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Changes in vitamin content of imitation meat nuggets formulated from oyster mushroom, flaxseed and amaranth grain in response to storage

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Abstract
In this study imitation meat nuggets were formulated from freeze dried oyster mushroom, flaxseed and amaranth grain and were analyzed for their vitamin A and C contents. The formulated imitation nuggets were stored for period of six months and changes in the vitamin A and C contents were recorded at an interval of 2 months. The study reported highest Vitamin A and Vitamin C contents in nuggets formulated from higher levels of freeze dried oyster mushroom in contrast to nuggets formulated from higher levels of flaxseed and amaranth grain flour. The imitation meat nuggets corresponding to T_2 (100:00:00:MP:PDF:AF) reflected highest mean vitamin A and C contents of 258.38 I.U and 4.51 mg/100g, respectively. The storage period decreased the vitamin A and C contents of imitation meat nuggets significantly. The mean vitamin A and C contents of imitation meat nuggets decreased from 117.85 to 95.65 I.U and 3.18 to 2.01 mg/100g, respectively during storage period of six months.

Keywords: Amaranth grain, imitation meat, oyster mushroom, flaxseed, amaranth

Introduction
Protein energy malnutrition is a serious problem faced by most of developing countries like India having about 800 million undernourished people (Bhise *et al.*, 2013) [3]. Furthermore most of the Indian population about 70-75 per cent is vegetarian and rest is non-vegetarian (Yadav and Kumar, 2006) [19]. Thus there is a need of time to develop a readily available less expensive alternative to meat and meat based products having potential to overcome protein energy related deficiencies and that can cater the needs of vegetarian segment as well. Imitation meat or meat analog or mock meat is a food product having physical attributes (texture, flavour, appearance) and chemical composition somewhat similar to those of meat or meat based products. These are usually developed from non-meat substrates like plant and fungal sources that are source of vitamins unlike meat and meat products. Imitation meats are less expensive and healthier replacements of meat and meat based products. These imitation meats can be formulated in the form of sheets, nuggets, patties or strips (Malav *et al.*, 2015) [8]. Oyster mushrooms an edible fruiting fungi popularly known as *dhingri* in India belongs to *Pleurotus* (Kong, 2004) [7]. These mushrooms are rich source of proteins (17-42%) containing all the essential amino acids (Deepalakshmi and Mirunalini, 2014) and at the same time are low in fat ranging from 0.8-7.0 per cent. Furthermore these mushrooms act as a good source of non-starchy carbohydrates and dietary fiber and are rich in minerals and vitamins (Randive, 2012) [12]. *Pleurotus* mushrooms apart from providing traditional nutrients offer health promoting benefits due to presence of biologically active substances like alkaloids, phenols, terpenes, antioxidants and therefore, are regarded as functional foods. Mushrooms also exhibit antioxidant, anti-diabetic, anti-carcinogenic and hepatoprotective properties (Thatoi and Singhdevsachan, 2014) [16]. They have fibrous structure that can mimic the texture of meat. The unique taste of mushrooms after cooking (Umami/meaty flavour) is somewhat similar to that of meat (Mau, 2005) [9]. Therefore, mushrooms can be well suited substrate for development of meat analogs. Flaxseed or Linseed having botanical name *Linum usitatissimmum* is a member of Linacceae family. The name "*Linum usitatissimmum"* is a latin word meaning "very useful" (Yograj *et al*., 2017) [20]. The seeds are nutritionally rich with about 41 per cent fat, 28 per cent total dietary fiber, 20 per cent protein, 7.7 per cent moisture and 3.4 per cent minerals (Verma and Mishra, 2014) [17]. The high fiber (both soluble and insoluble) content of flaxseeds help in regulating body weight by delaying gastric emptying and help in diminishing blood cholesterol and blood sugar levels (Yograj *et al*., 2017) [20]. 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 grain commonly known as *Rajgira*" and "*Ramdana*" meaning "*King seed*" and "*Seed sent by God*", respectively in India can be employed for improving malnutrition, enhancing food security, promoting rural development and can be utilized for developing nutraceuticals and functional foods. In recent years there has been an increasing interest in consumption of amaranth for human nutrition because of its high nutritive value and health benefits. On an average amaranth grain contains about 15-20 per cent of protein, 58-66 per cent of starch, 6-9 per cent of fiber and 6-8 per cent of highly unsaturated lipids (Solarov *et al*., 2007) [14]. Amaranth proteins are complete proteins because of the presence of all essential amino acids particularly its high lysine content (55.8 mg/g of total protein) comparable to that of milk and higher than wheat and maize (Bhat *et al*., 2015) [2]. In addition amaranth proteins also contain methionine, a sulphur containing amino acid which is the limiting amino acids in pulses (Sousa and Farfan, 2012) [15]. Amaranth grain is devoid of gluten and is easy to digest therefore, recommended as a diet for ill and fasting persons (Gambus *et al*., 2009) [4] and thus can be employed for preparation of meat alternatives.

Materials and method

The present study was carried out in division of Food Science and Technology, SKUAST-Jammu. The raw materials including fresh oyster mushroom, amaranth, flaxseed, deboned chicken breast and spice blend were obtained from local markets of Jammu.

Preparation of imitation meat nuggets

The fresh oyster mushroom after cleaning and slicing were subjected to freeze drying in a freeze dried (Martin Christ Type 101041) followed by grinding and sieving. Simultaneously the flaxseed and amaranth grain were cleaned and roasted. The roasted flaxseed was defatted using an oil expeller and the defatted meal was grinded and sieved while as roasted amaranth grains were grinded and sieved. The resulting freeze dried mushroom powder, defatted flaxseed, amaranth flour and spice blend were blended in different proportions (Table 1) for formulation of imitation nuggets as per procedure summarized in Fig. 1. The control chicken meat nuggets were formulated from deboned chicken breast.

Analysis of imitation meat nuggets

Vitamin A

A method described by (Srivastava and Kumar, 2002) was employed in estimation of β- carotene with slight modifications. The vitamin A content was calculated using formula:

$$\text{Vitamin A (I.U)} = \frac{\beta\text{-carotene } (\mu g/100g)}{0.6}$$

Vitamin C

Vitamin C content of imitation nuggets was determined according to method described by Nielsen (2017) using 2, 6, dichlorophenol indophenol dye.

Vitamin C content was calculated as:

$$\text{Vitamin C (mg/100g)} = \frac{\text{Titer value} \times \text{dye factor} \times \text{volume made up}}{\text{ml of filtrate taken for estimation} \times \text{weight of sample}} \times 100$$

Statistical analysis

The data obtained was statistically analyzed employing factorial completely randomized design using Opstat v.8.6 software.

Results and Discussion

Vitamin A

The vitamin A content of imitation meat nuggets was significantly affected by treatment and storage (Table 2). With the incorporation of partially defatted flaxseed and amaranth grain flour, mean vitamin A content of imitation meat nuggets decreased significantly from 258.38 to 10.58 and 258.38 to 2.66 (I.U.), respectively (Table 2). The imitation meat nuggets formulated from higher levels of freeze dried oyster mushroom reflected higher vitamin A content values in contrast to nuggets formulated from partially defatted flaxseed and amaranth grain flour. This might be due to higher amounts of vitamin A in freeze dried oyster mushroom in contrast to partially defatted flaxseed and amaranth grain flour.

With the advancement of storage period there was significant decrease in mean vitamin A content from 117.85 to 95.65 (I.U). This decrease might be due to non-oxidative changes (cis-trans isomeristion, epoxide formation or heat degradation of tissues or oxidative changes (Jan, 2018). Our results were in accordance with the findings of Nagarajaiah and Prakash (2015) [11] who also reported decrease in pro-vitamin A content during storage of carrot pomace incorporated cookies.

Vitamin C

The vitamin C content of imitation meat nuggets varied significantly with the treatment and storage (Table 3). The highest mean vitamin C content of 4.51 mg per 100g was recorded for T_2 (100:00:00::MP:PDF:AF) while as least mean vitamin C content of 0.36 mg per 100g was recorded for T_6 (00:100:00::MP:PDF:AF). The replacement of freeze dried oyster mushroom by partially defatted flaxseed and amaranth grain flour decreased the vitamin C content of imitation meat nuggets. The imitation meat nuggets formulated from higher levels of freeze dried oyster mushroom recorded more vitamin C content which might be because of higher vitamin C content of freeze dried oyster mushroom in comparison to amaranth grain and partially defatted flaxseed flour. Agaba (2012) [1] reported that inclusion of oyster mushroom increased the vitamin C content of Hamburger, confirming our results. An increased vitamin C content of gravy formulated from oyster mushroom in contrast to gravy formulated from button and milky mushroom was reported by Vidhya *et al*. (2018) [18] supporting our results.

During storage period of six months the mean vitamin C content of imitation meat nuggets decreased significantly from 3.18 to 2.01 mg per 100g. This might be because of the oxidation of L-ascorbic acid to dehydro-ascorbic acid and further to diketogulonic acid which is physiologically inactive (Munaza, 2018) [10]. Singh *et al*. (2003) [13] reported decrease in the ascorbic content of dehydrated mint from 46.66 to 18.90 mg per 100g during storage period of 60 days. Khursheed (2014) [6] also reported loss of vitamin C in high fiber meat balls during storage.

Table 1: Treatment details for formulation of imitation meat nuggets

Treatment	Deboned chicken breast (%)	Mushroom (%)	Flaxseed (%)	Amaranth (%)
T_1 (Control)	100	-	-	-
T_2	-	100	-	-
T_3	-	75	25	-
T_4	-	50	50	-
T_5	-	25	75	-
T_6	-	-	100	-
T_7	-	75	-	25
T_8	-	50	-	50
T_9	-	25	-	75
T_{10}	-	-	-	100
T_{11}	-	50	25	25
T_{12}	-	25	50	25
T_{13}	-	25	25	50

Table 2: Effect of treatment and storage on vitamin A (I.U.) content of meat analog nuggets

Treatment	Storage period (months)				Mean (Treatment)
	0	2	4	6	
T_1 (Control chicken nuggets)	133.26	125.35	115.16	105.10	119.72
T_2 (100:00:00::MP:PDF:AF)	272.47	264.34	253.78	242.94	258.38
T_3 (75:25:00::MP:PDF:AF)	207.85	199.89	190.13	179.43	194.33
T_4 (50:50:00::MP:PDF:AF)	143.24	136.24	127.01	115.02	130.38
T_5 (25:75:00::MP:PDF:AF)	78.63	72.72	64.95	57.76	68.52
T_6 (00:100:00::MP:PDF:AF)	14.01	11.98	9.29	7.02	10.58
T_7 (75:00:25::MP:PDF:AF)	181.18	174.38	166.46	156.06	169.52
T_8 (50:00:50::MP:PDF:AF)	137.99	130.32	121.10	110.89	125.08
T_9 (25:00:75::MP:PDF:AF)	70.75	61.86	52.74	43.45	57.2
T_{10} (00:00:100::MP:PDF:AF)	3.51	3.00	2.47	1.96	2.66
T_{11} (25:25:50::MP:PDF:AF)	72.49	67.82	61.08	54.91	64.08
T_{12} (50:25:25::MP:PDF:AF)	140.61	132.52	123.11	112.82	127.27
T_{13} (25:50:25::MP:PDF:AF)	76.00	71.09	64.17	56.09	67.36
Mean (Storage)	117.85	111.65	103.96	95.65	

Effects	$C.D_{(p=0.05)}$
Storage	0.99
Treatment	1.78
Storage × Treatment	3.56

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

Table 3: Effect of treatment and storage on vitamin C (mg/100g) content of meat analog nuggets

Treatment	Storage period (months)				Mean (Treatment)
	0	2	4	6	
T_1 (Control chicken nuggets)	4.14	3.68	3.19	2.67	3.42
T_2 (100:00:00::MP:PDF:AF)	5.25	4.77	4.28	3.74	4.51
T_3 (75:25:00::MP:PDF:AF)	4.00	3.56	3.07	2.56	3.30
T_4 (50:50:00::MP:PDF:AF)	2.74	2.30	1.83	1.44	2.08
T_5 (25:75:00::MP:PDF:AF)	1.50	1.29	1.07	0.83	1.17
T_6 (00:100:00::MP:PDF:AF)	0.44	0.38	0.34	0.29	0.36
T_7 (75:00:25::MP:PDF:AF)	4.51	4.11	3.67	3.21	3.88
T_8 (50:00:50::MP:PDF:AF)	3.76	3.32	2.83	2.29	3.05
T_9 (25:00:75::MP:PDF:AF)	3.01	2.65	2.25	1.75	2.42
T_{10} (00:00:100::MP:PDF:AF)	2.28	1.95	1.61	1.25	1.77
T_{11} (25:25:50::MP:PDF:AF)	3.25	2.86	2.43	1.95	2.62
T_{12} (50:25:25::MP:PDF:AF)	3.51	3.18	2.83	2.46	3.00
T_{13} (25:50:25::MP:PDF:AF)	2.98	2.57	2.15	1.72	2.36
Mean (Storage)	3.18	2.82	2.43	2.01	

Effects	$C.D_{(p=0.05)}$
Storage	0.02
Treatment	0.04
Storage × Treatment	0.08

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

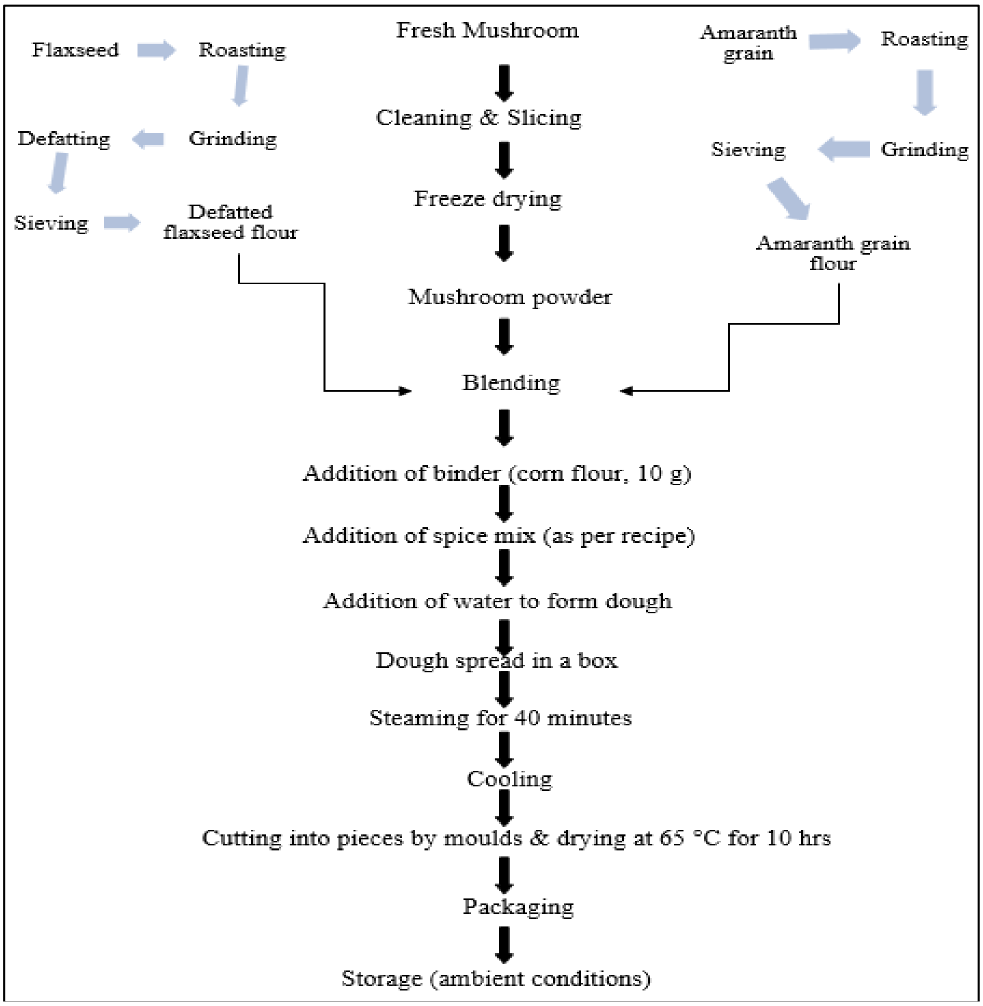

Fig 1: Flow chart for preparation of meat analog

Conclusion: The vitamin A content of imitation meat nuggets formulated from higher levels of oyster mushroom recorded higher vitamin A and C values in contrast to nuggets formulated from higher levels of partially defatted flaxseed. During storage of six months the mean vitamin A and C content of meat analog nuggets decreased from 117.85 to 95.65 I.U. and 3.18 to 2.01 mg per 100g, respectively

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