EFFECT OF *OCIMUM SANCTUM* EXTRACT ON STORAGE AND MICROBIAL QUALITY OF VACUUM PACKED CHICKEN NUGGETS

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ABSTRACT

The present study was undertaken to assess the antioxidant properties of *Ocimum sanctum* in enhancing the shelf-life of vacuum packed chicken nuggets. Meat products are very vulnerable to spoilage due to excessive fats and protein contents. The study was conducted on chicken nuggets fortified with 1, 2, and 3% extracts of *Ocimum sanctum* along with control to explore the potency of *Ocimum sanctum* on oxidative stability and storage quality of vacuum packed chicken nuggets on 0, 15th, 30th and 45th days. Extracts of *Ocimum sanctum* in desired percentage were prepared and incorporated in vacuum packed chicken nuggets. The chicken nuggets fortified with 3% of *Ocimum sanctum* were adjudged to the best among all, based on sensory attributes and were found safe for consumption till 45 days of refrigerated storage (4±1^{o}C) on the basis of pH, moisture, free fatty acid (FFA), thiobarbituric acid reacting substance (TBARS), microbiological profile, and sensory evaluation.

Key words**:** *Ocimum sanctum* extract, storage quality, vacuum packed chicken nuggets, free fatty acid (FFA), thiobarbituricacid reacting substance (TBARS)

Introduction

Today, 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 such extracts not only improves the sensory characteristics but also enhances self-life of meat products (Wojdylo *et al*., 2007).

Most of the diseases that are encountered today are lifestyle diseases which also includes degenerative diseases. The occurrence of these diseases is due to excessive formation of pro-oxidants in the body. To counter these pro-oxidants, the antioxidant are also secreted at cellular level but due to several reasons like genetic factors, dietary habit, physiological status, work load and environment pollution etc. There is excessive production of pro-oxidants at cellular level which leads to oxidative damage of the cell resulting in various lifestyle diseases (cancer, cardiovascular diseases, atherosclerosis, Alzheimer and Parkinson).

Therefore, in today's prospective natural antioxidants are essential dietary requirementsand development of value added meat products with incorporation of herbal extract as antioxidants helps in combating oxidative stress.

Ocimum sanctum Linn. (Family: Labiatae), locally known as 'Tulsi' in Hindi and 'Holy Basil' in English, is a herb which is found throughout India. The Indian herb *Ocimum sanctum* may serve as dietary antioxidant with various modes of action viz. anti-microbial agents, anti lipolytic agents etc. Therefore, the extract of *Ocimum sanctum* can elongate the self-life of meat product when incorporated at standardized and optimized level in emulsion based meat product. The extract of *Ocimum sanctum* also acts as antioxidant when consumed by humans and protect them from oxidative damage and age old cognitive decline (Deka *et al*., 2009). The aim of present study was to develop *Ocimum sanctum* extract incorporated vacuum packed chicken nuggets and to evaluate for its quality parameters during refrigeration storage.

Materials and Methods

Preparation of *Ocimum sanctum* extract

Fresh *Ocimum sanctum* sp. plants were collected and botanically authenticated. The leaves were separated and dried at 40^{o}C for 6 hours in hot air oven. Dried leaves were grinded into fine powder form, which was dissolved in 80% ethanol aqueous solution for four days by changing solution daily. The extract was filtered and evaporated to 10% dryness v/v using rotatory evaporator (Hannan *et al.,* 2008).

Preparation of chicken nuggets

Lean meat from broiler was cut and minced in mincer. The common salt, vegetable oil, refined wheat flour (maida), nitrite, sodium hexapolyphosphate, spice mixture and condiment mixture were added as per formulation. Meat emulsion for chicken nuggets was prepared in Sirman Bowl Chopper. Crushed ice was added and blended continuously for about 1.5 minutes. Refined vegetable oil, spice mixture, condiments and other ingredients were added and again mixed for 1.5 to 2 minutes to get the desired emulsion. Chicken nuggets were moulded in rectangular stainless steel boxes. The weighed quantity of batter or emulsion was stuffed in mould with parchment paper pre-smeared with refined soybean oil to avoid sticking. Mould was covered with lid and tied properly. The moulds were subjected to steam cooking for about 30 minutes in pressure cooker. The boxes were allowed to cool at room temperature after removal from pressure cooker. The brick shaped chicken nuggets so obtained were sliced and

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cut into pieces to get smaller nuggets. The formulation of chicken nuggets in (%) was standardized, optimized and used for preparation of chicken nuggets from broiler 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% *Ocimum sanctum* extracts was added in chicken nuggets (wt./wt.). In control chicken nuggets *Ocimum sanctum* extracts was no added. The nuggets were cooled and packed under vacuum packaging. These were stored in refrigerator (4±1°C) for evaluation of phisio-chemical and sensory parameter on 0, 15th, 30th and 45th day.

Analytical procedures

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.

Moisture contents

Ten gram of mashed sample was transferred in pre-weighed flat bottom aluminum moisture cup, which was transferred to hot air oven at 101±1 °C and kept for 16-18 hrs. Dried sample was then placed in desiccator having silica gel as desiccant. After 1 hr., the cup containing dried sample was weighed. Moisture content was calculated by applying the following formula:

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

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

Thiobarbituricacidreacting substance (TBARS)

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% Trichloro acetic acid (TCA) in a waring blender for 2 minutes and the resulting slurry 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% thiobarbituric acid (TBA) reagent. The contents of each test tube were thoroughly mixed and boiled for 35 minutes in boiling water bath and were then allowed to cool down. The absorbance was measured at 532 nm by spectrophotometer and TBA value was calculated by comparing the absorbance of test sample with a standard graph prepared by using known concentrations of malondialdehyde. For preparing standard graph, 0.3055 gm. of 1, 1, 3, 3 tetraethoxypropane (TEP) was dissolved in 100 ml of 95% absolute alcohol to obtain a concentration of 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 µg/ml of malonaldehyde and from that solution a standard graph was prepared by using different concentration of malondialdehyde.

Free fatty acid (FFA)

For the determination of free fatty acids, the method described by Koniecko (1979) was followed. Exactly 5 g nuggets blended for two minute with 30 ml of chloroform in presence of about 5g of anhydrous sodium sulphate. The mixture was filtered through Whatman No.1 filter paper into a 150 ml conical flask. About 2 to 3 drops of 0.2% phenolphthalein indicator were added to the chloroform extract, which was titrated against 0.1N alcoholic potassium hydroxide and consumed volume during titration was recorded. Percent free fatty acid was calculated as under and was also taken as absolute value in lipid profile.

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

Microbiological profile

Total plate count, psychotropic count, coliform count and yeast and mould count in the sample were determined by method described by American Public Health Association (1984). Ready made media (Hi-Media) were used for the analysis.

Sensory evaluation

The sensory evalution of fresh and stored sample was carried for different attributes viz. appearance, flavour, juiciness, texture and the overall acceptability of fresh and stored samples using 8 point descriptive scale (Seman *et al*., 1987) where 8 denotes “extremely desirable” and 1 denotes “extremely undesirable”. Seven members of the panel replicated the experiment thrice (n =21). Panelists were seated in a room free of noise and odours and suitably illuminated. Coded samples for sensory evaluation were prepared.

Statistical analysis

The results were analyzed statistically as per Snedecor and Cochran (2004). In significant effects, least significant differences were calculated at appropriate level of significance for pair wise comparison of treatment means.

Experimental design

The ethanolic-aqueous *Ocimum sanctum* extract was added in standardized formulation of chicken nuggets substituting proportionately (wt./wt.) at the level of 1, 2 and 3%. The products were vacuum packedand evaluated based on physio-chemical, sensory and microbiological profile on 0, 15th, 30th and 45th day kept under refrigeration storage at (4±1°C).

Results and Discussion

Physio-chemical parameters

The changes in physio-chemical profile of *Ocimum sanctum* extract fortified vacuum packed chicken nuggets at refrigeration temperature (4±1°C) are given in Table1.

pH

The pH of *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets was recorded as significantly low ($p<0.05$) as compared to control (Table1). The pH increased significantly on successive storage days irrespective

of levels of incorporation of *Ocimum sanctum* sp. extract in vacuum packed chicken nuggets including control. However, the inclination in pH level was significantly low in treated product as compared to control. It may be due to the fact that *Ocimum sanctum* sp. 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 *Ocimum sanctum* sp. powder (Lanjewar *et al*., 2000). The acidic pH of *Ocimum sanctum* sp. extract helpful in treatment of wilt disease of tomato plant reported by Murthy *et al.* (2014). Rojas and Brewer (2008) have reported similar findingson the effect of natural antioxidants on oxidative stability of frozen, vacuum-packed beef and pork.

Moisture

The moisture level recorded in *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets and control was comparable as the extract prepared contained similar moisture levels as present in meat emulsion. However, the moisture level observed in *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets and control decreased significantly ($p<0.05$) on successive refrigeration storage days. This fact is supported by the finding of Lanjewar *et al*. (2000); Shan *et al*. (2005) and Wojdylo *et al*. (2007). They 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.

Thiobarbituric acid reacting substances (TBARS)

The TBARS 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. The TBARS values decreased significantly ($p<0.05$) with increased in the level of incorporation of *Ocimum sanctum* sp. extract while it was found to be significantly ($p<0.05$) increased on successive storage days in *Ocimum sanctum* sp. extract incorporated vacuum packedchicken nuggets including control Table 1. The TBARS value indicated that control vacuum packed chicken nugget was not suitable for consumption on 45th day of refrigeration storage whereas all levels of *Ocimum sanctum* sp. extract incorporated vacuum packedchicken nugget were found to be suitable for consumption even on 45th day. This was an indicative of the fact that *Ocimum sanctum* sp. 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 *Ocimum sanctum* sp. 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 Ayo *et al*. (2005) who worked on properties of water, ethanol and methanol extract of *Ocimum sanctum* sp. 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. Fasseas (2007) reported that extract of various herbal plants causes reduction in TBA value and lipid oxidation. Rojas and Brewer (2008) had reported on similar line who worked on the effect of natural antioxidants on oxidative stability of frozen, vacuum-packed beef and pork. 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.

Free fatty acid (FFA)

The FFA value is quantitative indication of lipolysis in meat products. The FFA value of *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets recorded significantly low ($p<0.05$) in comparison to control on '0' day (Table 1). This was an indicative of the fact that *Ocimum sanctum* sp. extract had preventive effect on lipolysis and hence enhance its shelf-life. The FFA value had increased significantly ($p<0.05$) on successive refrigeration storage days in *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets including control (Table 1). This also suggested that *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets had better shelf life than control. It was due to the fact that urolic acid, apigenin, and luteolin present in extract of *Ocimum sanctum* sp. has anti-lipolytic activity due to which free fatty acid production was less. It is supported by the work of Yanpallewar *et al*. (2004) who worked on *Ocimum sanctum* sp. antioxidant and neuro-protective effect of *Ocimum sanctum* on transient cerebral ischaemia and long-term cerebral hypo perfusion. The present finding is 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 packed in vacuum and modified atmospheric packaging. Rojas and Brewer (2008) had reported on similar line who worked on the effect of natural antioxidants on oxidative stability of frozen, vacuum-packed beef and pork. The present finding is 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 *Ocimum sanctum* sp. extract fortified vacuum packed chicken nuggets at refrigeration temperature (4±1^{0}C) are presented in Table 2.

Total plate count

The total plate count was low in *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets than control on '0' day (Table 2). This was indicative of anti-microbial nature of *Ocimum sanctum sp*. extract. The total plate count was increased on successive refrigeration storage days in *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets including control (Table 2). The TPC value of control vacuum packed chicken nuggets was indicative that the product was not suitable for human consumption on 45th day of storage. The TPC value of *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets were found to be in the range of 3 $\log_{10}$ cfu/gm which was indicative of fact that *Ocimum sanctum* sp. extract incorporated vacuum packed 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 phospholipid bilayer cellular membrane and forming fatty acid hydro-peroxidase (Arques*et al*., 2008 and 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 products. 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 *Ocimum sanctum sp*. extract was having broad spectrum antimicrobial activity (Singh *et al*., 2012). Rojas and Brewer (2008) had reported on similar line who worked on the effect of natural antioxidants on oxidative stability of frozen, vacuum-packed beef and pork.

Psychrotrophic count

Psychrotropic counts were not detected till 15th day of refrigeration storage (Table 2). The psychrotropic were found to be lower on 30th and 45th day of refrigeration storage in *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets as compare to control. *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nugget was found to be suitable for human consumption even on 45th day of refrigeration storage (Table 2). It may be due the fact that the principle component urolic acid, apigenin, and luteolin present in *Ocimum sanctum* sp. 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 psychrotropic bacteria (Burt, 2007). The present result was also supported by finding of Rota *et al*. (2008) who suggested psychrotropic antimicrobial effect 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 (Table 2). 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 *Ocimum sanctum* sp. extract. Our present finding was supported by reports of Ben Sassi, *et al*., (2008); Graumann and Holley (2008), Ibrahim *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 molds count

The yeast and molds count was not detected till 15th day of storage but it was appeared in all product from 30th day onward (Table 2). The yeast and mold count of *Ocimum sanctum sp*. extract incorporated vacuum packed chicken nuggets was significantly lower than control on 30th and 45th day of storage (Table 2). It was also indicative of the fact that control vacuum packed chicken nugget was not suitable but *Ocimum sanctum* sp. extract incorporated vacuum packed 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. Moreover, these extract possess natural fungicidal effect against food borne fungi (Fisher and Phillips, 2008; Daferere *et al*., 2000 and Majhenic *et al*., 2007). Our finding was also supported by Razzaghi-Abyaneh *et al*. (2008) and El. Seedi *et al*. (2008) who reported that ethanolic extract of *Ocimum sanctum* sp. 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 and Lopez *et al*., 2007). Sanchez-Escalante *et al*. (2003) reported similarly while working on beef patties packed in modified atmosphere.

Sensory parameters

All the sensory attributes viz. color and appearance, flavour, texture, juiciness and overall acceptability was found to be lower on successive refrigeration storage in *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets including control (Table 3). The colour and appearance, flavour and juiciness of 3% *Ocimum sanctum* sp. extract incorporated chicken nuggets was found to be significantly higher than 1 and 2% *Ocimum sanctum* sp. extract incorporated and control vacuum packed chicken nuggets. The texture value of *Ocimum sanctum* sp. extract incorporated and control chicken nuggets were comparable with each other. The Overall acceptability of 3% incorporated *Ocimum sanctum* sp. extract in vacuum packed chicken nugget was higher than 1 and 2% *Ocimum sanctum* sp. extract incorporated and control vacuum packed chicken nuggets.

The vacuum packed control chicken nuggets have been quickly deteriorated on all parameters of sensory attributes as compared to *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets. The *Ocimum sanctum* sp. extract incorporated vacuum packed chicken nuggets was found to be acceptable on the basis of sensory attributes even on 45th day of refrigeration storage but the control vacuum packed chicken nuggets was rejected on 45th day of refrigeration storage.

The herbal extract has positive effect by inhibiting discoloration and off- odour formation in different meat product during refrigeration storage (Djenane *et al*., 2003; Nerin *et al*., 2006 and Camo *et al*., 2008). Natural antioxidant can positively affect colour and appearance parameter and maintained the original colour of product for longer duration during refrigeration storage (Djenane *et al*., 2003; Carpenter *et al*., 2007; Chouliara *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. Sanchez-Escalante *et al*. (2003) reported similarly while working on beef patties packed in modified atmosphere. 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 *Ocimum sanctum* sp. was used in preparation of value added vacuum packed chicken nuggets. The developed product exhibited significant ($p<0.05$) anti microbial, anti lipolytic and anti oxidant activity. The incorporation of *Ocimum sanctum* sp. extract (3%) in vacuum packed chicken nuggets has enhanced sensory scores as well as shelf life for a period of 45 days in refrigerated (4±1^{o}C) condition without any marked loss of physio-chemical,

Table 1:Mean±SE values of physico-chemical characteristics of vacuum packed chicken nuggets treated with different levels of *Ocimum sanctum* extract at refrigeration temperature

Treatments	0 Day	15th-DAY	30th-DAY	45th-0 DAY
pH				
Control (0%)	5.69±0.006^{Ad}	5.96±0.007^{Ac}	6.25±0.010^{Ab}	6.71±0.006^{Aa}
O. sanctum (1%)	5.66±0.015^{ABd}	5.85±0.008^{Bc}	6.10±0.009^{Bb}	6.47±0.009^{Ba}
O. sanctum (2%)	5.63±0.012^{BCd}	5.70±0.009^{Cc}	6.00±0.006^{Cb}	6.34±0.007^{Ca}
O. sanctum (3%)	5.58±0.016^{Cd}	5.61±0.026^{Dc}	5.83±0.014^{Db}	6.27±0.008^{Da}
Moisture				
Control (0%)	61.78±0.678^{a}	60.53±0.573^{ab}	59.95±0.332^{ab}	58.84±0.686^{b}
O. sanctum (1%)	61.93±0.332^{a}	60.77±0.328^{ab}	60.04±0.690^{bc}	58.92±0.547^{c}
O. sanctum (2%)	62.03±0.492^{a}	60.84±0.372^{ab}	60.06±0.370^{bc}	59.16±0.357^{c}
O. sanctum (3%)	62.35±0.517^{a}	61.43±0.492^{ab}	60.55±0.512^{bc}	59.59±0.621^{c}
TBARS (mg malonaldehyde/kg)				
Control (0%)	0.305±0.0011^{Aa}	0.431±0.0007^{Ab}	1.054±0.0007^{Ac}	1.309±0.0007^{Ad}
O. sanctum (1%)	0.300±0.0012^{Aba}	0.396±0.0011^{Bb}	0.676±0.0009^{Bc}	0.953±0.0015^{Bd}
O. sanctum (2%)	0.296±0.0018^{BCa}	0.365±0.0008^{Cb}	0.628±0.0010^{Cc}	0.896±0.0008^{Cd}
O. sanctum (3%)	0.291±0.0020^{Ca}	0.327±0.0009^{Db}	0.562±0.0011^{Dc}	0.782.±0.0014^{Dd}
FFA(% oleic acid)				
Control (0%)	0.0937±0.0003^{Ad}	0.1345±0.0007^{Ac}	0.2241±0.0004^{Ab}	0.3617±0.0005^{Aa}
O. sanctum (1%)	0.0917±0.0014^{Ad}	0.1142±0.0008^{Bc}	0.2041±0.0008^{Bb}	0.3195±0.0004^{Ba}
O. sanctum (2%)	0.0855±0.0013^{Bd}	0.0942±0.0007^{Cc}	0.1792±0.0007^{Cb}	0.2332±0.0007^{Ca}
O. sanctum (3%)	0.0811±0.0015^{Bd}	0.0751±0.0005^{Dc}	0.1309±0.0006^{Db}	0.2142±0.0015^{Ba}

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: Mean±SE values of microbiological characteristics of vacuum packed chicken nuggets treated with different levels of *Ocimum sanctum* extract at refrigeration temperature

Treatments	0 Day	15th-DAY	30th-DAY	45th-0 DAY
Total Plate Count (logcfu/g)				
Control (0%)	2.65±0.0052^{Ad}	3.69±0.0067^{Ac}	4.10±0.0110^{Ab}	5.05±0.0058^{Aa}
O.sanctum (1%)	2.34±0.0051^{Bd}	3.24±0.0092^{Bc}	3.80±0.0088^{Bb}	4.30±0.0079^{Ba}
O.sanctum (2%)	2.13±0.0043^{Cd}	3.05±0.0051^{Cc}	3.45±0.0113^{Cb}	3.68±0.0058^{Ca}
O.sanctum (3%)	1.80±0.0037^{Dd}	2.83±0.0069^{Dc}	3.00±0.0079^{Db}	3.44±0.0043^{Da}
Psychrotropic Count (logcfu/g)				
Control (0%)	ND	ND	1.49±0.0102^{Ab}	2.64±0.0152^{Aa}
O.sanctum (1%)	ND	ND	1.24±0.0122^{Bb}	2.16±0.0122^{Ba}
O.sanctum (2%)	ND	ND	0.88±0.0102^{Cb}	1.74±0.0112^{Ca}
O.sanctum (3%)	ND	ND	0.60±0.0012^{Db}	1.11±0.0112^{Da}
Coliform Count (logcfu/g)				
Control (0%)	ND	ND	ND	ND
O.sanctum (1%)	ND	ND	ND	ND
O.sanctum (2%)	ND	ND	ND	ND
O.sanctum (3%)	ND	ND	ND	ND
Yeast and Mould Count (logcfu/g)				
Control (0%)	ND	ND	2.31±0.0170^{Ab}	3.23±0.0081^{Aa}
O.sanctum (1%)	ND	ND	2.01±0.0082^{Bb}	2.81±0.0047^{Ba}
O.sanctum (2%)	ND	ND	1.71±0.0310^{Cb}	2.39±0.0076^{Ca}
O.sanctum (3%)	ND	ND	1.67±0.0075^{Db}	1.95±0.0222^{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 3: Mean±SE values of sensory attributes of vacuum packed chicken nuggets treated with different levels of *Ocimum sanctum* extract at refrigeration temperature

Treatments	STORAGE PERIOD(DAYS)			
	0 Day	15th-DAY	30th-DAY	45th-0 DAY
Colour and appearance				
Control (0%)	6.53 ± 0.095^{Ba}	5.93 ± 0.124^{Bb}	5.61 ± 0.083^{Bc}	3.97 ± 0.109^{Bd}
O.sanctum (1%)	6.74 ± 0.130^{Ba}	6.13 ± 0.120^{Bb}	5.78 ± 0.114^{Bc}	4.09 ± 0.106^{Bd}
O.sanctum (2%)	6.78 ± 0.082^{Ba}	6.22 ± 0.078^{Bb}	5.91 ± 0.115^{Bc}	4.17 ± 0.071^{Bd}
O.sanctum (3%)	7.18 ± 0.099^{Aa}	6.74 ± 0.068^{Ab}	6.35 ± 0.063^{Ac}	4.86 ± 0.075^{Ad}
Flavour				
Control (0%)	5.96 ± 0.115^{Ca}	5.94 ± 0.103^{Ca}	5.29 ± 0.068^{Cb}	4.13 ± 0.089^{Cc}
O.sanctum (1%)	6.22 ± 0.126^{Ca}	6.05 ± 0.131^{Ca}	5.39 ± 0.154^{Cb}	4.22 ± 0.075^{Cc}
O.sanctum (2%)	6.74 ± 0.113^{Ba}	6.51 ± 0.147^{Ba}	5.91 ± 0.147^{Bb}	5.01 ± 0.134^{Bc}
O.sanctum (3%)	7.35 ± 0.133^{Aa}	6.93 ± 0.144^{Ab}	6.32 ± 0.132^{Ab}	5.84 ± 0.131^{Ad}
Texture				
Control (0%)	7.41 ± 0.089^{Aa}	6.84 ± 0.108^{Ab}	5.56 ± 0.084^{Ac}	4.62 ± 0.101^{Ad}
O.sanctum (1%)	7.43 ± 0.112^{Aa}	6.87 ± 0.136^{Ab}	5.65 ± 0.091^{Ac}	4.67 ± 0.133^{Ad}
O.sanctum (2%)	7.52 ± 0.113^{Aa}	6.98 ± 0.127^{Ab}	5.73 ± 0.125^{Ac}	4.78 ± 0.132^{Ac}
O.sanctum (3%)	7.37 ± 0.079^{Aa}	6.79 ± 0.108^{Ab}	5.52 ± 0.088^{Ac}	4.65 ± 0.064^{Ac}
Juiciness				
Control (0%)	6.81 ± 0.103^{Ca}	6.34 ± 0.094^{Cb}	5.58 ± 0.124^{Cc}	4.89 ± 0.094^{Cd}
O.sanctum (1%)	7.05 ± 0.084^{BCa}	6.55 ± 0.105^{BCb}	5.86 ± 0.114^{BCc}	5.12 ± 0.076^{BCd}
O.sanctum (2%)	7.14 ± 0.113^{Ba}	6.67 ± 0.094^{Bb}	5.98 ± 0.096^{Bc}	5.23 ± 0.103^{Bd}
O.sanctum (3%)	7.46 ± 0.081^{Aa}	6.98 ± 0.091^{Ab}	6.42 ± 0.102^{Ac}	5.50 ± 0.051^{Ad}
Overall acceptability				
Control (0%)	6.86 ± 0.106^{Ca}	6.22 ± 0.072^{Cb}	5.41 ± 0.142^{Cc}	3.96 ± 0.118^{Cd}
O.sanctum (1%)	6.98 ± 0.117^{Ca}	6.34 ± 0.078^{Cb}	5.53 ± 0.110^{Cc}	4.13 ± 0.064^{Cd}
O.sanctum (2%)	7.27 ± 0.066^{Ba}	6.72 ± 0.115^{Bb}	5.90 ± 0.084^{Bc}	4.55 ± 0.117^{Bd}
O.sanctum (3%)	7.58 ± 0.092^{Aa}	7.17 ± 0.072^{Ab}	6.32 ± 0.091^{Ac}	5.01 ± 0.093^{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.

microbial and sensory quality. The result revealed the possible application of *Ocimum sanctum* sp. extract (3%) as a natural source of anti oxidant in development of vacuum packed chicken meat product with potential health benefits.

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