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Effect of black rice (*Oryza sativa L.*) flour on proximate composition, texture profile and microbiological qualities of chicken nuggets

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Abstract
Chicken nuggets prepared by incorporating three different levels of black rice flour along with other non-meat ingredients. The formulations prepared were – Control (0% Black Rice Flour), T_1 (1% Black Rice Flour), T_2 (3% Black Rice Flour) and T_3 (5% Black Rice Flour). Proximate composition of the study revealed significant (P>0.01) increase in the moisture and ash content, whereas the per cent crude protein and the per cent ether extract decreased significantly from the control to the treated groups. The texture profile analysis studies revealed a non-significant difference in all the parameters, which are hardness, chewiness, gumminess, springiness and cohesiveness. The microbiological studies revealed that products were acceptable up to fifteen days. Based on the results obtained in the study it might be concluded that chicken nuggets could be prepared satisfactorily on addition of up to 5% black rice flour without adverse effect on the quality of the products.

Keywords: Black rice flour, chicken nuggets, microbiological study, proximate composition, texture profile.

Introduction
Meat is considered as an integral component for healthy and well-balanced diet being a valuable source of high quality, easily digestible protein, essential micronutrients, fat, vitamins and minerals. In India, almost one third of the populations are vegetarian. However in the North Eastern Region of the country more than 90 percent people are non-vegetarian. Poultry meat is the fastest growing component to cater the need for global demand of meat. The total poultry population in India is 729.2 million (19th livestock census). During the last few decades, the consumption of poultry meat has increased tremendously. The main reasons for the increased consumption of poultry meat and meat products are that poultry meat is more favourable for processing in comparison with other type of meats because it has neutral flavour, consistent and good texture, and light colour. Also because of their healthy and nutritional value, relatively lower price as compared to other red meats, and absence of any cultural or religious effect [1].

Along with the increasing consumer demands for processed meat products, there is also an increase in consumer awareness regarding nutritional and hygienic status and use of various additives and preservatives, in processed meat products. Rice has been a staple food source for many Asian countries like China, Japan and India. Black rice is a type of the rice species *Oryza sativa L.* which is glutinous, packed with high level of nutrients and mainly cultivated in Asia. This rice is getting popular in recent years because of its high nutritive value and antioxidative properties. The dark colour of the black rice results from the high content of anthocyanins, a hydrophilic phenolic compound which is reported to possess remarkable antioxidant activities [2, 3] and is 10 times higher than in brown rice bran [4] and two fold stronger than blueberries with respect to antioxidant activities [5]. Chicken nuggets are the most readily acceptable ready-to-eat processed meat products that are either baked, steamed cooked or consumed after frying [6]. Since in recent past, due to several factors like increasing urbanisation and industrialization, rapid growth in information technology and proliferation of fast-food outlets, changing food habits and busy working schedule, processed meat products

are gradually becoming popular among the working middle classes in India. Mostly urban consumers prefer ready to eat and ready to cook type meat products with good quality and taste. Considering the above points the study was conducted to identify the effects of the incorporation of black rice flour as binders/ extenders at different levels for the preparation of chicken nuggets and to prepare a nutritious meat product added with functional properties of black rice.

Materials and Methods

Chickens were collected from nearby market and were scientifically slaughtered in the semi-mechanized poultry-dressing unit of the LPT Department, CVSc, AAU, Khanapara, and Guwahati.

Good quality black rice was purchased from local market of Imphal, Manipur and the black rice was ground in a grinder/ mixer to make black rice flour. The black rice flour was incorporated at 1. 3, 5% levels in the formulation by replacing lean meat.

Preparation of chicken meat nuggets

Deboning of the carcases was done within three hours of slaughter. Deboned meat along with heart, liver, gizzard, skin, fat harvested was packed separately in food grade polythene bags and then stored at 4±1°C temperature for 24 hours.

After 24 hours of storage, the deboned meat along with heart, gizzard, skin an fat was cut into small cubes of 3 cm size and then minced in a mechanical mincer through 4mm plate.

Curing ingredients viz. Salt (2%), sodium tripolyphosphate (0.3%) and sodium nitrite (150ppm) were added to the minced meat and thoroughly mix an stored at 4±1°C for next 24 hours to facilitate proper curing.

Preparation of meat emulsion

Different levels of black rice flour i.e. 1, 3, 5% along with other spices an condiments, soya bean flour, egg white, ice flakes were added to the minced meat and mixed thoroughly to prepare the meat emulsion whereas in the control sample black rice flour was absent.

Chicken nuggets were prepared by tightly filling the emulsions of different formulations in separate stainless steel moulds. The stainless steel moulds filled with the emulsions were cooked in hot water maintained at 80°C for 45 minutes. The cooked meat blocks were then given a cold shower and chilled in refrigerator. The chilled blocks were then cut into 3cm×1.5cm×1.5cm size nuggets and vacuum packed in HDPE pouches and stored under refrigeration.

Proximate Composition

The Moisture, Crude Protein (CP), Ether Extract (EE) and Total Ash (TA) of the products of the control and the treated groups were estimated as per the standard procedure laid down by the AOAC, 2000 [7].

Texture Profile Analysis

The texture profile of the nuggets (hardness, springiness, cohesiveness, chewiness and gumminess) was evaluated by a Food Texture Analyser (Make: Stable Micro Systems, UK, Model: TA-HD plus).

Microbiological Studies

The Colititre Count, Yeast and Mould Count and Total Viable Psychrotrophic Bacterial Count (TVPBC) of nugget samples were recorded as per the methods described by Harrigan and Mac Cancy [8].

Statistical analysis

A minimum of five batches of the products were prepared for the proposed study. The data obtained from the above study were analysed statistically by a software SAS (SAS 9.3 software).

Results and Discussions

Proximate Composition of Chicken nuggets

The results of Proximate Composition of chicken nuggets for both control and treated products are presented in Table 1.

Moisture

In the present study, per cent moisture value recorded in the control product was 59.94 ± 0.13 and in the treated formulation with 1% (T_1), 3% (T_2) and 5% (T_3) black rice flour, the values recorded were 60.68 ± 0.16, 61.31 ± 0.27 and 61.81 ± 0.23 respectively. The per cent moisture content of chicken nuggets incorporated with increasing levels of black rice flour exhibited a significantly (P>0.01) increasing trend from control to the treated formulations. The per cent increase in moisture value in the treated products might be due to the increase in the levels of black rice flour, which absorbs water during the emulsion preparation. These observations were in consistent with the findings of earlier workers [9] who reported a significant (P>0.05) increase in the moisture content with increasing levels of black rice flour in pork patties. In another study [10]. An increase (P>0.05) in the moisture content in pork sausage incorporated with the black rice flour was observed when compared with the control sample.

Crude Protein

In the present study, per cent protein recorded in the control product was 24.02 ± 0.18 and in the treated formulation with 1% (T_1), 3% (T_2) and 5% (T_3) black rice flour, the per cent protein recorded were 23.21 ± 0.09, 22.16 ± 0.23 and 21.98 ± 0.10 respectively. The per cent protein content for chicken nuggets incorporated with increasing levels of black rice flour was significantly (P>0.01) lower in all the treated samples compared to the control sample. Decrease in protein content might be due to the replacement of lean by black rice powder in the chicken nuggets formulation. Also because of moisture loss during heat processing, protein levels were higher in the control samples compared to the treated sausage samples with rice flour [11]. The present findings corroborate well with the findings of earlier workers, [9] who reported a decrease in protein content with the addition of black rice powder in pork sausage.

Ether Extract

The present study revealed that, the percent fat content recorded in the control product was 12.84 ± 0.07 and in the treated formulation with 1% (T_1), 3% (T_2) and 5% (T_3) black rice flour, the per cent fat recorded were 12.46 ± 0.14, 12.14 ± 0.26 and 11.59 ± 0.15 respectively. The per cent ether extract of chicken nuggets incorporated with increasing levels of black rice flour exhibited a significantly (P>0.01) decreasing trend in fat content from control to the treated formulations. This might be because the fat content of chicken nuggets was replaced by increasing levels of the black rice flour.

The result of the present study are in agreement with the findings of Park *et al*. (2017) [9] who reported a decrease in fat content with the increase in the black rice flour in pork patties and the samples formulated with 3% and 5% black rice flour showed a significantly (P>0.05) lower values than the control. Jebin et.al. (2012) [12] also reported that the mean

values of per cent ether extract decreased significantly (p>0.05) from control to products treated with glutinous rice flour in duck salami.

Total Ash

In the present study, per cent ash recorded in the control product was 1.91 ± 0.09 and in the treated formulation with 1% (T_1), 3% (T_2) and 5% (T_3) black rice flour, the per cent ash recorded were 2.09 ± 0.08, 2.26 ± 0.16 and 2.55 ± 0.19 respectively. The total ash content of the chicken nuggets incorporated with increasing levels of black rice flour revealed a significantly (P>0.05) increasing trend from control to the treated formulations. The increase in the ash content might be attributed to higher ash content in added black rice flour which represents the mineral content of rice [13]. The present findings were in agreement with the earlier reports [9] which revealed a significant (P>0.05) increase in the ash percentage with the increasing black rice powder content in pork patties. Similar findings of the present study, a minor increase in the total ash content in duck meat salami treated with glutinous rice flour was recorded when compared to control product. [12]

Texture Profile Analysis (TPA)

The results of mean values for texture profile scores (hardness, springiness, cohesiveness, chewiness and gumminess) of black rice incorporated chicken nuggets for both control and treated products are presented in Table 2.

Hardness

In the present study, the hardness values recorded in the control product was 2.247 ± 0.53 and in the treated formulation with 1% (T_1), 3% (T_2) and 5% (T_3) black rice flour, the hardness values recorded were 2.515 ± 0.69, 2.631 ± 0.80 and 2.708 ± 0.51 respectively. The black rice treated products had higher hardness values than the control one. However, the difference was found to be non-significant. The change in hardness values might be due to the incorporation of black rice flour in the treated formulations. The present finding was in close agreement with the findings of some other workers [14] who reported that the addition of black rice flour resulted in increased hardness in sausages.

Springiness

In the present study, springiness values recorded in the control product was 0.43 ± 0.02 and in the treated formulations with 1 % (T_1), 3% (T_2) and 5% (T_3) black rice flour the springiness value recorded were 0.45 ± 0.03, 0.45 ± 0.02 and 0.47 ± 0.03 respectively. The chicken nuggets treated with different levels of black rice flour had higher springiness values compared to the control product. However, the difference was found to be non-significant. These observations were in close agreement with the findings of Park et.al. (2017) [9] who observed no significant difference in the springiness value with the incorporation of black rice flour in pork patties.

Cohesiveness

Cohesiveness values recorded in the present study, in the control product was 0.71 ± 0.09 and in the treated formulations with 1 % (T_1), 3% (T_2) and 5% (T_3) black rice flour were 0.67 ± 0.05, 0.60 ± 0.08 and 0.57 ± 0.06 respectively. The chicken nuggets treated with different levels of black rice flour had lower cohesiveness values compared to the control product. However, the difference was found to be non-significant. Similar to the findings of the present study, some previous workers also reported that cohesiveness value was significantly (P>0.05) reduced in duck meat sausage with the addition of rice flours [15].

Chewiness

In the present study, chewiness values recorded in the control product was 0.460 ± 0.10 and in the treated formulations with 1 % (T_1), 3% (T_2) and 5% (T_3) black rice flour the chewiness value recorded were 0.447 ± 0.13, 0.442 ± 0.13 and 0.438 ± 0.03 respectively. The chicken nuggets treated with different levels of black rice flour had lower chewiness values compared to the control product. However, the difference was found to be non-significant. Park et.al.(2016) [10] reported that the chewiness values were significantly (P>0.05) lower in the pork patties incorporated with the black rice flour than the control sample.

Gumminess

In the present study, the gumminess values recorded in the control product was 0.958 ± 0.12 and in the treated formulations with 1 % (T_1), 3% (T_2) and 5% (T_3) black rice flour the gumminess value recorded were 0.952 ± 0.60, 0.930 ± 0.01 and 0.919 ± 0.01 respectively. The chicken nuggets treated with different levels of black rice flour had lower gumminess values in comparison to the control product. However, the difference was found to be non-significant. Similar findings were reported by several workers, Park et.al.(2016) [10] reported that the gumminess were significantly (P>0.05) lower in the pork sausage extended with black rice flour than the control group. Jebin *et al*. (2012) [15] also reported that the gumminess value was lower (p>0.05) in the rice flour treated duck sausage than the control sample.

Microbiological Qualities

Colititre Value (MPN)

The colititre values obtained in the study for both control and the samples with black rice flour, besides, storing of the chicken nuggets up to 15th day of storage were found to be nil. The negative result obtained in the present study might be due to the destruction of these bacteria during cooking much above their death point of 57°C [16]. Further hygienic practices followed during the preparation of chicken nuggets could also be the reason for the absence of coliform count. [17] Present findings corroborated well with the reports of Kumar and Sharma (2003) **[18]** who recorded absence of coliforms in low-fat pork patties throughout 7 days storage period. The present findings are also in well accord with the reports of Malav et.al.(2014) [19] who reported that coliforms were not detected in control and treated products of chicken meat blocks extended with lentil flour during the storage period of 15th days.

Yeast and Mould Count

The mean values for yeast and mould count of chicken nuggets incorporated with different levels of black rice flour at different storage period are presented in Table 3. In the present study, the yeast and mould counts were not detected up to 5th day of storage. However they appeared on day 10 onwards and followed a significantly (P>0.01) increasing trend in all the treated products as well as the control. However, no significant differences were observed in between the control and treated chicken nuggets with increasing levels of black rice flour. The detection of yeast and mould counts on day 15th onwards could be due to post processing

contamination. The present study is in well agreement with the findings of Singh *et al*. (2011) [20] who reported that yeast and mould were detected during the last day (30) of storage of chicken snacks due to the availability of nutrients in meat.

Total Viable Psychotropic Bacterial Count (TVPBC)

The mean values of TVPBC of chicken nuggets incorporated with different levels of black rice flour at different storage period are presented in Table 4. There were no significant differences in the TVPBC of chicken nuggets due to incorporation of increased levels of black rice flour. The total psychotropic count of chicken nuggets recorded a significantly (P>0.01) increasing trend from 1st - 15th day of storage. A detectable count on progressive storage period, while nil count on preceding observations might be attributed to the fact that bacteria generally need some lag phase before active multiplication is initiated [16]. Absence of psychotrophs in the initial stages of cold storage may be due to cooking of the product at high temperature followed by storage at low temperature resulting in retardation of microbial growth due to temperature shock [21]. The present findings corroborated well with the reports of Zargar *et al*. (2014), [22] who also reported a significant (P>0.01) increase in the total psychrophilic count in chicken sausages incorporated with pumpkin.

Table 1: Effect of Incorporation of Black Rice Flour on Proximate Composition (%) of Chicken Nuggets (Mean ± Se)

Group	Moisture	Protein	Fat	ASH
Control	59.94^{a}±0.13	24.02^{a}±0.18	12.84^{a}±0.07	1.91^{a}±0.09
T_1	60.68^{b}±0.16	23.21^{b}±0.09	12.46^{ab}±0.14	2.09^{a}±0.08
T_2	61.31^{c}±0.27	22.16^{c}±0.23	12.14^{b}±0.26	2.26^{ab}±0.16
T3	61.81^{c}±0.23	21.98^{c}±0.10	11.59^{c}±0.15	2.55^{b}±0.19

n = 5

Mean with superscript bearing different alphabet (small) column wise differ significantly

Table 2: Effect of Incorporation of Black Rice Flour on Texture Profile Analysis (Force in Kg) Of Chicken Nuggets (Mean ± Se)

TPA	Control	Black Rice Flour		
	C_T (0%)	T_1 (1%)	T_2 (3%)	T_3 (5%)
Hardness	2.247^{a}±0.53	2.515^{a}±0.69	2.631^{a}±0.80	2.708^{a}±0.51
Chewiness	0.460^{a}±0.10	0.447^{a}±0.13	0.442^{a}±0.13	0.438^{a}±0.03
Gumminess	0.958^{a}±0.12	0.952^{ab}±0.06	0.930^{ab}±0.01	0.919^{b}±0.01
Springiness	0.432^{a}±0.03	0.453^{a}±0.02	0.458^{a}±0.02	0.470^{a}±0.03
Cohesiveness	0.710^{a}±0.09	0.670^{a}±0.05	0.600^{a}±0.08	0.570^{a}±0.06

n = 5

Mean with superscript bearing different alphabet (small) row wise differ significantly

Table 3: Effect of Incorporation of Black Rice Flour on Yeast and Mould Count (Log Cfu/G) Of Chicken Nuggets (Mean ± Se)

Day	Control	Black Rice Flour		
	C_T	T_1	T_2	T_3
1	-	-	-	-
5	-	-	-	-
10	$^{A}1.81^{a}$±0.01	$^{A}1.83^{a}$±0.02	$^{A}1.80^{a}$±0.01	$^{A}1.82^{a}$±0.01
15	$^{B}1.97^{a}$±0.02	$^{B}1.96^{a}$±0.01	$^{B}1.98^{a}$±0.02	$^{B}1.93^{a}$±0.02

n = 5

Mean with superscript bearing different alphabet (small) row wise differ significantly

Mean with superscript bearing different alphabet (capital) column wise differ significantly

Table 4: Effect of Incorporation of Black Rice Flour on Total Viable Psychrotrophic Bacterial Count (Log Cfu/G) Of Chicken Nuggets (Mean ± Se)

Day	Control	Black Rice Flour		
	CT	T1	T2	T3
1	-	-	-	-
5	$^{A}2.47^{a}$±0.01	$^{A}2.44^{a}$±0.01	$^{A}2.46^{a}$±0.02	$^{A}2.45^{a}$±0.01
10	$^{B}3.09^{a}$±0.02	$^{B}3.06^{a}$±0.01	$^{B}3.08^{a}$±0.01	$^{B}3.04^{a}$±0.03
15	$^{C}3.22^{a}$±0.01	$^{C}3.20^{a}$±0.01	$^{C}3.24^{a}$±0.01	$^{C}3.23^{a}$±0.02

n = 5

Mean with superscript bearing different alphabet (small) row wise differ significantly

Mean with superscript bearing different alphabet (capital) column wise differ significantly

Conclusion

Chicken nuggets with incorporation of black rice can be prepared successfully, which could add functional properties of black rice to the final product. Black rice flour added chicken nuggets were found to be of high nutritional value, optimum textual properties and acceptable microbiological qualities. Thus, it may be concluded that chicken meat products can be developed satisfactorily with addition of black rice flour up to 5 percent level without any adverse effect on the quality of the processed products.

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