006 ^{Aa} |
| <i>O. sanctum</i> (1%) | 0.091±0.0063 ^{Ad} | 0.115±0.0009 ^{Bc} | 0.205±0.0009 ^{Bb} | 0.320±0.0005 ^{Ba} |
| <i>O. sanctum</i> (2%) | 0.085±0.0140 ^{Bd} | 0.095±0.0008 ^{Cc} | 0.180±0.0008 ^{Cb} | 0.234±0.0009 ^{Ca} |
| <i>O. sanctum</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.

Table 2. Changes in microbiological profile of *Ocimum sanctum* extract fortified chicken nuggets at refrigeration temperature (4±1°C). (Mean ±SE)*.

| TREATMENTS | 0-day | 7 th day | 14 th -day | 21 st -day |
|---|---------------------------|---------------------------|---------------------------|---------------------------|
| Total Plate Count (log₁₀cfu/g) | | | | |
| <i>O. sanctum</i> (Control) | 2.70±0.0076 ^{Ad} | 3.74±0.0088 ^{Ac} | 4.15±0.0114 ^{Ab} | 5.10±0.0076 ^{Aa} |
| <i>O. sanctum</i> (1%) | 2.35±0.0073 ^{Bd} | 3.29±0.0111 ^{Bc} | 3.85±0.0076 ^{Bb} | 4.35±0.0096 ^{Ba} |
| <i>O. sanctum</i> (2%) | 1.80±0.0066 ^{Cd} | 3.10±0.0076 ^{Cc} | 3.50±0.0101 ^{Cb} | 3.72±0.0076 ^{Ca} |
| <i>O. sanctum</i> (3%) | 1.79±0.0057 ^{Dd} | 2.88±0.0080 ^{Dc} | 3.15±0.0060 ^{Db} | 3.49±0.0060 ^{Da} |
| Psychrophilic count (log₁₀cfu/g) | | | | |
| <i>O. sanctum</i> (Control) | ND | ND | 1.59±0.011 ^{Ab} | 2.74±0.0152 ^{Aa} |
| <i>O. sanctum</i> (1%) | ND | ND | 1.34±0.360 ^{Bb} | 2.26±0.0125 ^{Ba} |
| <i>O. sanctum</i> (2%) | ND | ND | 0.98±0.013 ^{Cb} | 1.84±0.0116 ^{Ca} |
| <i>O. sanctum</i> (3%) | ND | ND | 0.70±0.007 ^{Db} | 1.21±0.0116 ^{Da} |
| Coliform count (log₁₀cfu/g) | | | | |
| <i>O. sanctum</i> (Control) | ND | ND | ND | ND |
| <i>O. sanctum</i> (1%) | ND | ND | ND | ND |
| <i>O. sanctum</i> (2%) | ND | ND | ND | ND |
| <i>O. sanctum</i> (3%) | ND | ND | ND | ND |
| Yeast and Mold count (log₁₀cfu/g) | | | | |
| <i>O. sanctum</i> (Control) | ND | ND | 2.73±0.0101 ^{Ab} | 3.60±0.0110 ^{Aa} |
| <i>O. sanctum</i> (1%) | ND | ND | 2.40±0.0114 ^{Bb} | 3.20±0.0076 ^{Ba} |
| <i>O. sanctum</i> (2%) | ND | ND | 2.09±0.1344 ^{Cb} | 2.79±0.0107 ^{Ca} |
| <i>O. sanctum</i> (3%) | ND | ND | 1.71±0.0105 ^{Db} | 2.34±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.

olo et al., 2007; Lanjewar et al., 2009) who worked on Indian holy basil (*Ocimum sanctum* sp), oregano (*Organum vulgare* L.), rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia officinalis* L.) in various emulsion based meat products.

TBA value: The TBA value is quantitative indication

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

| TREATMENTS | STORAGE PERIOD (DAYS) | | | |
|------------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| | 0 Day | 7 th -DAY | 14 th -DAY | 21 th -0DAY |
| Colour and appearance | | | | |
| <i>O.sanctum</i> (Control) | 6.69±0.100 ^{Ca} | 6.07±0.099 ^{Cb} | 5.59±0.107 ^{Dc} | 4.95±0.131 ^{Ad} |
| <i>O.sanctum</i> (1%) | 7.02±0.116 ^{Ba} | 6.52±0.106 ^{Bb} | 6.02±0.080 ^{Bc} | 5.40±0.131 ^{Bd} |
| <i>O.sanctum</i> (2%) | 7.45±0.108 ^{Aa} | 6.95±0.083 ^{Ab} | 6.42±0.086 ^{Ac} | 5.97±0.080 ^{Ad} |
| <i>O.sanctum</i> (3%) | 6.38±0.076 ^{Da} | 5.78±0.106 ^{Db} | 5.28±0.112 ^{Cc} | 4.54±0.137 ^{Dd} |
| Flavour | | | | |
| <i>O.sanctum</i> (Control) | 6.52±0.094 ^{Ba} | 5.92±0.130 ^{Bb} | 5.59±0.074 ^{Bc} | 3.95±0.108 ^{Bd} |
| <i>O.sanctum</i> (1%) | 6.73±0.131 ^{Ba} | 6.11±0.119 ^{Bb} | 5.76±0.112 ^{Bc} | 4.07±0.105 ^{Bd} |
| <i>O.sanctum</i> (2%) | 6.76±0.081 ^{Ba} | 6.21±0.073 ^{Bb} | 5.88±0.113 ^{Bc} | 4.14±0.070 ^{Bd} |
| <i>O.sanctum</i> (3%) | 7.14±0.098 ^{Aa} | 6.73±0.061 ^{Ab} | 6.33±0.061 ^{Ac} | 4.83±0.076 ^{Ad} |
| Texture | | | | |
| <i>O.sanctum</i> (Control) | 7.40±0.088 ^{Aa} | 6.83±0.105 ^{Ab} | 5.57±0.086 ^{Ac} | 4.61±0.102 ^{Ad} |
| <i>O.sanctum</i> (%) | 7.42±0.110 ^{Aa} | 6.88±0.137 ^{Ab} | 5.64±0.092 ^{Ac} | 4.66±0.130 ^{Ad} |
| <i>O.sanctum</i> (2%) | 7.50±0.912 ^{Aa} | 6.97±0.126 ^{Ab} | 5.71±0.127 ^{Ac} | 4.76±0.136 ^{Ac} |
| <i>O.sanctum</i> (3%) | 7.38±0.076 ^{Aa} | 6.76±0.101 ^{Ab} | 5.52±0.087 ^{Ac} | 4.59±0.062 ^{Ac} |
| Juiciness | | | | |
| <i>O.sanctum</i> (Control) | 6.80±0.100 ^{Ca} | 6.33±0.105 ^{Cb} | 5.59±0.127 ^{Cc} | 4.88±0.090 ^{Cd} |
| <i>O.sanctum</i> (1%) | 7.04±0.083 ^{BCa} | 6.54±0.108 ^{BCb} | 5.85±0.115 ^{BCc} | 5.11±0.074 ^{BCd} |
| <i>O.sanctum</i> (2%) | 7.16±0.110 ^{Ba} | 6.66±0.099 ^{Bb} | 5.97±0.094 ^{Bc} | 5.21±0.100 ^{Bd} |
| <i>O.sanctum</i> (3%) | 7.47±0.080 ^{Aa} | 6.97±0.094 ^{Ab} | 6.40±0.101 ^{Ac} | 5.52±0.050 ^{Ad} |
| Overall acceptability | | | | |
| <i>O.sanctum</i> (Control) | 6.85±0.104 ^{Ca} | 6.21±0.073 ^{Cb} | 5.40±0.144 ^{Cc} | 3.97±0.116 ^{Cd} |
| <i>O.sanctum</i> (1%) | 6.97±0.116 ^{Ca} | 6.33±0.079 ^{Cb} | 5.54±0.113 ^{Cc} | 4.16±0.062 ^{Cd} |
| <i>O.sanctum</i> (2%) | 7.26±0.065 ^{Ba} | 6.73±0.117 ^{Bb} | 5.93±0.086 ^{Bc} | 4.57±0.115 ^{Bd} |
| <i>O.sanctum</i> (3%) | 7.54±0.090 ^{Aa} | 7.16±0.071 ^{Ab} | 6.33±0.093 ^{Ac} | 5.00±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.

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 *O. sanctum* extract while it was found to be significantly (p<0.05) increased on successive storage days in *O. sanctum* extract incorporated chicken nuggets including control. The TBA value indicated that control chicken nugget was not suitable for consumption on 21st day of refrigeration storage whereas all levels of *O. sanctum* extract incorporated chicken nugget was found to be suitable for consumption even on 21st. This was an indicative of the fact that *O. sanctum* extract had preventive effect on lipid peroxidation and hence enhance its shelf-life. This was due to the fact that mainly urolic acid, apigenin, and luteolin present in *O. sanctum* acted as anti-lipolytic factor due to which the shelf life of meat product was enhanced. This finding was in congruence with the finding of (Alo *et al.*, 2012) who worked on properties of water, ethanol and methanol extract of *O. sanctum*. The plant

extract had positive effect on lipid oxidation by reducing the production of 2-TBA and malondialdehyde formation in herbal extract incorporated meat product during refrigeration storage. The extract of various herbal plants and found out reduction in TBA value and lipid oxidation (Fasseas *et al.*, 2007). Present findings were also supported that the incorporation of phenols and flavonoids in pork product reduced production of malondialdehyde and lowered TBA value (Tanabe *et al.*, 2002).

FFA: The FFA value is quantitative indication of lipolysis in meat products. The FFA value of *O. sanctum* extract incorporated chicken nuggets recorded significantly lower (p>0.05) value in comparison to control product. This was an indicative of the fact that *O. sanctum* 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 *O. sanctum* extract incorporated chicken nuggets including control. This also suggested that *O. sanctum* extract incorporated chicken nuggets had better shelf life than control. It was due to the fact

that urolic acid, apigenin, and luteolin present in extract of *O. sanctum* has anti-lipolytic activity due to and which free fatty acid production was less. It was supported by the work of (Yanpallewar *et al.*, 2007) who worked on *O. sanctum* antioxidant and neuro-protective effect of *O. sanctum* on transient cerebral ischemia and long-term cerebral hypo perfusion. Our present finding was also supported by the finding of (Djenane *et al.*, 2003) who concluded that surface application of extract of various herbs had positive effect on oxidative stability of beef steaks packaged in vacuum and modified atmospheric packaging. 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 on oxidative stability by producing fewer amounts of free fatty acids during refrigeration storage.

Microbiological characters: The changes in microbiological profile of *O. sanctum* extract fortified chicken nuggets at refrigeration temperature ($4\pm 1^{\circ}\text{C}$) depicted in Table-2.

Total plate count: The total plate count was lowered in *O. sanctum* extract incorporated chicken nuggets than control. This was indicative of anti-microbial nature of *O. sanctum* extract. The total plate count was increased on successive refrigeration storage days in *O. sanctum* extract incorporate chicken nuggets including control. The TPC value of control chicken nuggets was indicative that the product was not suitable for human consumption on 21st day of storage. The TPC value of *O. sanctum* .extract incorporated chicken nuggets were found to be in the range of $3 \log_{10} \text{cfu/gm}$ which was indicative of fact that *O. sanctum* . extract incorporated chicken nuggets were suitable for consumption even on 21st 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 phospholipid bilayer cellular membrane and forming fatty acid hydro-peroxidase (Arques *et al.*, 2008; Burt *et al.*, 2007). 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. The herbal extract had antimicrobial activity and when incorporated in meat product could elongate its shelf life during refrigeration storage. The urolic acid, apigenin, and luteolin active principal content in *O sanctum*. extract was having broad spectrum antimicrobial activity (Alo *et al.*, 2012).

Psychrotrophic count: Psychrotrophic count was not detected till 7th day of refrigeration storage. The psychrophilic were found to be lower on 14th and 21st day of refrigeration storage in *O. sanctum*. extract incorporated chicken nuggets as compare to control. It was also observed that control chicken nugget was not found to be suitable for consumption on 21st day. However, *O. sanctum*. extract incorporated chicken nugget

was found to be suitable for human consumption even on 21st day of refrigeration storage. It may be due the fact that the principle component urolic acid, apigenin, and luteolin present in *O. sanctum*. extract had significant antimicrobial effect at refrigeration temperature. The apigenin, and luteolin interacted with phospholipid bilayer of microbial cell wall and cell membrane and disrupted it. It also defunct electron transport system, ion gradient and other enzyme dependent cellular mechanism of psychrotrophic bacteria (Burt, 2004). The present result was also supported by finding (Rota *et al.*, 2008) who suggested Psychrotrophic antimicrobial affect of essential oils and extracts of herbal plants.

Coliform count: The coliform were not detected at any day of refrigeration storage in any of the 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 urolic acid, apigenin, and luteolin present in *O. sanctum*. 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; 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 7th day of storage but it was appeared in all product from 14th day onward. The yeast and mould count of *O. sanctum*. extract incorporated chicken nuggets was significantly lower than control on 14th and 21st day of storage. It was also indicative of the fact that control chicken nugget was not suitable but *O. sanctum* extract incorporated chicken nuggets was found to be suitable for human consumption even on 21st day of refrigeration storage. The appearance of yeast and moulds on 14th day may be due to the fact that yeast and moulds requires incubation period of approximately 10 days. Moreover these extract possess natural fungicidal effect against food borne fungi (Fisher and Phillips, 2008; Daferere *et al.*, 2000; Majhenic *et al.*, 2007). Our finding was supported by (Razzaghi-Abyaneh *et al.*, 2008; El. Seedi *et al.*, 2008) who reported the ethanolic extract of *O. sanctum* and other herbs significantly reduces yeast and moulds count in various meat product. It was also effective against mycotoxin (Friedman, 2007; Musyimi *et al.*, 2008; Kong *et al.*, 2007; Lopez *et al.*, 2007).

Sensory parameters: The changes in the sensory attributes of aerobically packaged *O. sanctum*. extract fortified Chicken nuggets during refrigerated storage at $4\pm 1^{\circ}\text{C}$ 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 *O. sanctum* extract incorporated chicken nuggets including control. The color and appearance, flavour and juiciness of 3% *O. sanctum* extract incorporated

chicken nuggets was found to be significantly higher than 1% and 2% *O. sanctum* extract incorporated and control chicken nuggets. The texture value of *O. sanctum* extract incorporated and control chicken nuggets were comparable with each other. The Overall acceptability of 3% incorporated *O. sanctum* extract in chicken nugget was higher than 1% and 2% *O. sanctum* extract incorporated and control chicken nuggets.

The control chicken nuggets have been quickly deteriorated on all parameters of sensory attributes as compared to *O. sanctum* extract incorporated chicken nuggets. The *O. sanctum* extract incorporated chicken nuggets was found to be acceptable on the basis of sensory attributes even on 21st day of refrigeration storage but the control chicken nuggets was rejected on 21st day of refrigeration storage.

The herbal extract has positive effect by inhibiting discoloration and off-odor formation in different meat product during refrigeration storage (Djenane *et al.*, 2003; Nerin *et al.*, 2006; Camo *et al.*, 2008). 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; Chouliaris *et al.*, 2007; Nerin *et al.*, 2006 and Simitzis *et al.*, 2008). The herbal extract can act as a very good flavoring agent. It can also act as a binding agent. The sensory character can also be enhanced with herbal extract incorporation in various meat products (Chaves *et al.*, 2008).

Conclusion

The ethanolic: aqueous (80:20) extract of *O. sanctum* was used in preparation of value added chicken nuggets. The developed product exhibited significant ($p > 0.05$) anti microbial, anti lipolytic and anti oxidant activity. The incorporation of *O. sanctum* extract (3%) in value added chicken nuggets has enhanced sensory scores as well as shelf life. The result revealed the possible application of *O. sanctum* extract (3%) as a natural source of anti oxidant in development of value added chicken meat product with potential health benefits.

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