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Effects of Incorporation of Black Rice *(Oryza sativa* L. indica) Extract on Nutritional, Antimicrobial, and Antioxidant properties of Duck Meat Nuggets

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ABSTRACT

Duck Meat Nuggets were prepared by incorporating Black Rice Extract (BRE) as natural antioxidants to find the best formulation with superior antioxidant properties and shelf life. Four formulations were prepared viz. Control (0% BRE), T1 (0.5% BRE), T2 (0.9% BRE), T3 (1.3% BRE). Five batches of duck meat nuggets of each formulation were prepared and the final products were evaluated for physicochemical, antioxidant, and microbiological qualities. All parameters under the proximate composition, viz. Moisture, crude protein (CP), ether extract, and total ash (TS) content showed a non-significant ($p<0.05$) difference between control and BRE-treated products. The total plate count (TPC) of the control and BRE-treated products increased significantly ($P<0.01$) with increasing storage time up to 15 days. However, the control products had the highest TPC throughout the storage period. The total number of viable psychrophilic bacteria (TVPBC) was not detected until the 5th day of storage. After day 5, TVPBC increased significantly ($P<0.01$) in all control and BRE-treated products through day 15 of storage. The incorporation of black rice extracts reduced TVPBC significantly ($P<0.01$) in the treated samples than in the control sample. Colititre count, yeast and mold count and Staphylococcus count were negative for all formulations up to the 15th day of storage. It was noticed that higher concentrations of BRE had high total phenolic content. The addition of BRE significantly ($P<0.01$) increased antioxidant activity in the treated samples compared to the control. One of the challenges faced during the study was the determination of the optimal concentration of Black Rice Extract (BRE) to be incorporated into the duck meat nuggets. The researchers had to carefully select and evaluate different concentrations of BRE to find the formulation that provided the best antioxidant properties and extended shelf life for the product. Duck meat nuggets with 1.3% BRE (T3) had the highest antioxidant activity value and had an extended shelf life of up to 15 days.

Keywords: *Antioxidant, Black rice extract, duck meat, nuggets, phenolic content, proximate composition, natural antioxidants , syanthetic antioxidants , antimicrobial agents*

INTRODUCTION

Poultry meat is a very popular choice for animal protein worldwide due to its low price and availability. After chicken and turkey, duck meat is the third most commonly produced poultry meat in the world. Duck's popularity as a source of poultry meat is more pronounced in Asian countries compared to other parts of the world. Asian countries contribute 84.2% of all duck meat produced worldwide [11]. Due to its high nutrient content with optimal essential amino acids, the right fatty acid composition with a high proportion of polyunsaturated fatty acids, and a balanced ratio of omega-6 and omega-3, duck meat is consumed as a nutritional food. Duck meat is unique and tasty, easy to prepare, and use in various dishes [17]. Thus, duck meat has shown less juiciness and more toughness, which are the hidden reasons behind the negative response to duck meat by consumers. In addition, due to the higher content of unsaturated fatty acids, duck meat is more susceptible to lipid oxidation [7]. Meat and meat products are highly perishable because of the lack of inherent antioxidants and the high nutrients available. The use of natural antioxidants to improve the antioxidative defense may be an alternative method of preventing oxidation and quality deterioration of freshly processed meat and meat products [8, 21, 18]. Even at low concentrations, the antioxidants can retard the oxidation of easily oxidizable biomolecules, such as lipids and proteins in meat products [27]. However, on these health-conscious days, the use of synthetic antioxidants, such as butylated hydroxyl toluene (BHT) and butylated hydroxyl l anisole (BHA), is found to be low as many researchers reported many health risks to man due to the use of synthetic antioxidants. Many researchers and scientists have used different herbs, fruits, and vegetables having antioxidant properties and are used in meat product development, and they reported that they had found positive results. Among natural

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antioxidants, black rice can be used as a source of antioxidants in meat and meat products, which consists of two main anthocyanins, i.e. cyanidin and peonidin, which react similarly as the antioxidant agents that reduce the density of lipoprotein and reduce the nitric oxide formation [34]. The extraction methods and solvents affect the antioxidant activity of natural antioxidants in many conditions [33]. Ethanol and water are the most frequently used extraction solvents because they are edible and safe. Ethanol is used as a suitable solvent [29]. Since meat products and rice must be thermally processed before consumption, the thermal degradation of phenolic compounds is a significant problem in using natural pigments in the meat industry. Therefore, to retain more bioactive compounds in the end products use of β-cyclodextrin was reported [9]. Based on the above facts, the present study is planned to evaluate the different quality parameters of duck meat nuggets by adding antioxidants from black rice extract as replacement food additives for inhibiting lipid oxidation.

MATERIALS AND METHODS

Preparation of products

The duck meat nuggets were prepared as per a basic formulation (Table 1.). Local ducks were purchased from the Beltola market of Guwahati city and were slaughtered in the laboratory of the department of LPT, CVSc, A.A.U., Khanapara, Guwahati-781022. The carcasses were stored at a refrigeration temperature (4±1ºC). After 24 hrs of storage at a refrigeration temperature of the carcasses, deboning was done manually, maintaining hygienic conditions in the laboratory. The required portion of meat was packed in an LDPE bag and stored at 4±1ºC temperature. The deboned meat, heart, and gizzard were cut into small cubes and then minced in a mechanical mincer through a 4-millimeter pore size plate. After mincing of meat, all the curing ingredients, i.e. salt, sodium tripolyphosphate, and sodium nitrite (Table 4.1), were added to the minced meat. For proper curing, all the ingredients and meat were mixed thoroughly and stored at refrigeration temperature (4±1ºC) for another 24 hours to facilitate adequate curing.

Preparation of black rice extract: Good quality black rice was purchased from the nearby supermarket in Guwahati city. Black rice extract (BRE) was prepared according to the method described by [26], with slight modifications. After being purchased from the market, black rice was adequately cleaned and then ground in a mechanical grinder and made of black rice flour. Then a weighed portion, i.e.100g of black rice flour, was soaked in 250 ml of 80% ethanol for 4 hours. After 4 hours, the extract was filtered through Whatman No.1 filter paper. Then with the help of a vacuum rotary evaporator, the filtered extract solution was evaporated, and the initial volume of the extract solution was reduced. After that, the remaining extract solution was poured into sterilized Petri dishes and placed in an incubator at 37ºC until all the ethanol and water were evaporated. After that, with the help of a sterilized spatula, the extract was scraped out from the petri dish, and with a pestle & mortar, the black rice extract was made into fine powder form. The finely prepared black rice extract was stored at -20ºC for future use.

Preparation of meat emulsions

Three different meat batters were prepared, incorporating different concentrations of black rice extract. After initial trials with different concentrations, based on organoleptic acceptability, BRE at 0.5%, 0.9%, and 1.3% were incorporated in duck nuggets of T1, T2, and T3 groups, respectively. A control group of nuggets was also prepared following the same procedure but without incorporating black rice extract. The percentages of meat and non-meat ingredients are given in (Table 1). To prepare meat emulsion, all seasonings, i.e., spices and condiments and other non-meat ingredients, were added to the cured duck meat, and black rice extract of different concentrations in the treated formulations and all the ingredients were mixed thoroughly to make the emulsion. In preparation of meat emulsion, the emulsion stability was determined and compared to the control nuggets. The emulsions were then stuffed into a stainless steel mold and cooked with the steam cooking method (80ºC for 45min). Duck meat blocks so obtained were cooled, sliced, and cut into the shape of nuggets.

Proximate composition

The proximate composition of duck meat sausages was estimated as per the standard method [2]. The crude protein content of the samples was determined by the Micro Kjeldahl method by KEL PLUS KES 6L (Make: Pelican Equipment, Chennai), and fat contents was determined by Soxhlet methods (Make: Pelican Equipment, Chennai; Model: KEL PLUS CLASSIC DX). The moisture content was assessed at 105°C under normal pressure by the drying method, whereas crude ash content was determined by placing the samples in a muffle furnace and operated at 525°C for 10-12 hours until white ash was obtained.

Microbiological qualities

Total Viable Count (T.V.C.)

Enumeration of the total viable plate count of the sausage samples was done in standard plate count agar medium by following the pour plate technique as per the standard method [3].

Total Viable Psychrophilic Bacterial Count (TVPBC.)

The Total viable psychrophilic bacterial counts of sausages were determined as per the standard method [3].

Coliform Count

Coliform counts were enumerated by following the standard technique [15]. It was done by inoculating 1ml of the diluents in Endo agar followed by incubating at 37ºC for 24h. The average number of colonies counted was then expressed as the presence or absence of coliforms in samples.

Staphylococcus Count

Staphylococcus counts were made at similar time intervals as in Total plate count by inoculating the appropriate dilution of the sample in Mannitol Salt Agar and incubating at 37ºC up to 24hrs. The yellow-colored colony indicates the presence of the Staphylococcus organism [15].

Yeast and Mould Counts

Yeast and mold counts of the nuggets sample were made at similar time intervals as that of the total plate count by

inoculating the appropriate dilution of the sample on Rose Bengal Agar Base and incubating at 37ºC up to 72h [15].

Total Phenolic Content

The total phenolic content of the Black Rice Extract was determined by the spectrophotometric method using the Folin-Ciocalteu reagent by following the procedure described by [24]. 10 mg of the extract was weighed, inserted into a 10.0 ml volumetric flask, added 6.0 ml methanol, was shaken until dissolved, diluted with methanol to the marked line, and shaken homogenously (obtained extract stock solution with concentration 1000µg/ml). 5ml and 9ml of extract stock solution were taken, inserted into a 10.0 ml volumetric flask, diluted with methanol to the marked line, and shaken homogenously (obtained solution with extract concentration 500µg/ml and 900µg/ml). With the same procedure, 13mg of extract was dissolved in 10 ml of methanol to obtain 1300µg/ml extract concentration. 0.5 ml of each extract solution was taken and inserted into a 10.0 ml volumetric flask. Then, it was added with 7.5 ml water and 0.5 ml of FC solution, homogenized with vortex for 1 min, diluted with sodium carbonate solution to the marked line, and shaken homogenously. The solution was left until the operating time, measured the absorbance at 725nm wavelength.

The total phenolic content of the extracts was calculated from the regression equation of calibration curve (Y = 0.004x + 0.108; R2 = 0.993) and expressed as mg gallic acid equivalents (GE) per gram of sample.

Antioxidant Activity (DPPH free radical scavenging activity)

The antioxidant activity was determined by DPPH free radical scavenging activity method. The antioxidant activity test was based on the method described by [10] and the antioxidant activity of nuggets was determined as a radical scavenging activity of DPPH according to [12] with slight modification.

Three (3) g of nugget sample was mixed with 10 ml methanol and homogenized for 30 min at room temperature. The homogenate was centrifuged at 3000 g and 4ºC for 15 min to collect the clear supernatant. In a test tube, 200 µl of 0.5 mM DPPH solution was taken, and 100 µl of nugget extracts were added to this and incubated in the dark for 20 min at room temperature. The absorbance was measured at 517 nm using a UV-Vis Spectrophotometer.

The DPPH scavenging activity was calculated by the following equation:

$$\text{DPPH scavenging activity (\%)} = \frac{[Ac - As]}{Ac} \times 100$$

Where "Ac" is the absorbance of the control (DPPH with methanol), "As" is the absorbance of the sample.

Statistical Analysis

The data obtained in the study were analysed statistically following the standard statistical method by employing SAS 9.3 software. Data were presented using basic descriptive statistics, viz. mean and standard error. Comparison of different groups and storage days were analyzed using the Two-way Analysis of Variance technique.

RESULTS AND DISCUSSION

Proximate composition

Moisture, Crude Protein (CP), ether extract and Total Ash (TS) percent content showed a non-significant ($p < 0.05$) difference between the control and the treated products incorporated with different levels of BRE (Table 2). The present study's findings were in close agreement with the study of [25] and they found a non-significant difference between control and black rice extract-treated products in terms of proximate composition with the incorporation of 0.4, 0.8 and 1.2% black rice water extract in beef patties. Similarly, [5] observed a non-significant difference between control and roselle extract-treated frankfurter-type sausage in moisture, fat and ash content. [3] also found a similar result with the addition of BHT, pomegranate peel aqueous extract (PPAE), and pomegranate aril bagasse powder aqueous extract (PABAE) in chicken patties. The black mulberry water extract (BMWE) treatment had no significant effect ($P > 0.05$), with control and treated products on moisture, fat, and ash values of beef patties [32]. In corroboration with the present study, some earlier workers also reported similar non-significant differences ($P > 0.05$) in values for moisture, protein, fat, and ash content among the treatments, showing that neither red pitaya extract nor sodium erythorbate affected the composition of the pork patties [6].

MICROBIOLOGICAL QUALITIES

Total Plate Count (TPC)

The mean value for TPC showed that the incorporation of black rice extract significantly ($P < 0.01$) decreased the TPC in the treated samples on day 1 than in the control sample (Table 3). Bioactive and phenolic compounds, i.e. anthocyanin having antimicrobial properties present in black rice extract, might be the reason for the lower TPC value in the treated duck nuggets [4].

The TPC (Table 3) of control and BRE-treated products increased significantly ($P < 0.01$) with the advancement of the storage period up to 15 days. However, throughout the storage period, control products had the highest TPC. Similar observations to the present study have been reported by earlier workers [19] in chicken products incorporated with pomegranate peel extract, pork burgers incorporated with red grape pomace extract [13], beef meatloaf with the incorporation of olive leaf, blueberry, and Zizyphus jujuba extracts [14] and beef patties with the addition of black mulberry extract [32]. [30] reported that the addition of pomegranate by-product extracts significantly increased ($P < 0.05$) the TPC of control and treated patties in all the treatments with the increase in storage duration.

Total Viable Psychrophilic Bacterial Count (TVPBC.)

The TVPBC was not detected until the 5th day of storage. After the 5th day, the TVPBC increased significantly ($P < 0.01$) in all the control and BRE-treated products up to the 15th day of storage. The incorporation of black rice extract significantly ($P < 0.01$) decreased the TVPBC in the treated sample throughout the storage period (Table 4). This might be due to the inhibitory effect of bioactive and phenolic compounds, i.e. anthocyanin present in the black rice extract, which lowers the growth of bacteria in the treated products [4].

The initial absence of TVPBC during storage could be due to the

lower metabolic rate of these microbes at low pH, thus retarding log phage. This detection of psychrophiles after initial absence could be because bacteria generally need some lag phase before starting active multiplication in the log phase [20] which is similar to the report of [21] in pork patties with phyto-extract.

A similar observation that agrees well with the present study was reported by [31] using Jamun fruit extract in chicken patties. They did not detect psychrophilic bacterial count until the 6th day of refrigerated storage of patties. On the 9th day, it appeared for the first time (1.42 log cfu/g), which increased significantly ($P < 0.05$) during the propagation of the storage period. In another study, it was reported that the addition of pomegranate by-product extract significantly increased ($P < 0.05$) the TVPBC value of control and treated chicken patties in all the treatments with an increase in storage duration [30]. The rate of growth was less in pomegranate by-products and their extract. The inhibitory effect of bioactive and phenolic compounds present in pomegranate peel, bagasse, and their extracts resulted in significantly lower TVPBC in treated patties than in control at the end of storage.

Coliform Count, Yeast and Mould Count, and Staphylococcus Count

The study showed that coliform counts, yeast and mold counts and Staphylococcus counts were not detected in control or black rice extract-treated products during 15 day storage period. It might be due to the good sanitary condition of the raw material and hygienic processing or manufacturing conditions in the laboratory. Similar to the present study, in an earlier study, faecal coliforms were not detected in any control and different concentrations of pomegranate peel extract-treated chicken lollipop [19]. In beef sausage with red dragon fruit extract, a similar result was found for E.coli [23]. Up to 14 days of storage, the coliform count has not detected in watermelon rind extract-treated products in pork patties incorporating watermelon rind extract [20]. Onion peel extract showed an antibacterial effect in the case of staphylococcus count in pork sausage [22].

TOTAL PHENOLIC CONTENT OF BLACK RICE EXTRACT

The results for the total phenolic content of different black rice extract concentrations are presented in (Table 5). The present study observed that a higher concentration of black rice extract had a high amount of total phenolic content.

Similar findings were observed with different concentrations of red dragon fruit peel extract [23]. [16] reported highest black rice ethanol extract had higher amounts of total phenolic compounds than the red sorghum hull.

DPPH FREE RADICAL SCAVENGING ACTIVITY

The mean values for antioxidant activity showed that incorporating black rice extract significantly ($P < 0.01$) increased the antioxidant activity in the treated sample than in the control sample. The T3 sample had the highest antioxidant activity value, and the control sample had the significantly lowest one on day 1. The result might be due to the phenolic content and flavonoid, i.e. anthocyanin present in black rice extract, which have potent antioxidant properties.

The findings of the present study agree well with an earlier study where it was reported that the polyphenols and anthocyanin present in black rice are effective antioxidants that can limit oxidative changes in beef meat patties [25]. Anthocyanin and phenolics can prevent the formation of fatty free radicals by inhibiting the free radical formation and blocking radical chain reactions in the oxidation process [28]. In one more study, it was also observed that the incorporation of capsicum, carrot, spinach, purple cabbage and oyster mushroom in chicken sausage had shown that purple cabbage rich in anthocyanin had the highest antioxidant activity than other vegetables [1].

The results of the present study revealed that the antioxidant activity (Table 6) of control and BRE-treated products decreased significantly ($P < 0.01$) with an increased storage period of up to 15 days. However, control products had the lowest antioxidant activity value throughout the storage period, and T3 products had the highest antioxidant activity value on day 15. A similar study revealed decreased antioxidant activity in beef patties treated with black rice water extract during refrigeration storage [25]. It was also reported that Red pitaya extract incorporated in pork patties revealed gradually decreased antioxidant activity during the storage period [6].

CONCLUSION

Based on the results obtained from studies on the various parameters in this investigation, it may be concluded that duck meat nuggets can be prepared by incorporating Black Rice Extract. Duck meat nuggets with 1.3% BRE (T3) had the highest antioxidant activity value. These results indicate that Black Rice Extract can be used as a promising natural alternative to synthetic colorants, antioxidants, and antimicrobial agents in meat products.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Table 1. FORMULATION OF INGREDIENTS FOR PREPARATION OF DUCK MEAT NUGGETS

Name of ingredients	Quantity (%)
Duck meat	75.0
Vegetable oil	8.0
Ice cubes	10.0
Liquid egg white	3.0
Corn flour	4.0
Total	**100.0**
Spices	1.5
Condiments	3.0
Salt	1.5
STPP	0.3
Sodium nitrite	0.2
Black rice extract**	C -0%, T_1-0.5%, T_2- 0.9%, T_3-1.3%
β-cyclodextrin	1.0

Table 2. PROXIMATE COMPOSITION (%) OF DUCK MEAT NUGGETS (MEAN ± SE.) INCORPORATED WITH DIFFERENT CONCENTRATIONS OF BLACK RICE EXTRACT

Parameter	Duck meat nuggets with Different Concentrations of BSE			
	CS	T1S	T2S	T3S
Moisture	63.78 ±0.41	63.76 ±0.41	63.76 ±0.41	63.74±0.41
Ether Extract	11.52±0.15	11.53 ±0.15	11.55±0.15	11.57 ±0.15
Protein	19.15±0.01	19.16 ±0.01	19.18±0.01	19.19±0.01
Ash	3.37±0.17	3.41±0.16	3.42 ±0.16	3.43±0.16

Table 3. TOTAL PLATE COUNT (log cfu/g) OF DUCK MEAT NUGGETS (MEAN ± SE.) INCORPORATED WITH DIFFERENT CONCENTRATIONS OF BLACK RICE EXTRACT

Day	Duck meat nuggets with Different Concentrations of BSE.			
	C	T_1	T_2	T_3
1	$^{A}2.82\pm0.06^{a}$	$^{A}2.69\pm0.05^{ab}$	$^{A}2.58\pm0.04^{b}$	$^{A}2.49\pm0.04^{bc}$
5	$^{B}3.79\pm0.03^{a}$	$^{B}3.66\pm0.05^{b}$	$^{B}3.52\pm0.03^{bc}$	$^{B}3.40\pm0.04^{c}$
10	$^{C}4.63\pm0.07^{a}$	$^{C}4.48\pm0.05^{b}$	$^{C}4.37\pm0.04^{bc}$	$^{C}4.24\pm0.04^{c}$
15	$^{D}5.83\pm0.02^{a}$	$^{D}5.65\pm0.03^{b}$	$^{D}5.44\pm0.03^{c}$	$^{D}5.35\pm0.03^{cd}$

Means with dissimilar superscripts in a row (small letter) differ significantly, P < 0.01.
Means with dissimilar superscripts in a column (capital letter) differ significantly, P < 0.01.

Table 4. TOTAL VIABLE PSYCHROPHILIC COUNT (log cfu/g) OF DUCK MEAT NUGGETS (MEAN ± SE.) INCORPORATED WITH DIFFERENT CONCENTRATION OF BLACK RICE EXTRACT

Day	Duck meat nuggets with Different Concentrations of BSE			
	CS	T_1S	T_2S	T_3S
1	-	-	-	-
5	-	-	-	-
10	$^{A}1.80\pm0.06^{a}$	$^{A}1.64\pm0.05^{ab}$	$^{A}1.58\pm0.06^{ab}$	$^{A}1.49\pm0.07^{b}$
15	$^{B}2.76\pm0.05^{a}$	$^{B}2.66\pm0.04^{ab}$	$^{B}2.57\pm0.04^{b}$	$^{B}2.48\pm0.03^{b}$

Means with dissimilar superscripts in a row (small letter) differ significantly, P < 0.01.
Means with dissimilar superscripts in a column (capital letter) differ significantly, P < 0.01.

Table 5. TOTAL PHENOLIC CONTENT OF DIFFERENT CONCENTRATION OF BLACK RICE EXTRACT

Sample	Sl No.	Absorbance (AU.)	Concentration (µg/ml)	Total phenolic (mg/g)
Black Rice Extract	1	0.231	25.21309	307.5 mg/g
	2	0.356	50.6027	620.0 mg/g
	3	0.457	71.32063	872.5 mg/g

Table 6. DPPH FREE RADICAL SAVENGING ACTIVITY (%) OF DUCK MEAT NUGGETS INCORPORATED WITH DIFFERENT CONCENTRATIONS OF BLACK RICE EXTRACT

Day	Duck meat nuggets with Different Concentrations of BSE.			
	CS	T_1S	T_2S	T_3S
1	$^{A}22.39 \pm 0.48^{a}$	$^{A}31.26 \pm 0.03^{b}$	$^{A}31.79 \pm 0.04^{bc}$	$^{A}32.13 \pm 0.04^{c}$
5	$^{AB}22.15 \pm 0.46^{a}$	$^{AB}31.06 \pm 0.09^{b}$	$^{A}31.28 \pm 0.10^{bc}$	$^{A}31.38 \pm 0.11^{bc}$
10	$^{B}19.18 \pm 0.44^{a}$	$^{B}28.14 \pm 0.23^{b}$	$^{B}28.25 \pm 0.21^{b}$	$^{B}28.37 \pm 0.21^{bc}$
15	$^{C}17.16 \pm 0.40^{a}$	$^{C}26.21 \pm 0.11^{b}$	$^{C}26.28 \pm 0.11^{b}$	$^{C}26.39 \pm 0.10^{b}$

Means with dissimilar superscripts in a row (small letter) differ significantly, P < 0.01
Means with dissimilar superscripts in a column (capital letter) differ significantly, P < 0.01

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