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IMPACT OF STORAGE ON QUALITY PARAMETERS OF MEAT ANALOG NUGGETS FORMULATED FROM OYSTER MUSHROOM, FLAXSEED AND AMARANTH

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ABSTRACT

The present study was envisaged to formulate meat analog nuggets from Oyster mushroom, flaxseed and amaranth and characterize its quality and shelf stability under ambient conditions. The water absorption index, fat absorption index, water activity and total microbial count of meat analog nuggets were analyzed for six months at two months interval. The mean water activity, oil absorption index and microbial count of meat analog nuggets increased from 0.558 to 0.604, 2.60 to 2.75 g g^{-1} and 0.172 × 10^4 to 2.863 × 10^4 cfu g^{-1}, respectively, during six months storage. However, the water absorption index of meat analog nuggets decreased from 5.06 to 4.03 g g^{-1} during six months storage. The study revealed higher values of water and oil absorption index in meat analog nuggets formulated from higher levels of freeze-dried Oyster mushroom; while the incorporation of defatted flaxseed decreased water activity of meat analog nuggets. The microbial load of meat analog nuggets was within the permissible limits of BIS (4 × 10^4 cfu mL^{-1}) throughout storage period of six months.

Keywords: Meat analog, microbial load, mushroom, oil absorption index, water absorption index, water activity

INTRODUCTION

Meat and Meat-based products are nutrient dense foods due to increased bioavailability of iron and vitamin B_{12} in comparison to plant and plant-based foods. Animal proteins are generally highly qualitative due to proper balance of all essential amino acids in them than in vegetable proteins (Salcedo-Chavez *et al.*, 2002). However, animal protein foods are more expensive than vegetable protein foods which restricts the meat consumption to developed countries. The meat and meat-based foods, being concentrated sources of saturated fatty acids, cholesterol and sodium, are considered as the principle dietary cause of several diseases like hypercholestremia, obesity and other cardiovascular diseases deteriorating the healthy image of these foods (Joshi and Kumar, 2015). The high prices, relative scarcity, negative health effects corresponding to meat and meat-based foods and increasing health awareness among consumers have encouraged the development of novel healthier alternative meat-products which may offer better nutritional profile at relatively low price.

Meat analog or mock meat is a food product having physical attributes (texture, flavor and appearance) and chemical composition somewhat similar to those of meat or meat-based products. Meat analogs are less expensive and healthier replacements of meat and meat-based products. Meat analogs can be formulated in the form of sheets, nuggets, patties or strips (Malav *et al*., 2015). The developing countries like India are facing acute shortage of high biological value proteins because the cost of meat is not only very high but frequently not readily available. In India about 75% population is vegetarian and rest is non-vegetarian. Protein energy malnutrition is a serious problem faced in almost all developing countries including India which has about 800 million people undernourished (Bhise and Kaur, 2013). Hence, there is a need to develop a readily available but less expensive alternative to meat and meat-based products having potential to overcome protein energy-related deficiencies which may confer health benefits due to the presence of bioactive components. These meat analogs can cater to the needs of vegetarian segment as well.

Oyster mushrooms (*Pleutrotus* sp.) are edible fruiting fungi (Kong, 2004) which possess low fat content (0.8-7.0%) depending upon the species involved. These can serve as a good source of non-starchy carbohydrates and dietary fiber, minerals and vitamins (Randive, 2012). Oyster mushrooms apart from providing traditional nutrients offer health promoting benefits due to the presence of biologically active substances such as alkaloids, phenols, terpenes, antioxidants, etc. and therefore, are regarded as functional foods (Thatoi and Singhdevsachan, 2014). They have fibrous structure that can mimic the texture of meat and impart unique taste after cooking (meaty flavour) (Mau, 2005). Therefore, mushrooms may be regarded as well-suited substrate for the development of meat analogs.

Flaxseed or linseed (*Linum usitatissimmum*) belongs family Linacceae. The consumption of flaxseed as a food stuff dates back to ancient times (Yograj *et al*., 2017). The seeds are nutritionally rich with about 41% fat, 28% total dietary fiber, 20% proteins, 7.7% moisture and 3.4% minerals (Verma and Mishra, 2014). Flaxseed being a rich source of polyunsaturated fatty acids especially α-linolenic acid, polyphenols-like lignans and dietary fiber aids minimizes the disease-risks and act as a nutraceutical thereby help in the prevention and treatment of diseases (Otlis and Cagnidi, 2006). High meat prices have prompted the food industry to produce non-meat proteins and flax can serve a good substrate for the formulation of same. Amaranth (*Amaranthus* sp.), commonly known as pigweed, is a primitive crop belonging to the order Caryophyllales, family Amaranthaceae (Fatinah *et al*., 2012). In recent years there has been increased interest in consumption of amaranth for human nutrition because of its high nutritive value and health benefits. On an average amaranth grain contains 15-20% protein, 58-66% starch, 6-9% fiber and 6-8% highly unsaturated lipids (Solarov *et al*., 2007). Amaranth proteins are complete proteins as have all essential amino acids particularly lysine (55.8 mg g^{-1} total protein) as compared to milk, wheat and maize (Bhat *et al*., 2015). In addition, amaranth proteins have sulphur containing methionine which is a limiting amino acid in pulses (Sousa and Farfan, 2012). Hence, amaranth grain can be used for improving malnutrition, enhancing food security, promoting rural development and for developing nutraceuticals and functional foods. The present study was aimed to formulate meat analog nuggets from Oyster mushroom, flaxseed and amaranth and characterize their quality and shelf stability under ambient conditions.

MATERIALS AND METHODS

The present study was carried out in the Division of Food Science & Technology, SKUAST-Jammu. The flaxseed, amaranth grain, Oyster mushrooms, deboned chicken and spice blend were procured from local markets of Jammu.

Preparation of raw materials

The flaxseed and amaranth grains were screened for stones and other extraneous materials followed by roasting for 3 min in a microwave oven at 450 watts separately. The roasted flaxseed grains were partially defatted in an oil expeller to reduce oil content of grain (double press). The partially defatted meal was cooled, ground in a grinder followed by sieving through 80-100 mesh sieve to obtain flaxseed flour. The amaranth grain flour was obtained by grinding and sieving the roasted grains. The Oyster mushrooms were subject to freeze drying in a freeze drier (Martin Christ Type 101041) with chamber temperature of -60°C, under vacuum (<13 Pa) and a condenser temperature of -50°C. The chicken breast (broiler hens, 6 weeks old) was used for the preparation of control food after it was defatted and deboned manually.

Preparation of meat analog

The formulation of meat analog nuggets was developed as per standardized procedure of Nisha (2008) with slight modifications (Fig. 1). The flaxseed flour, amaranth grain flour and freeze Oyster mushroom powder were blended separately in different ratios with each other along with 10 g corn flour and spice blend (6 g ginger, 22 g garlic, 10 g black pepper, 6 g cinnamon and 6 g salt).

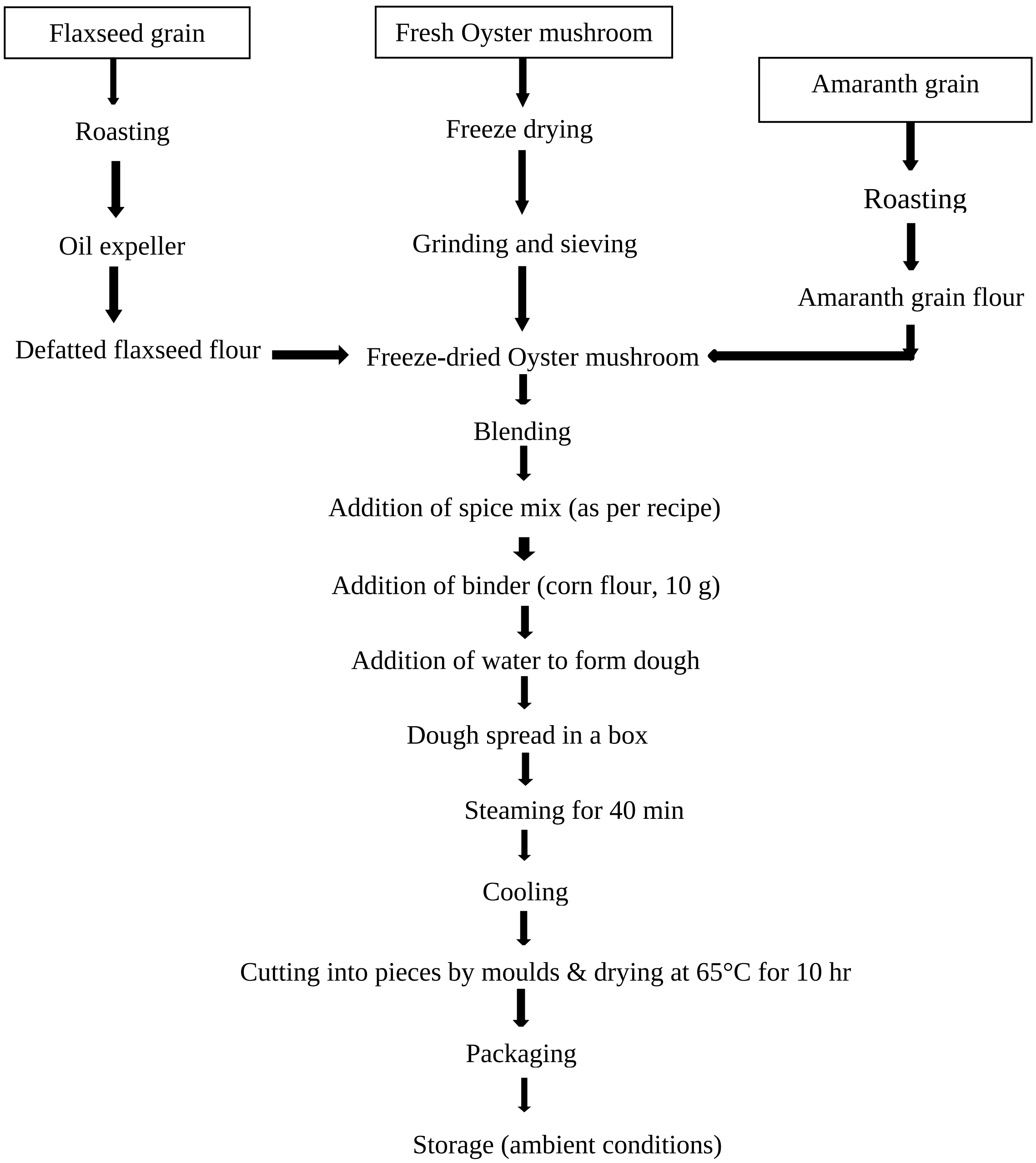

Fig. 1: Flow chart for preparation of meat analog

Analysis of quality parameters

Water absorption index (WAI): WAI was calculated as per Julianti *et al*. (2017) using the formula:

$$\text{Water absorption index (g g}^{-1}\text{)} = \frac{\text{Weight of sediment (g)}}{\text{Weight of dry solids(g)}}$$

Oil absorption index (OAI): Oil absorption index was determined as per Julianti *et al*. (2017) and oil absorption index calculated as:

$$\text{Oil absorption index (g g}^{-1}\text{)} = \frac{\text{Weight of sediment (g)}}{\text{Weight of raw material (g)}}$$

Water activity: Water activity was measured using an Aqualab water activity meter (model: 3TE) and readings were corrected at 20°C (AOAC, 2005).

Microbiological analysis: Spread plate technique described by Palczar and Chan (1991) was followed for total microbial count. The sample (1 g) was aseptically transferred to test tubes containing 9 mL sterile water and mixed vigorously. After mixing, 1 mL mixture was again transferred to test tubes containing 9 mL sterile water for further serial dilution. The process was continued until 4^{th} diluents (10^{-4}). Nutrient agar was inoculated with 0.1 mL diluted sample (10^{-4}), by spread plating technique and incubated at 37°C for 24 h. Colonies were counted and multiplied by dilution factor.

Statistical analysis

The experimental data was analyzed using factorial complete randomized design by employing Opstat v.8.6 software. The critical differences were calculated at 5% confidence level.

RESULTS AND DISCUSSION

Water absorption index

The water absorption index (WAI) measures the volume occupied by granules or starch polymers after swelling in excess of water. It determines the ability of flour to absorb water and swell for improved consistency in food. It is desirable to improve yield and give body to the food (Abiodun *et al*., 2014). The highest mean WAI of 5.66 g g^{-1} was recorded in T_2 (100:00:00 :: MP:PDF:AF) while T_1 (control chicken nuggets) observed lowest mean WAI of 1.7 g g^{-1} (Table 1). The meat analog

Table 1: Effect of treatment and storage on water absorption index (g g^{-1}) of meat analog nuggets

Treatments	Storage period (months)				Mean
	0	2	4	6	
T_1 (Control chicken nuggets)	2.19	1.91	1.56	1.15	1.70
T_2 (100:00:00::MP:PDF:AF)	6.13	5.87	5.52	5.11	5.66
T_3 (75:25:00::MP:PDF:AF)	5.94	5.64	5.30	4.91	5.45
T_4 (50:50:00::MP:PDF:AF)	5.75	5.48	5.12	4.71	5.27
T_5 (25:75:00::MP:PDF:AF)	5.56	5.26	4.92	4.51	5.06
T_6 (00:100:00::MP:PDF:AF)	5.38	5.11	4.76	4.36	4.90
T_7 (75:00:25::MP:PDF:AF)	5.14	4.86	4.52	4.11	4.66
T_8 (50:00:50::MP:PDF:AF)	4.87	4.58	4.21	3.81	4.37
T_9 (25:00:75::MP:PDF:AF)	4.61	4.31	3.98	3.57	4.12
T_{10} (00:00:100::MP:PDF:AF)	4.36	4.08	3.77	3.37	3.90
T_{11} (25:25:50::MP:PDF:AF)	5.05	4.79	4.46	4.05	4.59
T_{12} (50:25:25::MP:PDF:AF)	5.49	5.19	4.83	4.41	4.98
T_{13} (25:50:25::MP:PDF:AF)	5.31	5.02	4.73	4.32	4.85
Mean	5.06	4.78	4.44	4.03	
$CD_{0.05}$	Storage = 0.02;	Treatment = 0.03;	Storage × Treatment = ns		

MP: Oyster mushroom powder; PDF: Partially defatted flaxseed flour; AF: Amaranth grain flour

nuggets formulated from higher levels of freeze-dried Oyster mushroom depicted higher WAI in contrast to the nuggets formulated from higher levels of partially defatted flaxseed and amaranth grain flour. The presence of higher hydrophilic constituents such as crude fiber and proteins in freeze-dried Oyster mushroom in contrast to partially defatted flaxseed and amaranth grain flour might be responsible for higher WAI of meat analog nuggets formulated from higher levels of freeze-dried Oyster mushroom (Lopera-Cardona *et al*., 2016). Ishara *et al*. (2018) reported increase in WAI of composite flour with the increase in incorporation levels of *Pleurotus ostreatus* powder quite similar to our results. The decrease in WAI of cookies with increase in incorporation levels of amaranth grain has previously been reported by Sindhuja *et al.* (2005).

With the progression of storage period, the mean WAI of meat analog nuggets decreased from 5.06 to 4.03 g g^{-1} with a non-significant interaction effect between treatment and storage. The reason might be the decrease in crude fiber content and increase in hydrophobic amino acid residues during storage (Kumar and Thakur, 2017). Similar results were reported by Adebowale *et al*. (2017) and Abiodun *et al*. (2014) while studying the effect of storage on functional properties of water yam flour and trifoliate yam flour, respectively.

Oil absorption index

The oil absorption index of food is an important parameter determining the ability of proteins to physically bind fat by capillary action and plays role in flavour retention and enhances the mouth feel of foods (Kumar and Saini, 2016). The highest mean oil absorption index of 4.52 g g^{-1} was recorded for T_2 (100:00:00::MP:PDF:AF) while as least value of 1.25 g g^{-1} was recorded for T_1 (control chicken nuggets) {Table 2}. The supplementation of partially defatted flaxseed and amaranth decreased the oil absorption index of meat analog nuggets while meat analog nuggets prepared from higher levels of freeze-dried Oyster mushroom observed higher values of oil absorption index. The presence of non-polar amino acids in oyster mushroom powder might be responsible for increased oil absorption index (Sulieman *et al*., 2017). This might also be because of polar or hydrophilic amino acids present in partially defatted flaxseed and amaranth grain and a higher fat content that depicts an inverse relation with oil absorption index (Umezuruike and Nwabueze, 2017). This trend is supported by Azeez *et al*. (2018) who reported an increase in oil absorption index of bread with increase in supplementation levels of *Pleurotus ostreatus* mushroom. Bhise and Kaur (2013) reported a decrease

Table 2: Effect of treatment and storage on oil absorption index (g g^{-1}) of meat analog nuggets

Treatments	Storage period (months)				Mean
	0	2	4	6	
T_1 (Control chicken nuggets)	1.21	1.23	1.26	1.30	1.25
T_2 (100:00:00::MP:PDF:AF)	4.42	4.48	4.55	4.63	4.52
T_3 (75:25:00::MP:PDF:AF)	3.74	3.80	3.87	3.94	3.84
T_4 (50:50:00::MP:PDF:AF)	3.01	3.04	3.08	3.14	3.07
T_5 (25:75:00::MP:PDF:AF)	2.30	2.33	2.38	2.44	2.36
T_6 (00:100:00::MP:PDF:AF)	1.55	1.59	1.63	1.68	1.61
T_7 (75:00:25::MP:PDF:AF)	3.69	3.73	3.77	3.83	3.76
T_8 (50:00:50::MP:PDF:AF)	2.92	2.96	3.03	3.12	3.01
T_9 (25:00:75::MP:PDF:AF)	2.16	2.19	2.22	2.29	2.22
T_{10} (00:00:100::MP:PDF:AF)	1.43	1.45	1.49	1.54	1.48
T_{11} (25:25:50::MP:PDF:AF)	2.20	2.23	2.26	2.31	2.25
T_{12} (50:25:25::MP:PDF:AF)	2.98	3.03	3.09	3.16	3.07
T_{13} (25:50:25::MP:PDF:AF)	2.24	2.27	2.32	2.40	2.31
Mean (Storage)	2.60	2.64	2.69	2.75	
$CD_{0.05}$	Storage = 0.01; Treatment = 0.02; Storage × Treatment = ns				

MP: Oyster mushroom powder; PDF: Partially defatted flaxseed flour; AF: Amaranth grain flour

in oil absorption index of *chappati* with increase in the addition of flaxseed flour. Sneha and Haripriya (2018) also reported a decrease in oil absorption index of *dosa* mix with the addition of amaranth grain flour thus supporting the present study.

The mean oil absorption index of meat analog nuggets increased from 2.60 to 2.75 g g^{-1} during 6 months of storage period. The increase in oil absorption index during storage might be due to increase in hydrophobic/non-polar amino acid residues which form complex structures (Shobha *et al*., 2014). Our results are quite consistent with the findings of Obiegbuna *et al*. (2014) who reported increase in oil absorption index of cocoyam flours during storage period of seven months.

Water activity

The water activity content of meat analog nuggets varied significantly with variation in the ingredients and was found in the range of 0.507 to 0.627 (Table 3). The water activity values of meat analog nuggets decreased with increase in the incorporation levels of partially defatted flaxseed quite similar to Sharma *et al*. (2014) who too reported a decrease in water activity levels of restructured mutton chops with the addition of partially defatted flaxseed. Khouryieh and Aramouni (2013) observed a decrease in water activity of cereal bars with increase in addition of flaxseed flour. The increased moisture content of amaranth grain flour and freeze-dried Oyster mushroom in contrast to partially defatted flaxseed might be responsible for increased water activity of nuggets formulated from higher levels of amaranth grain flour and mushroom powder. Wahyono *et al*. (2018) reported an increase in water activity of noodles enriched with *Pleorotus ostreatus* powder similar to our findings.

During six months of storage, the water activity content of meat analog nuggets increased from 0.558 to 0.604. The gain in moisture by meat analog nuggets during storage was probably due to the absorption of moisture from environment and also due to the hygroscopic nature of sample might be responsible for increase in water activity content. Our findings were also supported by Wong and Lim (2016) who also reported an increase in water activity of spray-dried papaya (*Carica papaya* L.) from 0.16 to 0.28 during storage period of 2 months which might be due to the higher relative humidity thus more absorption of moisture by the fruit powder during storage.

Table 3: Effect of treatment and storage on water activity (a_w) of meat analog nuggets

Treatments	Storage period (months)				Mean
	0	2	4	6	
T_1 (Control chicken nuggets)	0.604	0.619	0.630	0.656	0.627
T_2 (100:00:00::MP:PDF:AF)	0.552	0.566	0.582	0.594	0.574
T_3 (75:25:00::MP:PDF:AF)	0.539	0.552	0.567	0.582	0.560
T_4 (50:50:00::MP:PDF:AF)	0.523	0.537	0.551	0.566	0.544
T_5 (25:75:00::MP:PDF:AF)	0.504	0.519	0.536	0.553	0.528
T_6 (00:100:00::MP:PDF:AF)	0.482	0.499	0.515	0.533	0.507
T_7 (75:00:25::MP:PDF:AF)	0.569	0.582	0.596	0.613	0.590
T_8 (50:00:50::MP:PDF:AF)	0.576	0.590	0.604	0.618	0.597
T_9 (25:00:75::MP:PDF:AF)	0.597	0.615	0.628	0.648	0.622
T_{10} (00:00:100::MP:PDF:AF)	0.612	0.626	0.641	0.661	0.635
T_{11} (25:25:50::MP:PDF:AF)	0.580	0.594	0.608	0.622	0.601
T_{12} (50:25:25::MP:PDF:AF)	0.566	0.578	0.591	0.610	0.586
T_{13} (25:50:25::MP:PDF:AF)	0.549	0.559	0.578	0.590	0.569
Mean	0.558	0.572	0.587	0.604	
$CD_{0.05}$	Storage = 0.003; Treatment = 0.005; Storage × Treatment = ns				

MP: Oyster mushroom powder; PDF: Partially defatted flaxseed flour; AF: Amaranth grain flour

Table 4: Effect of treatment and storage on microbial count (× 10^4 cfu g^{-1}) of meat analog nuggets

Treatments	Storage period (months)				Mean
	0	2	4	6	
T_1 (Control chicken nuggets)	0.231	1.078	2.106	3.216	1.658
T_2 (100:00:00::MP:PDF:AF)	0.169	0.901	1.832	2.843	1.436
T_3 (75:25:00::MP:PDF:AF)	0.149	0.875	1.758	2.731	1.378
T_4 (50:50:00::MP:PDF:AF)	0.136	0.869	1.707	2.602	1.329
T_5 (25:75:00::MP:PDF:AF)	0.118	0.838	1.654	2.512	1.281
T_6 (00:100:00::MP:PDF:AF)	0.100	0.799	1.599	2.410	1.227
T_7 (75:00:25::MP:PDF:AF)	0.181	0.911	1.883	2.898	1.468
T_8 (50:00:50::MP:PDF:AF)	0.200	0.972	2.001	3.001	1.544
T_9 (25:00:75::MP:PDF:AF)	0.207	1.154	2.034	3.069	1.616
T_{10} (00:00:100::MP:PDF:AF)	0.245	1.250	2.202	3.310	1.752
T_{11} (25:25:50::MP:PDF:AF)	0.190	0.941	1.993	3.031	1.539
T_{12} (50:25:25::MP:PDF:AF)	0.161	0.893	1.803	2.834	1.423
T_{13} (25:50:25::MP:PDF:AF)	0.151	0.891	1.781	2.767	1.398
Mean	0.172	0.952	1.873	2.863	
$CD_{0.05}$	Storage = 0.027; Treatment = 0.048; Storage × Treatment = 0.097				

MP: Oyster mushroom powder; PDF: Partially defatted flaxseed flour; AF: Amaranth grain flour

Microbial quality of meat analog nuggets

The microorganisms present in meat analog samples reveal the sanitary quality of raw materials, processing and storage (Nazir, 2018). The results showed that the microbial load of meat analog nuggets was significantly affected by treatments (Table 4). The highest mean total microbial count of 1.752 × 10^4 cfu g^{-1} was recorded in T_{10} (00:00:100::MP:PDF:AF); whereas the lowest value of 1.227 × 10^4 cfu g^{-1} was found in T_6 (00:100:00::MP:PDF:AF). Initially, chicken nuggets (T_1) recorded microbial load of 0.231×10^4 cfu g^{-1} that increased to 3.216 × 10^4 cfu g^{-1} during 6 months of storage. The supplementation of amaranth grain flour enhanced microbial load of meat analog nuggets which might be owing to higher moisture content in amaranth grain flour favouring the microbial growth. Similar tour results Hozova *et al*. (2000) reported an increase in microbial load of biscuits with addition of amaranth grain flour. The lower water activity of partially defatted flaxseed flour in contrast to amaranth grain flour and freeze-dried Oyster mushroom might be responsible for lower microbial load of meat analog nuggets formulated from higher levels of partially defatted flaxseed. Sharma *et al*. (2014) reported decrease in microbial load of mutton chops with increase in supplementation of flaxseed flour.

With the advancement of storage period the total microbial count of meat analog nuggets increased significantly from 0.172 x × 10^4 to 2.863 × 10^4 cfu g^{-1}. The increase in microbial count during storage may be due to increase in moisture content of meat analog nuggets that provides favourable environment for microbial growth (Chakroborty and Chakroborty, 2017). However, the values obtained for meat analog nuggets were within the acceptable BIS (Bureau of Indian Standards) limits and recommendations for products of this nature (4 × 10^4 cfu mL^{-1}). Nisha (2008) also reported an increase in microbial load during storage of green gram-based meat analog.

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