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Effect of Rosemary (*Rosmarinus officinalis*), Oregano (*Origanum vulgare*), Thyme (*Thymus vulgaris*) on the physico-chemical, microbial and sensory attributes of chicken nuggets

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ABSTRACT

A comparative study was undertaken to evaluate the antimicrobial and antioxidant efficacy of Rosemary oil (RO), Oregano oil (OO), and Thyme oil (TO) on physico-chemical, microbial and sensory attributes of chicken nuggets. Chicken nuggets were formulated with the addition of a pre-standardized level of 0.1% essential oils of RO (T_1), OO (T_2), TO (T_3), along with a control. Fresh chicken nugget developed were analyzed for various physico-chemical (emulsion & product pH, emulsion stability, product yield, shear force value, proximate analysis, calorific value, DPPH scavenging activity, total phenolic content, fatty acid composition) and sensory parameters. The physic-chemical, proximate analysis, fatty acid composition and sensory attributes of treatment groups were significantly ($p<0.05$) higher than control. In order to evaluate the antimicrobial efficacy of essential oil, the aerobically packed chicken nuggets were subjected to storage studies viz., product pH, free fatty acids, DPPH scavenging activity, thiobarbituric acid reactive substances value, tyrosine value, microbiological and sensory parameters for 35day's at 7day's interval under refrigerated condition. As the storage period progresses the quality attributes gradually and significantly ($p<0.05$) decreases, but were well within the limits of acceptability. Thus it is concluded that among the three different essential oils used, 0.1% level of thyme oil added to chicken nuggets was found to be optimum and it can be effectively stored upto 28day's under refrigerated conditions (4±1°C) without marked loss in storage quality.

Keywords: *Chicken nuggets, rosemary oil, oregano oil, thyme oil, antimicrobial and antioxidant efficacy*

Introduction

Chicken meat provides highly versatile muscle food with low in fat and calories than meat from other species, but rich in B-complex vitamins, essential amino acids, USFA, phospholipids and minerals. It is a very well - recognized nutritious food due to its flavour, tenderness, ease of digestion and delicacy. So, it can be processed into a novel, tasty, appealing, convenient and ready-to-eat nuggets, which can be more widely acceptable by the consumer [1]. The addition of antimicrobial chemical preservatives can better protect the meat and it's product from microorganisms. Because of the awareness among the consumers, they prefer natural food without any chemical preservatives. Nowadays, it has been proven that the best alternative for chemical preservatives are the oils of spices, which can stabilize the meat from microbial deterioration. These spices are commonly used as flavor enhancers of food. In addition to flavoring foods, essential oils of spices are rich source of biologically active compounds and have been shown to possess antibacterial, antifungal, antiviral and antiprotozoal actions besides beneficial effects on the cardiovascular and immune system [2].

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Rosemary *(Rosmarinus officinalis)*, extract has potent antioxidant activity mainly due to the presence of several phenolic diterpenes, such as carnosic acid, carnosol, rosmanol, rosmariquinone and rosmaridiphenol [3]. This herbal spice is used in cuisines as dried leaves not only to improve or modify the flavor of food but also to prevent its deterioration [4]. Oregano (*Origanum vulgare*) is well known for its antioxidative and antimicrobial properties mainly due to its polyphenol contents; carvacrol, thymol, p-cymene and terpinene. Even though oregano essential oil has a great potential as a natural antioxidant, little is known about the optimal level to prevent oxidative changes of meat product while maintaining other quality parameters such as flavour. Thyme (*Thymus vulgaris*) has number of critical compounds such as the phenols: thymol (44-60%) and carvacrol (2.2 -4.2 %) [5]. Most commonly the leaves of wild, green and lemon thyme are used as a flavoring agent in the meat cooking process.

Hence, the essential oils of spices have the greatest advantage to cater to the demands of the consumer for the natural preservatives. In this context, the present investigation has been designed to elicit the antimicrobial and antioxidant efficacy of rosemary, oregano and thyme oil on physico-chemical, microbial and sensory attributes of chicken nuggets and their storage stability.

Materials and Methods

Procurement of materials for the preparation of chicken Nuggets

Dressed broiler chicken were procured from the retail outlets in the vicinity of VCRI, Tirunelveli town, TN. The carcasses were trimmed off the visible adipose and connective tissues and hygienically deboned in the meat processing unit of the department. The deboned meat was minced in a meat mincer and stored at (-18±2°C) in LDPE pouches until further use. Commercially available refined oil was used in preparation. The condiments paste used contained fresh onion, garlic and ginger in the 1:1:1 ratio. All the spices were purchased from local market of Tirunelveli town. Certified food grade essential oil (Rosemary, Oregano & Thyme) was purchased and used.

Preparation of chicken nuggets

Based on the preliminary trials, the basic formulation of the chicken nuggets was standardized. For 1kg lean chicken meat, the following ingredients were added at the rate of salt 2%, vegetable oil 5%, dry spices mix 2.5%, wet condiments 2.5%, refined wheat flour 3%, ice flakes 5%. The meat emulsion was prepared by using bowl chopper. The emulsion was filled into parchment paper lined stainless steel moulds and cooked in a steam cooker for 40 minutes till it reaches, the internal temperature (80±2°C). The blocks were allowed to cool at room temperature after removal from pressure cooker and then cut into nuggets of (4×1.5×1.5cm) and packed in polyethylene pouches and stored (4±1°C) for further studies.

Physico-chemical Properties

The pH of emulsion and product was measured by using a digital pH meter as per [6]. Weight of raw and cooked chicken nuggets were recorded to calculate the product yield. Standard procedures were used for the determination of emulsion stability [7] shear force value [8], proximate analysis [9], DPPH scavenging activity [10], total phenolic content [11], fatty acid composition [12].

Storage studies

The product was evaluated for physico-chemical parameters, microbial and sensory attributes. The free fatty acid (FFA) content of the chicken nuggets was determined by [13]. Thiobarbituric acid reactive substances value was measured by a method of [14]. Tyrosine value was estimated by modified method of [15]. Total plate count, psychrophilic count, coliform count, staphylococcal count and yeast and mould count was enumerated as per the procedure by [16].

Sensory Evaluation

The sensory evaluation of chicken nuggets was performed by panel of eight semi-trained members based on 8 points hedonic scale, wherein 8 denoted "extremely acceptable" and 1 denoted "extremely unacceptable" for sensory attributes viz., appearance, flavour, spiciness, juiciness, texture and overall acceptability [17].

Statistical Analysis

The data generated were subjected to one way-ANOVA and critical difference as per the procedure of [18] and means were compared by using Dunkan's multiple range tests [19].

Results and Discussion

Physico-chemical characteristics:

The mean and SE values of various physico-chemical characteristics of chicken nuggets incorporated with essential oils along with control are presented in Table 1.

Table 1: Effect of incorporation of rosemary, oregano and thyme oil on physico-chemical characteristics and proximate analysis of chicken nuggets

Parameters	C	T_1	T_2	T_3
		RO (0.1%)	OO (0.1%)	TO (0.1%)
Emulsion pH	6.05±0.05	6.07±0.05	6.08±0.05	6.09±0.04
Product pH	6.16±0.05	6.17±0.05	6.19±0.05	6.21±0.05
Emulsion stability (%)	95.74±0.28	96.31±0.40	96.48±0.50	96.87±0.37
Product yield (%)	94.14±0.57	94.64±0.65	95.06±0.66	95.33±0.61
Shear force value (kg/cm^2)	0.6 1±0.05	0.48±0.03	0.44±0.04	0.41±0.04
Moisture (%)	64.14±0.84	64.45±0.77	64.12±0.94	64.55±0.62
Crude Protein (%)	19.22±0.90	19.65±0.59	19.36±1.02	19.56±0.93
Crude Fibre (%)	0.31±0.06^a	0.49±0.11^b	0.53±0.09^b	0.52±0.04^b
Ether Extract (%)	5.12±0.37	5.09±0.63	5.01±0.51	4.92±0.39
Total Ash (%)	2.40±0.07	2.45±0.36	2.43±0.27	2.39±0.13
Calorific value (kcal/kg)	1756.83±67.58	1787.17±41.38	1779.83±58.71	1784.33±58.07
DPPH scavenging activity of essential oils	-	17.33±1.67^a	81.14±0.68^c	34.66±1.15^b
DPPH scavenging activity of essential oils in product	20.61±1.27^a	28.24±1.76^b	39.11±2.05^c	55.67±1.27^d
Total phenolic content in product	606.50±4.62^a	662.50^b±4.81^b	728.67±4.94^c	796.67±5.57^d

Note: Means bearing different superscripts in a row differ significantly (P<0.05)

pH and proximate analysis: Inclusion of essential oils resulted in non-significant (p>0.05) increases in pH values of both emulsion and product. Conversely, [20] observed that the addition of clove oil decreased the product pH. The possible reasons for this contradiction may be due the difference in the concentration (2%) and interaction of a combination of essential oils. Upon cooking, pH showed an increase in values among all the treatments and control. This finding was consistent with the observation of [21] who found no difference in the pH of control and test antioxidants like grape seed, bearberry and rosemary extracts incorporated raw and cooked pork meat products. Emulsion stability and product yield was found to be similar for both control and treatment. Shear force value (kg/cm^2) is a measure that indicates the toughness or tenderness of the product. The treatment had recorded a non-significantly lower shear force value than control. The result was in coincidence to [22] who observed a significant decrease in shear force value in rosemary extract-incorporated chicken nuggets. Proximate analysis of the chicken nuggets had revealed non-significant increasing trend except crude fibre and ether extract. The per cent moisture content of control and treated nuggets was found to be similar. The mean per cent of protein content in the chicken nuggets was found to range from 19.22 to 19.65 in the present investigation. The per cent fat content in the chicken nuggets (control) was slightly higher than in treatment. The per cent fat content ranged from 4.92 to 5.12.

The per- cent crude fibre content was found significantly higher in (T3) when compared to other treatments and control. The per cent total ash content of the control and treated nuggets was found to be similar. A similar result was observed in ginger essential oil-added beef patties by [23].

Fatty acid composition: In the present study, essential oils significantly ($p<0.05$) influenced the percent palmitoleic acid, linoleic acid, saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acid. Study recorded MUFA, PUFA in thyme oil treated chicken nuggets than other treatment and control. The overall fatty acid content was significantly ($p<0.05$) higher in thyme oil-treated chicken nuggets than the other treatment. Among the fatty acid of treatment and control, linoleic acid had recorded highest value whereas myristic value had recorded lowest value, respectively.

Table 2: Effect of incorporation of rosemary, oregano and thyme oil on fatty acid composition of chicken nuggets

Name of fatty acids	C	T_1 REO (0.1%)	T_2 OO (0.1%)	T_3 TO (0.1%)
Myristic (%)	0.38±0.03^a	0.49±0.03^b	0.56±0.03^c	0.61±0.03^d
Palmitic (%)	12.37±0.50^a	15.23±0.60^b	16.12±0.72^c	17.32±0.77^d
Palmitoleic (%)	1.38±0.12^a	1.57±0.11^b	1.65±0.09^c	1.71 ±0.07^d
Stearic (%)	3.89 ±0.11^a	4.24±0.15^b	4.59±0.18^c	4.93±0.19^d
Oleic (%)	30.89±1.58	28.59±2.12	29.95±1.36	28.31±5.14
Linoleic (%)	40.54±3.64^a	45.18±3.73^b	47.64±3.57^c	48.94±3.8^d
Linolenic (%)	0.64±0.03^a	0.74±0.04^b	0.77±0.04^c	0.80±0.04^d
Arachidonic (%)	0.46±0.03^a	0.68±0.06^b	0.79 ±0.04^c	0.94±0.06^d

Note: Means bearing different superscripts in a row differ significantly ($p<0.05$).

Sensory attributes: The mean and SE values of sensory evaluation of chicken nuggets incorporated with different essential oil are presented in Table 3.

Table 3: Effect of incorporation of rosemary, oregano and thyme oil on sensory attributes of chicken nuggets

Sensory attributes	C	T_1 RO (0.1%)	T_2 OO (0.1%)	T_3 TO (0.1%)
Appearance	6.87±0.10	6.97±0.11	7.05±0.12	7.15±0.11
Flavour	6.52±0.21^{bc}	6.35±0.17^a	6.45±0.18^b	6.97±0.11^c
Spiciness	6.39±0.21	6.43±0.19	6.48±0.16	6.51±0.15
Texture	7.17±0.07	7.12±0.08	7.19±0.13	7.22±0.13
Juiciness	6.93±0.16^b	6.89±0.17^a	7.01±0.16^{ab}	7.12±0.15^c
Overall acceptability	6.42±0.17^b	6.25±0.09^a	6.32±0.18^a	6.87±0.09^b

Note: Means bearing different superscripts in a row differ significantly ($p<0.05$)

The mean value for appearance did not differ significantly ($p>0.05$) between treatments and control. However, there was a gradual increase in sensory scores of appearance with the different essential oil incorporated in chicken nuggets. Flavor and overall acceptability scores of treated nuggets were found to be significantly ($p<0.05$) higher than control nuggets and other treated nuggets. The texture and juiciness scores increased numerically in treated nuggets. However, no significant difference ($p>0.05$) was found between the treatments and control. Nuggets incorporated with 0.1% thyme oil (T_3) had scored the highest flavor, juiciness, and overall acceptability value.

Storage study: The mean and SE values of various physic-chemical, microbial and sensory attributes of chicken nuggets incorporated with three different essential oil (T_1) RO - (0.1%); (T_2) OO - (0.1%) and (T_3) TO - (0.1%) stored under refrigerated condition are represented in Table 4, 5 & 6.

Table 4: Effect of incorporation of essential oils on physico-chemical parameters of chicken nuggets stored at refrigerated condition (4±1°C)

	Storage days					
Treatments	**0**	**7**	**14**	**21**	**28**	**35**
Product pH						
C	6.20±0.03^A	6.24±0.03^{AB}	6.28±0.02^B	6.36±0.02^{cC}	6.38±0.02^{bC}	6.44±0.01^{bC}
T1	6.22±0.03^A	6.24 ±0.03^A	6.29±0.02^{AB}	6.34±0.02^{bcB}	6.35±0.02^{abB}	6.36±0.02^{aB}
T2	6.21±0.03^{AB}	6.19±0.03^A	6.24±0.02^{ABC}	6.28±0.02^{aBC}	6.30±0.02^{aC}	6.31±0.02^{aC}
T3	6.19±0.03^A	6.21±0.03^A	6.25±0.02^{AB}	6.29±0.02^{abBC}	6.33±0.02^{abBC}	6.35±0.02^{aC}
Free fatty acids (% oleic acid)						
C	0.22±0.03	0.23±0.03	0.24±0.03	0.25±0.03	0.27±0.03	0.29±0.03
T1	0.22±0.03	0.22±0.03	0.23±0.03	0.24±0.03	0.26±0.03	0.27±0.03
T2	0.21±0.03	0.22±0.03	0.23±0.03	0.24±0.03	0.26±0.03	0.27±0.03
T3	0.21±0.03	0.22±0.03	0.23±0.03	0.24±0.02	0.26±0.03	0.27±0.03
DPPH scavenging activity (%)						
C	31.52±1.49^{aC}	31.05±1.08^{aBC}	29.28±0.50^{aBC}	28.13±0.56^{aB}	24.73±0.48^{aA}	22.24±0.51^{aA}
T1	40.30±2.85^{bB}	39.27±2.97^{bB}	36.65±1.74^{bAB}	35.26±1.55^{bAB}	34.16±1.34^{bAB}	32.49±1.24^{bA}
T2	46.50±2.09^{bcB}	45.17±1.88^{bcB}	44.49±1.59^{cB}	41.54±1.62^{cAB}	38.83±1.33^{cA}	37.35±1.29^{cA}
T3	48.82±2.3^{cC}	47.06±2.13^{cBC}	45.57±1.56^{cBC}	42.67±1.12^{cAB}	39.87±0.90^{cA}	38.06 ±0.98^{cA}

TBARS (mg malonaldehye/kg)						
C	0.25±0.04^{bA}	0.39±0.07^{bAB}	0.52±0.04^{bB}	0.72±0.06^{bC}	0.82±0.06^{bC}	1.05±0.02^{bD}
T1	0.18±0.03^{abA}	0.21±0.04^{aA}	0.27±0.06^{aAB}	0.27±0.06^{aAB}	0.30±0.06^{aAB}	0.41±0.05^{aB}
T2	0.16±0.02^{aA}	0.18±0.02^{aA}	0.22±0.02^{aAB}	0.23±0.03^{aAB}	0.27±0.02^{aB}	0.40±0.04^{aC}
T3	0.14±0.02^{aA}	0.16±0.03^{aA}	0.21±0.03^{aAB}	0.22±0.03^{aAB}	0.27±0.03^{aB}	0.40±0.05^{aC}
Tyrosine value (mg/100gm)						
C	13.15±0.84^{A}	22.04±0.93^{bB}	25.22±1.19^{bC}	26.42±1.06^{bC}	27.29±1.10^{bC}	27.70±1.32^{bC}
T1	11.54±0.60^{A}	11.53±0.66^{aA}	12.90±0.77^{aAB}	14.60±0.82^{aBC}	15.87±0.77^{aC}	20.68±0.71^{aD}
T2	11.04±0.68^{A}	11.36±0.69^{aA}	12.27±0.64^{aB}	13.18±0.91^{aAB}	15.08±0.64^{aB}	17.97±0.69^{aC}
T3	11.37±0.62^{A}	11.48±0.74^{aA}	12.46±0.88^{aA}	13.40±0.72^{aAB}	15.58±0.96^{aB}	18.34±1.05^{aC}
Note: Means bearing different upper case letter superscripts in a row and lower case letter superscripts in a column differ significantly (p<0.05)						

Physico-chemical properties:

pH: The mean pH values showed a significant ($p<0.05$) increasing trend from day 0 to day 35 in case of control as well as treated samples. This is in agreement with the reports of [24] in pork patties, [25] in chicken patties, [26] in goat meat patties, [27] in chicken nuggets, [28] in chicken meat balls and [29] in fresh chicken sausages. The pH of treated products had recorded lower values throughout the storage period as compared to control. This might be due to antimicrobial effect of rosemary, oregano and thyme oil.

Free fatty acid (% oleic acid): FFA increased non-significantly ($p>0.05$) from day 0 to 35 in all chicken nuggets preparations. The lower FFA content of the treated products might be attributed to the antioxidant properties of the rosemary, oregano and thyme oil. A non-significant increasing trend was recorded with storage in case of control and treated nuggets. The result of the present study is following [30] in chicken nuggets. FFA content of the products was well below the threshold value i.e. 1.8% [31].

DPPH scavenging activity: The DPPH scavenging activity of essential oil (rosemary, oregano and thyme oil) in the product showed there was a significant difference ($p<0.05$) of control and treated nuggets, whereas treated nuggets were comparable. Higher DPPH scavenging activity in treated products might be attributed to the presence of various antioxidants such as phenolic acids, phenolic diterpenes, flavonoids, monoterpenes and volatile oils in essential oils [32].

TBARS value: The TBARS values followed a trend similar to FFA values with a significantly ($p<0.05$) increasing trends towards the advancement of the storage period. TBARS concentration in all the chicken nuggets (treatment) during the entire storage study were well below the threshold level of lipid oxidation (1-2mg malonaldehyde/kg) suggested by [33] which indicates the antioxidant activity of essential oils in the chicken nuggets [5]. TBARS values greater than 1 were observed in control at 35th day of the storage period. This could be due to lipid hydrolysis, oxidative rancidity and secondary products formation at refrigeration temperature [34].

Tyrosine value: Tyrosine value is an indicator of proteolysis in meat and meat products due to bacterial action [35]. In the present study, there was a significant ($p<0.05$) increase in tyrosine value noticed in both control and treatment. When a comparison was made between control and treatment, (T_3) product had recorded a much lower tyrosine value.

The changes observed in microbiological parameters of the aerobically packed chicken nuggets (T_1) RO - (0.1%); (T_2) OO - (0.1%) and (T_3) TO - (0.1%) in LDPE pouches stored under refrigerated storage condition at 7 days interval are represented in Table 5.

Determination of microbiological counts is obvious to determine the resistance of the product to spoilage.

Total plate count: The mean value of Total plate count (log_{10}cfu/g) increased significantly ($p<0.05$) as the storage days progressed well within the acceptable threshold limit. When comparison was made between control and treatment, the lower microbial count was noted in (T_3) treated samples which might be due to the presence of antimicrobial compounds in essential oils. Similar to these findings, [36] reported that increase in total plate counts while studying the quality attribute of chicken nuggets extended with rice flour. Similar load of SPC (log 3.47-4.4/gm) in low fat chicken nuggets during a storage study (4±1°C) for 20 days was reported by [37].

Table 6: Mean (± S.E.) values of the microbial load of aerobically packed essential oils and their combination incorporated chicken nuggets stored at refrigerated temperature (4±10C)

Storage days						
Treatments	**0**	**7**	**14**	**21**	**28**	**35**
Total viable count (log_{10} cfu/gm)						
C	2.82±0.25^{bA}	3.31±0.23^{bAB}	3.58±0.22^{bBC}	3.79±0.19^{bBCD}	4.05±0.13^{bCD}	4.23±0.13^{bD}
T1	2.12±0.03^{aA}	2.32±0.10^{aAB}	2.46±0.17^{aABC}	2.82±0.22^{aBCD}	2.94±0.24^{aCD}	3.09±0.24^{aC}
T2	2.05±0.05^{aA}	2.19±0.08^{aAB}	2.36±0.10^{aAB}	2.48±0.18^{aAB}	2.64±0.17^{aBC}	2.97±0.20^{aC}
T3	2.02±0.07^{aA}	2.13±0.05^{aAB}	2.28±0.06^{aABC}	2.40±0.12^{aBC}	2.60±0.15^{aCD}	2.90±0.19^{aD}
Staphylococcal count (log_{10} cfu/gm)						
C	2.04±0.13^{A}	2.24±0.14^{bAB}	2.41±0.12^{BC}	2.53±0.08^{BC}	2.63±0.04^{C}	2.66±0.07^{cC}
T1	1.46±0.22^{A}	1.64±0.24^{abAB}	1.88±0.24^{AB}	1.99±0.21^{AB}	2.09±0.18^{AB}	2.26±0.13^{abB}
T2	1.43±0.18^{A}	1.58±0.18^{aAB}	1.85±0.21^{AB}	1.93±0.22^{AB}	2.04±0.23^{AB}	2.25±0.23^{abB}
T3	1.41±0.25	1.62±0.22^{ab}	1.78±0.27	1.90±0.27	1.99±0.27	2.18±0.31^{a}
Coliform count (log_{10} cfu/gm ^{A})						
C	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected

T1	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
T2	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
T3	Not detected	Not detected	Not detected	Not detected	Not detected	Not detected
Psychrophillic count (log_{10} cfu/gm)						
C	Not detected	Not detected	1.39±0.23	1.64±0.25^{A}	2.12±0.26^{AB}	2.68±0.15^{bB}
T1	Not detected	Not detected	1.15±0.13	1.34±0.24	1.65±0.17	2.58±0.08^{a}
T2	Not detected	Not detected	1.09±0.20	1.28±0.24^{B}	1.60±0.28^{B}	2.37±0.10^{aB}
T3	Not detected	Not detected	1.10±0.12	1.25±0.17^{AB}	1.57±0.20^{AB}	2.32±0.09^{aB}
Yeast and mould count (log_{10} cfu/gm)						
C	Not detected	Not detected	1.40±0.31^{A}	1.77±0.29^{AB}	2.24±0.23^{BC}	2.64±0.19^{bC}
T1	Not detected	Not detected	1.09±0.25^{A}	1.43±0.27^{AB}	1.79±0.26^{AB}	2.07±0.20^{aB}
T2	Not detected	Not detected	1.03±0.24^{A}	1.31±0.20^{AB}	1.75±0.20^{AB}	2.01±0.17^{abB}
T3	Not detected	Not detected	1.04±0.27	1.36±0.31	1.77±0.27	2.01±0.19^{ab}
Note: Means bearing different upper case letter superscripts in a row and lower case letter superscripts in a column differ significantly ($p<0.05$)						

Psychrophilic count: Psychrophilic counts (log_{10}cfu/g) were not observed on day 0 and day 7 in any of the chicken nuggets preparations. Psychotropic colonies appeared on day 14th of refrigerated storage. This appearance of psychrotrophs after such a long gap might be caused by sufficient heat treatment during cooking which drastically injured and killed the psychotropic population reducing the number of surviving injured and resistant ones to a non-countable limits [35]. All the groups revealed a non-significant increase in counts with an increase in storage days. (T_3) had showed significantly ($p<0.05$) lower counts than other treated and control. Among the treatment groups, thyme oil treated group showed a significantly ($p<0.05$) lower count than rosemary and oregano - treated group. However, psychrophilic counts in all three treated groups remained significantly ($p<0.05$) lower than control 35 day of storage. Natural preservatives such as thyme oil showed antimicrobial activity against psychrophilic bacteria in chicken nuggets.

Coliform count: No coliform count (log_{10}cfu/g) were detected in any of the preparation during the entire storage period. Coliform are an important source of faecal contamination. The absence of coliforms during storage depicts that heat processing and subsequent hygienic handling and packaging was effective controlling coliform growth in chicken nuggets.

Yeast and Mould count: Yeast and Mould (log_{10} cfu/g) counts were not observed in control and treated chicken nuggets upto 7 days of refrigerated storage condition. Though the groups showed a gradual and significant ($p<0.05$) increase in counts from 14th day onward but no significant differences were observed between treated and control samples on all the days of storage.[38] reported that aqueous and ethanolic extracts of cardamom and star anise showed same antimicrobial effect against yeast and mould. The appearance of yeasts and moulds could be due to chemical and enzymatic activities that breakdown fat, protein and carbohydrates of meat product resulting in slime formation [39].

***Staphylococcal count**:* The staphylococcal count (log_{10} cfu/g) for control and treated (rosemary, oregano and thyme) group ranged between (1.46 to 2.26, 1.43 to 2.25 and 1.41 to 2.18), respectively for 0 day to 35 day of storage. Both treatment groups contained significantly ($p<0.5$) lower staphylococcal count than control on 0 day. Though the groups showed a gradual and significant ($p<0.05$) increase in counts from 0 to 35 day, the values for both treated groups remained significantly ($p<0.05$) lower than control throughout the storage period. However all the microbial counts were well within the acceptable limits and much below the threshold values for spoilage [35].

The changes observed in sensory attributes of the aerobically packed chicken nuggets (T_1) RO - (0.1%); (T_2) OO - (0.1%) and (T_3) TO – 0.1%) in LDPE pouches stored under refrigerated storage condition at 7 days interval are represented in Table 6.

Sensory attributes: The mean and SE values of various sensory attributes of aerobically packed chicken nuggets containing different essential oil viz., T_1, T_2 and T_3 are presented in Table 6.

Appearance: The appearance scores showed a significant ($p<0.05$) decreasing trend throughout the period of storage for all products. This decreasing trend might be due to pigment and lipid oxidation and non-enzymatic browning resulting from Millard's reaction [40]. Significantly ($p<0.05$) higher scores were recorded for the treated products (T2) in comparison to control on all days of storage. This might be attributed to the color pigments and phenolic compounds of essential oil which are said to have antioxidant and antimicrobial properties.

Flavour: The scores for flavor decreased significantly ($p<0.05$) for all products as the period of storage progressed. Fat oxidation and proteolysis by microbial activity in stored chicken nuggets could be the reason for getting lower flavor scores. The results of the present study were in agreement with the findings of [41] who found the reduction of flavor scores in chevon sausage incorporated with different concentrations (0.25 - 0.75%) of *Tribulus terrestris* under storage condition.

Spiciness: Spiciness scores of with no significant ($p>0.05$) difference among the treatments where T3 had the lowest score.

Texture: A significant ($p<0.05$) decreasing trend was observed with storage for control as well as treated products (T1, T2 and T3) which might be due to loss of moisture, breakdown of fat and degradation of proteins by bacterial action [35]. The nuggets incorporated with thyme oil (0.1%) (T2) showed significantly higher scores for texture. These higher scores of treated products could be attributed to the antimicrobial and antioxidant properties of the extract.

Juiciness: The scores for juiciness decreased significantly ($p<0.05$) for control as well as treated products (T_1, T_2 and T_3) with storage. This might be due to the continuous moisture loss from the products during the storage period. The results were in agreement with (40) who observed similar juiciness scores in chevon sausage incorporated with *Tribulus terrestris* during refrigerated storage.

Overall acceptability: The mean scores decreased significantly ($p<0.05$) with the advancement of the storage period for all products. This might be reflective of the decline in scores for appearance, flavor, juiciness and texture. Significantly ($p<0.05$) higher scores were observed for the thyme
oil -treated samples. [42] also observed an increase in the overall acceptability scores of meat products with the addition of some natural antioxidants.

Table 7: Mean (± S.E.) values of the sensory attributes of aerobically packaged essential oils and their combination incorporated chicken nuggets stored at refrigerated temperature (4 ±10C)

	Storage days					
Treatments	**0**	**7**	**14**	**21**	**28**	**35**
			Appearance			
C	6.87±0.18^{C}	6.74±0.16^{BC}	6.43±0.17^{ABC}	6.27±0.15^{AB}	6.03±0.14^{A}	Spoiled
T1	6.88±0.17^{bB}	6.77±0.16^{bB}	6.44±0.15^{abAB}	6.25±0.14^{abA}	6.07±0.16^{abA}	5.94±0.19^{aA}
T2	6.95±0.19^{cC}	6.88±0.18^{cC}	6.52±0.16^{bcBC}	6.29±0.14^{abABC}	6.13±0.16^{abAB}	5.98±0.15^{aA}
T3	6.89±0.19^{C}	6.74±0.17^{C}	6.45±0.14^{BC}	6.22±0.15^{AB}	6.05±0.16^{AB}	5.88±0.14^{A}
			Flavour			
C	6.85±0.14^{D}	6.69±0.11^{CD}	6.38±0.12^{BC}	6.12±0.10^{AB}	6.00±0.10^{bA}	Spoiled
T1	6.77±0.15^{C}	6.68±0.18^{BC}	6.29±0.14^{B}	6.06±0.10^{AB}	5.92±0.10^{aAB}	5.79±0.11^{abA}
T2	6.82±0.17^{B}	6.70±0.16^{B}	6.37±0.14^{AB}	6.20±0.13^{A}	6.11±0.16^{aA}	5.99±0.14^{bA}
T3	6.69±0.15^{C}	6.63±0.16^{C}	6.23±0.14^{B}	5.97±0.11^{AB}	5.73±0.10^{aA}	5.62±0.11^{aA}
			Spiciness			
C	6.78±0.17	6.68±0.18	6.65±0.20	6.55±0.21	6.31±0.23	Spoiled
T1	6.39±0.20	6.31±0.21	6.27±0.23	6.18±0.22	6.16±0.22	6.01±0.25
T2	6.54±0.19	6.41±0.22	6.39±0.23	6.31±0.26	6.22±0.27	6.06±0.28
T3	6.38±0.19	6.26±0.20	6.21±0.17	6.12±0.26	6.04±0.29	5.71±0.28
			Texture			
C	7.01±0.18^{BC}	6.93±0.20 ^{BC}	6.78±0.20^{BC}	6.38±0.20^{AB}	6.16±0.20^{A}	Spoiled
T1	6.62±0.18^{B}	7.01±0.18 ^{B}	6.28±0.19^{AB}	6.19±0.20^{AB}	6.08±0.21^{AB}	6.00±0.22^{A}
T2	6.83±0.17^{B}	7.04±0.17^{B}	6.68±0.19^{AB}	6.53±0.25^{AB}	6.37±0.26^{AB}	6.16±0.25^{A}
T3	6.55±0.16^{B}	6.80±0.21^{AB}	6.19±0.22^{AB}	6.10±0.23^{AB}	5.95±0.23^{AB}	5.84±0.17^{A}
			Juiciness			
C	6.75±0.17^{B}	6.58±0.13^{B}	6.41±0.17^{AB}	6.24±0.18^{AB}	6.03±0.18^{A}	Spoiled
T1	6.47±0.15^{B}	6.36±0.15^{AB}	6.32±0.16^{AB}	6.14±0.16^{AB}	6.02±0.17^{AB}	5.92±0.17^{A}
T2	6.52±0.18	6.44±0.18	6.38±0.18	6.19±0.17	6.03±0.16	5.96±0.17
T3	6.39±0.16^{B}	6.28±0.18^{AB}	6.19±0.17^{AB}	6.13±0.16^{AB}	5.99±0.15^{AB}	5.87±0.13^{A}
			Overall acceptability			
C	6.78±0.13^{bB}	6.62±0.14^{bB}	6.23±0.14^{bAB}	6.09±0.14^{bAB}	5.91±0.17^{A}	Spoiled
T1	5.90±0.15^{aB}	5.90±0.20^{aB}	5.73±0.16^{aAB}	5.69±0.13^{AB}	5.56±0.14^{A}	5.48±0.13^{A}
T2	6.33±0.22^{abB}	6.33±0.14^{abB}	6.17±0.17^{abAB}	6.13±0.15^{AB}	6.04±0.13^{A}	5.99±0.22^{A}
T3	6.21±0.21^{abB}	6.21±0.17^{abB}	6.17 ±0.17^{abAB}	6.08±0.16^{AB}	6.04±0.14^{A}	5.91±0.22^{A}
Note: Means bearing different upper case letter superscripts in a row and lower case letter superscripts in a column differ significantly (p<0.05)						

CONCLUSION

From the observations recorded in this study it can be concluded that among the three different essential oils used, 0.1% thyme oil incorporated chicken nuggets (T_3) has higher physico-chemical characteristics, DPPH scavenging activity, superior microbial and sensory attributes than control and other treatment (T_1 and T_2), and such products can be stored for 28 days under refrigeration (4±1˚C) without any deleterious effects on the keeping qualities.

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