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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

The study was conducted during January, 2021–December, 2021 at Dept. of Livestock Products Technology, College of Veterinary Science, Khanapara, Assam, India to evaluate the different quality parameters of duck meat nuggets by adding black rice extract (BRE). Duck meat nuggets were developed using black rice extract as natural antioxidants. This innovative approach not only enhanced the nutritional profile of the nuggets but also contributed to their enhanced flavour, making them a healthy processed food with improved antioxidant properties and shelf life (Gaps required in enhanced the healthy processed food with improved). Four formulations were prepared: Control (0% BRE), Treatment 1 (0.5%), Treatment 2 (0.9%), and Treatment 3 (1.3%). Five batches of duck meat nuggets of each formulation had been evaluated for physicochemical, antioxidant, and microbiological properties. Moisture, crude protein, ether extract, and total ash indicated no significant difference ($p<0.05$) between control and BRE-treated items. As storage duration increased up to 15 days, the total plate count (TPC) of the control and BRE-treated products considerably increased ($p<0.01$). However, control products had the highest TPC during storage. Total Viable Psychrophilic Bacterial Count (TVPBC) increased ($p<0.01$) in all control and BRE-treated items after the 5th day of storage till the 15th day. TVPBC was significantly ($p<0.01$) lower in the BRE treated samples than in the control sample. Coliform, yeast, mould and Staphylococcus counts were negative for all formulations up to the 15th day. The addition of BRE significantly ($p<0.01$) increased antioxidant activity in the treated samples compared to the control. Duck meat nuggets with 1.3% BRE (Treatment 3) had the highest antioxidant activity and a 15-day shelf life.

KEYWORDS: Antioxidant, black rice extract, duck meat, nuggets, phenolic

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1. INTRODUCTION

Duck meat consumption worldwide has surged rapidly; overall production in the past decade (2012–2021) has increased from 4.7 mt to 6.2 mt. (Anonymous, 2023). Over the past decade (2012–2022), Asia has emerged as the global leader in duck meat production, accounting for 86.4% of the total, followed by Europe with 9.7% (Anonymous, 2021a). With optimal essential amino acids, a high proportion of PUFAs and a balanced ratio of omega-6 and omega-3, duck meat is consumed as a nutritious food due to its unique taste, and ease of preparation in various dishes (Ismoyowati and Sumarmono, 2017). On the other hand, duck meat has shown less juiciness and more toughness, which are the hidden reasons behind the negative response to duck meat by the consumers. In addition, due to the higher content of unsaturated fatty acids, duck meat is more susceptible to lipid oxidation (Biswas et al., 2019). Duck fat, abundant in unsaturated fatty acids (particularly oleic acid), offers physical, thermal, and oxidative stability of final processed products compared to other animal fats and vegetable oils. (Shin at al., 2023). Oxidation is one of the main factors associated with the reduction or degradation of quality of meat products without a microbial reaction. (Lee et al., 2020). The meat and meat products are highly perishable due to their lack of inherent antioxidants and the high nutrient content. Several synthetic antioxidants have been used to successfully prevent lipid oxidation in the meat industry, even at low concentrations, the antioxidants can retard the oxidation of easily oxidisable biomolecules, such as lipids and proteins in meat products (Rather et al., 2016). However, at present due to increased health-consciousness, the use of synthetic antioxidants, such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA), is found to be low as many researchers reported many health risks to man due to the use of synthetic antioxidants. Therefore, there has been a growing interest in natural antioxidants. (Reddy et al., 2018). The use of natural antioxidants to improve the anti-oxidative quality may be an alternative method of preventing oxidation and quality deterioration of processed meat and meat products (Brettonnet et al., 2010, Kumar et al., 2015 and Jiang et al., 2016). Among natural 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 (Zawistowski and Kitts, 2003). Anthocyanins have been described as compounds that prevent or inhibit the oxidation by scavenging free radicals and reducing the oxidative stress. (Tena and Asuero, 2020). The extraction methods and solvents affect the antioxidant activity of natural antioxidants in many conditions (Turkmen et al., 2006). Ethanol and water are the most frequently used extraction solvents because they are edible and safe. Ethanol is used as a suitable solvent (Shah et al., 2014). Since meat products and rice must be thermally processed before consumption, the thermal degradation of phenolic compounds is a significant problem in using natural pigment in the meat industry. Therefore, to retain more bioactive compounds in the end products use of β-cyclodextrin were reported by Cheng et al. (2019). Based on the above facts, the present study was 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.

2. MATERIALS AND METHODS

2.1. Preparation of products

The study was conducted from January, 2021–December, 2021 at Dept of Livestock Products Technology, College of Veterinary Science, Khanapara, Assam, India. 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 Livestock Products Technology, College Veterinary Science, A.A.U., Khanapara, Guwahati (781 022) India. The carcasses were stored at refrigeration temperature (4±1°C). After 24 hrs of storage at refrigeration temperature, the carcasses were manually deboned, maintaining hygienic conditions in the laboratory. The required portion of meat was packed in an LDPE bag and stored at 4±1°C temperature. The deboned meat, heart, and gizzard were cut into small cubes and then minced in a mechanical mincer through a 4-millimetre pore size plate. After mincing the meat, all the curing ingredients, i.e. salt, sodium tripolyphosphate and sodium nitrite (Table 4), were added to the minced meat. For proper curing, all the ingredients and meat were mixed thoroughly and stored at refrigeration temperature (4±1°C) for another 24 hrs to facilitate adequate curing.

2.2. 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 Rahman, et al. (2016) with slight modification. After being purchased from the market, black rice was adequately cleaned and then ground in a mechanical grinder to make the black rice flour. Then a weighed portion i.e.100 g of black rice flour was soaked in 250 ml of 80% ethanol for 4 hrs. After 4 hrs, the extract was filtered through Whatman No. 1 filter paper. Then with the help of a rotary vacuum evaporator, the filtered extract solution was evaporated, and the initial volume of extract solution was reduced. After that, the remaining extract solution was poured into sterilized Petri

dishes and placed in an incubator at 37°C 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 and mortar, the black rice extract was made into fine powder form. The finely prepared black rice extract was stored at -20°C for future use.

2.3. 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 T_1, T_2 and T_3 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. After preparation of meat emulsion, the emulsion stability was determined and compared to the control nuggets. The emulsions were then stuffed into stainless steel moulds and cooked with the steam cooking method (80°C for 45 min). Duck meat blocks so obtained were cooled, sliced and cut into the shape of nuggets.

2.4. Proximate composition

The proximate composition of duck meat sausages was estimated as per the standard method (Anonymous, 2020). 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 hrs until white ash was obtained.

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	100.00
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

2.5. Microbiological qualities

2.5.1. 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 (Anonymous, 2021).

2.5.2. Total viable psychrophilic bacterial count (TVPBC.)

The Total viable psychrophilic bacterial counts of sausages were determined as per the standard method (Anonymous 2021a).

2.5.3. Coliform count

Coliform counts were enumerated by following the standard technique (Harrigan, and McCance, 1976). It was done by inoculating 1 ml of the diluents in Endo agar followed by incubating at 37°C for 24 hrs. The average number of colonies counted was then expressed as the presence or absence of coliforms in samples.

2.6. 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 37°C up to 24 hrs. The yellow-colored colony indicates the presence of the Staphylococcus organism (Harrigan, and McCance, 1976).

2.7. 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 37°C up to 72 hrs (Harrigan, and McCance, 1976).

2.8. 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 Nerdy et al. (2018). 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 1000 μg ml^{-1}). 5 ml and 9 ml 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 500 μg ml^{-1} and 900 μg ml^{-1}). With the same procedure, 13 mg of extract was dissolved in 10 ml of methanol to obtain 1300 μg ml^{-1} 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 725 nm wavelength.

The total phenolic content of the extracts was calculated from the regression equation of calibration curve (Y=0.004x+0.108; R^2=0.993) and expressed as mg gallic acid equivalents (GE) per gram of sample.

2.9. 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 Desmiaty et al. (2017)and the antioxidant activity of nuggets was determined as a radical scavenging activity of DPPH according to Fratianni et al. (2010) 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 4°C 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:

DPPH scavenging activity (%)={(Ac–As)/Ac}×100

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

2.10. 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.

3. RESULTS AND DISCUSSION

3.1. Proximate composition

Moisture, Crude Protein (CP), ether extract and Total Ash per cent content showed a non-significant (p<0.05) difference between 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 Prommachart et al. (2020) 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, (Baez et al., 2020) observed a non-significant difference between control and roselle extract-treated frankfurter type sausage in moisture, fat and ash content. Sharma and Yadav (2020) 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.

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	T_1S	T_2S	T_3S
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

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 (Turan and Simsek, 2021) .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 (Bellucci et al., 2021).

3.2. Microbiological qualities

3.2.1. 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 (Aziz et al., 2018).

The TPC (Table 3) of control and BRE treated products increased significantly (p<0.01) with the advancement of

Table 3: Total plate count (log cfu g^{-1}) of duck meat nuggets (mean±se.) incorporated with different concentrations of Black rice extract

Days	Duck meat nuggets with different concentrations of BSE			
	C	T_1	T_2	T_3
4	^{A}2.82±0.06^{a}	^{A}2.69±0.05^{ab}	^{A}2.58±0.04^{b}	^{A}2.49±0.04^{bc}
5	^{B}3.79±0.03^{a}	^{B}3.66±0.05^{b}	^{B}3.52±0.03^{bc}	^{B}3.40±0.04^{c}
10	^{C}4.63±0.07^{a}	^{C}4.48±0.05^{b}	^{C}4.37±0.04^{bc}	^{C}4.24±0.04^{c}
15	^{D}5.83±0.02^{a}	^{D}5.65±0.03^{b}	^{D}5.44±0.03^{c}	^{D}5.35±0.03^{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^{-1}) of duck meat nuggets (mean±se.) incorporated with different concentration of Black rice extract

Days	Duck meat nuggets with Different concentrations of BSE			
	C	T_1	T_2	T_3
1	-	-	-	-
5	-	-	-	-
10	^{A}1.80±0.06^{a}	^{A}1.64±0.05^{ab}	^{A}1.58±0.06^{ab}	^{A}1.49±0.07^{b}
15	^{B}2.76±0.05^{a}	^{B}2.66±0.04^{ab}	^{B}2.57±0.04^{b}	^{B}2.48±0.03^{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, $p<0.01$

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 (Kanatt et al., 2010) in chicken products incorporated with pomegranate peel extract, pork burgers incorporated with red grape pomace extract (Garrido et al., 2011), beef meatloaf with the incorporation of olive leaf, blueberry and Zizyphusjujuba extracts (Gok and Bor, 2012) andbeef patties with the addition of black mulberry extract (Turan and Simsek, 2021). Sharma and Yadav (2020) 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.

3.2.2. 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 (Aziz and Karboune, 2018). 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 (Kumar, et al., 2018). This is similar to the report of Kumar et al. (2015) in pork patties with phyto-extract.A similar observation that agreed well with the present study was reported by Talukder et al. (2020), 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^{-1}), 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 (Sharma and Yadav, 2020). The rate of growth was less in pomegranate by-products and their extract. 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.

3.2.3. Coliform count, yeast and mould count and staphylococcus count

The study showed that coliform counts, yeast and mould 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 (Kanatt et al., 2010), in beef sausage with red dragon fruit extract, a similar result was found for *E. coli* (Manihuruk et al., 2017). Up to 14 days of storage, the coliform count was not detected in watermelon rind extract-treated products in pork patties incorporating watermelon rind extract (Kumar et al., 2018). Onion peel extract showed an antibacterial effect in the case of staphylococcus count in pork sausage (Lee et al., 2015).

3.3. 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 (Manihuruk et al., 2017). Hussein (2015) reported highest black rice ethanol extract had higher amounts of total phenolic compounds than the red sorghum hull.

Table 5: Total phenolic content of different concentration of Black rice extract

Sample	Sl. No.	Absorbance (AU.)	Concentration (μg ml^{-1})	Total phenolic (mg g^{-1})
Black rice extract	1	0.231	25.21309	307.5
	2	0.356	50.6027	620.0
	3	0.457	71.32063	872.5

3.4. 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 T_3 sample had the highest antioxidant activity value, and the control sample had a 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 had potent antioxidant properties. The findings of the present study agreed well with an earlier study where it was reported that the polyphenols and anthocyanin present in black rice were effective antioxidants that can limit oxidative changes in beef meat patties (Prommachart et al., 2020). Anthocyanin and phenolics could prevent the formation of fatty free radicals by inhibiting the free radical formation and blocking radical chain reactions in the oxidation process (Rice-Evans., 1997). (Gaps required in phenolicscouldprevent) 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 (Ahmad et al., 2020).

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 T_3 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 (Prommachart et al., 2020). It was also reported that Red pitaya extract incorporated in pork patties revealed gradually decreased antioxidant activity during the storage period (Bellucci et al., 2021).

Table 6: DPPH free radical scavenging activity (%) of duck meat nuggets incorporated with different concentrations of black rice extract

Days	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

4. CONCLUSION

Duck meat nuggets could be prepared by incorporating Black Rice Extract. Duck meat nuggets with 1.3% BRE (T_3) had the highest antioxidant activity value.

5. ACKNOWLEDGEMENT

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