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RESEARCH ARTICLE

Development and evaluation of sensorial and antioxidant properties of functional black rice *kheer*

Bhavika Dhingra and Amrita Poonia*

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Abstract: The present investigation involved preparation of *kheer* by replacing white rice with black rice @ 40g (K1), 30g (K2) and 20g (K3). Coconut sugar was added @15 g in all the combinations. Standardized pasteurized milk (4.5% fat and 8.5% SNF) was used with black rice grains. Cleaned rice was soaked in water (rice to water ratio as 1:2) and cooked at 93°C till the water was completely absorbed. *Kheer* prepared using 20g of rice (K3) was selected as optimized product on the basis of sensory evaluation. The developed black rice *kheer* was acceptable upto 12 days of storage at refrigerated temperature. Optimized *kheer* contained 58.45±0.04% moisture, 5.11±0.26% fat, 72.57±0.51% total carbohydrate and total phenolic content 54.46±0.56 (mg of gallic acid equivalents/g dry weight). During storage at refrigerated temperature (4±2°C), the pH decreased significantly while acidity increased. The SPC increased from 1.32±0.3 to 6.89 ± 0.23 (log CFU/g) at the end of 12 days storage in optimized black rice *kheer*. This study can be utilized to find out the suitability of black rice which is known to have many functional properties, to the non-consumers of black rice and to add variety to the diets of black rice consumers.

Keywords: *Kheer*, Black rice, Functional, Total phenolic content, Traditional dairy product

Department of Dairy Science and Food Technology,
Institute of Agricultural Sciences,
Banaras Hindu University, Varanasi, Uttar Pradesh, India

Amrita Poonia (✉)
Department of Dairy Science and Food Technology,
Institute of Agricultural Sciences, Banaras Hindu University, Varanasi-221005, Uttar Pradesh, India
Email: amrita12@bhu.ac.in

Introduction

Now a day's black rice (*Oryza sativa* L.) is widely used among the health-conscious consumers as a 'functional food' due to its nutritional profile. Black rice is good source of protein, iron, antioxidants and vitamin E (Kumar and Murali, 2020). Black rice is a potential functional food ingredient owing to its useful composition such as very low fat and sugar, gluten free and high protein (Sushmitha and Reddy, 2020). Priya et al. (2019) compared the nutritional profile of black rice with red, brown and white rice and found that black rice has the highest amount of fat (2.0g/100g), protein (8.50g/100g), tocopherols (12.54 mg/100g), thiamine (0.46 mg/100g), riboflavin (0.403 mg/100g) and zinc (3.16 mg/100g). Chanu et al. (2016) reported that black rice is free from gluten and has higher protein per cent (11.40), fat per cent (3.20), crude fibre per cent (1.64), whereas zinc (6.2mg/100g) and manganese (2.56%) content is higher in Chakhaoamubi and Poireitonchakhao variety of black rice, respectively.

Black rice has been regarded as an excellent source of anthocyanins, which can reduce the risk of cancer, obesity and its compounds has anti- ageing, antiviral, anti-inflammatory effects (Yamuangmorn and Prom-u-Thai, 2021). Research has shown that black rice has many health benefits. Anthocyannins are the characteristic compound which is responsible for its popularity throughout the world. More than 90% of the total anthocyanins are constituted by cyanidin-3-glucoside in black rice, followed by peonidin-3-glucoside (Zhang et al. 2010). Balasubramaniam et al. (2019) reported the health benefits of black rice such as its antioxidant activity, anti-proliferative, anticancer, anti-diabetes, anti-atherosclerosis and cholesterol lowering activity. Sterols and triterpenoids found in black rice have potential anticancer properties (Dias et al. 2017).

Kheer is a very popular traditional dairy product consumed all over India. It is considered as nutritious dairy dessert. But its production is limited to household level and unorganized sector only. Coconut sugar has been reported to have glycemic index of about 35 (Kusumawaty et al. 2012). It is a good source of minerals like zinc, iron, calcium, phosphorous, potassium and magnesium. Hebbar et al. (2015) also reported that coconut sugar is good source of vitamins, such as vitamin C, B-complex, antioxidants,

polyphenols and dietary fibres. Recently, coconut sugar become very popular among health conscious consumers.

In this study, black rice in different proportions was added to replace the white rice for preparation of *kheer*. The major objective of this study was to popularize the consumption of black rice in the areas where it is not consumed. There are some studies on the enrichment of *kheer* with millets, quinoa, pumpkin etc., but there are no studies on the *kheer* preparation using black rice. Hence, the present study was undertaken to prepare and evaluate *kheer* by incorporating different proportions of black rice in traditional white rice-based *kheer*.

Materials and Methods

Black rice (Chahao) was procured from market of Shalimar Bagh, New Delhi. Organic coconut sugar of Tropicoco Kokos Natural was purchased through online shopping site (Flipkart). Standardized & pasteurized milk (with fat: 4.5% and SNF:8.5%) was procured from local market of Varanasi, Uttar Pradesh. Chemicals used in the analysis were procured from different sources Hi -Media Laboratories Pvt. Ltd., Mumbai, India; Sigma Chemicals Co. St. Louise, M.O., USA; Fisher scientific, Mumbai, India and Merck Specialists Pvt. Ltd., Mumbai, India.

Preparation of rice powder

Rice was cleaned and milled to a fine powder using mixer grinder and then the powder was transferred to low density polyethylene (LDPE) packages of size 12 cm x 9 cm, sealed and vacuum packed for further study.

Nutritional composition and antioxidant activity of black rice

By following the method of AOAC (2000), moisture and fat content was calculated by taking 5.0 g of sample. The ash content of finely ground sample of black rice was estimated by following the method of AOAC 2000. Iron and zinc were analyzed using Atomic Absorption Spectroscopy (AAS) (Thermo Fisher Scientist-IN) (Mowuta, and Mayangsari, 2022). Sample of rice flour was extracted by method of Sutharut and Sudarat (2012). Total phenolic content (TPC) was determined using the modified method of Folin-Ciocalteau method (Lavelli et al. 2016). The DPPH radical scavenging assay was performed according to the method of (Locatelli et al. 2009).

Preparation of black rice *kheer*

Firstly, rice was soaked (1part of rice:2 part of water) for half an hour and cooked in a cooking pan with occasionally stirring with a wooden spoon till the water was completely absorbed/ evaporated. The flowchart for preparation of black rice *kheer* is given in (Figure 1). The proportions of ingredients produced by the levels of black rice addition were considered as treatments given below (Figure 2). The proportions used were decided based on the preliminary trials. Notations for control and different treatments are shown below:

K_0 = White rice (12.5g) + Sugar (25g) + Toned Milk (500ml) as control

K_1 = Black Rice (40g) + Coconut Sugar (15g) + Toned Milk (500ml) + Cardamom pod (1)

K_2 = Black Rice (30g) + Coconut Sugar (15g) + Toned Milk (500ml) + Cardamom pod (1)

K_3 = Black Rice (20g) + Coconut Sugar (15g) + Toned Milk (500ml) + Cardamom pod (1)

Sensory evaluation

The samples were evaluated for their sensory attributes i.e. flavour, colour, texture/body, and overall acceptability by semi - trained panel of 20 judges using 9 point hedonic scale. A score of 5.5 and above was considered acceptable. The scores rated by the panel of judges were then statistically analyzed. The samples were code numbered to avoid identification and bias. Based on sensory score K_3 sample was found most acceptable and therefore it has been selected for further study.

Chemical composition analysis of black rice *kheer*

By following the method of AOAC (2000), moisture content was calculated by taking 5g of sample. After the determination of moisture, the left residue was taken for calculation the total solid content.

Determination of total phenolic content

The TPC of extracts was determined using the Folin-Ciocalteu's phenol reagent. 0.5 ml of diluted extract was added to 2.5ml of 0.2N Folin-Ciocalteau reagent and placed for 5 minutes. 2ml of 75g/L of Na_2CO_3 was then added. The above solution was then kept for incubation at room temperature for 2 hours. Absorbance was measured at 760nm using a 1cm cuvette UV-1800 spectrometer (Shimadzu, Japan). Gallic acid (0-800mg/L) was used to produce a standard calibration curve. The total phenolic content was expressed in mg of Gallic acid equivalent (GAE)/100ml of extract after applying the dilution factor (Stankovic, 2011). The free radical scavenging activity was determined by using DPPH assay with modified method of (Brand-Williams et al. 1995).

Statistical Analysis

The data obtained during the course of investigation were subjected to statistical analysis. One-way analysis of variance (ANOVA) was applied to analyze test of significance.

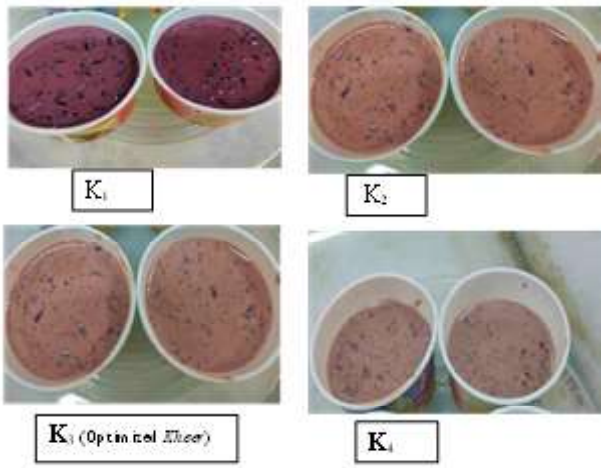


Fig.1 Diagrammatic representation of different combinations of black rice *kheer*

Shelf- life of black rice *kheer*

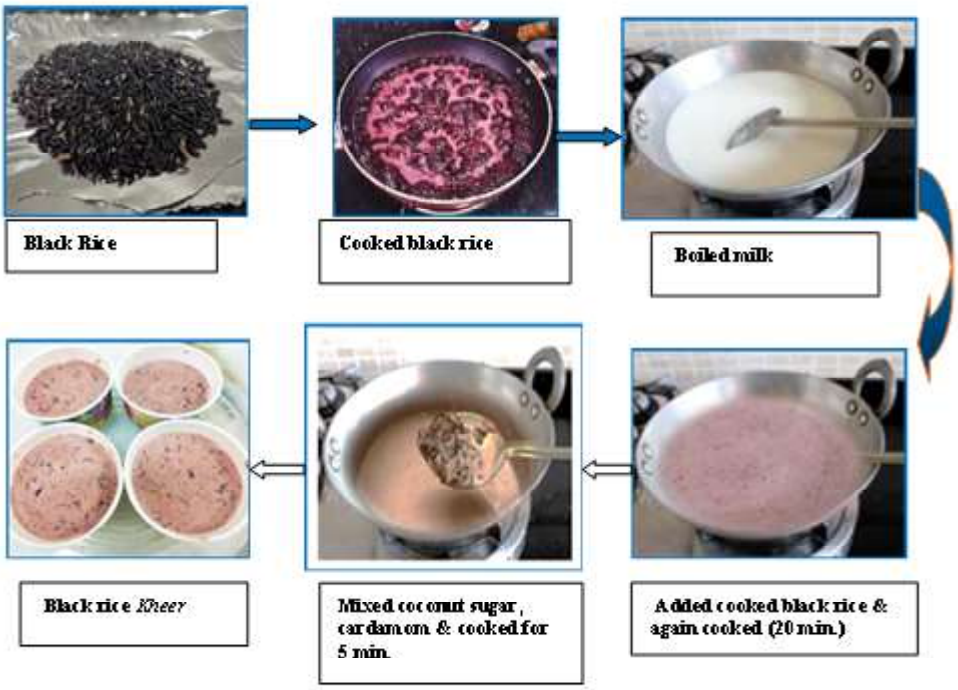
Based on the sensory characteristics of the different combinations K_3 was selected for shelf- life study with control sample of white rice *kheer. Kheer* was packed in polystyrene cups of 100 g capacity and stored at 4±2°C in refrigerator. The pH, acidity and microbial parameters of black rice *kheer* were determined at an interval of 2 days for a period of 12 days.

Results and Discussion

The ash content was high in black rice (2.80%). The amount of ash content indicates the levels of minerals present in the food sample. Similar results were reported by (Colasanto et al. 2021). The authors reported that the moisture content of black rice was 11.70%, total dietary fiber, proteins, and ashes were 10.8%, 10.5% and 1.95% on dry weight (d.w.), respectively. The study conducted by Thomas et al. (2013) reported that fat content of black rice was 0.70% but in the present study the fat content was 1.90%. The difference in fat content may be due to the cultivation and regional difference (Kang et al. 2011). Reported mineral content of black rice was zinc (6.20mg/100g) and iron (3.50 mg/100g).

Plant phenolic are important constituents that contribute to functional quality, colour and flavour and have significant roles in both as singlet oxygen quencher and free radical scavengers, helping to minimize molecular damage (Tanvir et al. 2017). Phenolic contents were expressed as mg gallic acid equivalents per gram of black rice extract. Using the standard curve, total phenolic content of the extract was determined. The total phenolic content of the black and white rice extracts was found to be 75.59±7.28 and 11.94±0.97 mg GAE/g of black rice extract & control extract, respectively. The results indicate that the black rice extract contains higher total phenols as compare to the white rice extracts. The phenolic constituents of rice are mainly distributed in rice pericarp (Paiva et al. 2014). This may be the reason for higher phenolic contents in black rice than the white rice. The main form of phenolic compounds present in rice is bound form (Saikia et al. 2012). The free radical scavenging activities of the extracts were determined by using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. DPPH in oxidized form gives a deep violet color in ethanol. An antioxidant compound donates the electron to DPPH thus causing its reduction and in reduced from its color changes from deep violet to yellow (Shirazi et al. 2014).

Fig. 2 Flow chart for preparation of functional black rice *Kheer*



Based on % DPPH assay, the antioxidant capacity of the extracts were found to be 56.23±3.56 & 11.67±1.18 for black rice and white rice extract, respectively. From Table 1, it can be concluded that antioxidant capacity of black rice is much more than that of white rice. These results were comparable with the observations given by Sampong et al. (2011) and Walter et al. (2013) i.e. DPPH value ranged from 59.02 to 75.52% for black rice varieties. The lowest value of antioxidant activity was observed in white rice. It may be due to reduction in polyphenol content as the concentration of the total soluble phenolic contents was related to lower antioxidant activity (Walter et al. 2013). Jun et al. (2012) evaluated the antioxidant activities and phenolic compounds of pigmented rice (black, red, and green rice) and brown rice brans and reported similar results i.e. black and red rice bran shows high antioxidant activities and contains high amount of phenolic compounds. Indeed, black could be better raw materials for manufacturing the food with high antioxidant activity.

Sensory attributes of black rice *kheer*

Sensory evaluation was done to assess the overall acceptability of the product. The product was prepared under 3 different variations of black rice which was tested by a panel of 20 judges. The sensory parameters were color & appearance, flavor, body & texture and overall acceptability. Table 2, shows that sensory score was found to be highest in the sample K3 that is in the range of 7.7 to 9.80. According to the sensory evaluation the K3 sample containing 20 gram black rice was found to have good color and appearance, taste and flavor and body and texture. The sensory score was in the range of 7.21 to 7.81 of K1 sample followed by K2 sample that has a sensory score ranging from7.38 to 8.54. Considering one attribute at a time the color and appearance was highly scored for sample K3 than K2 than K1. Statistical analysis of sensory data for color and flavor reveals that there is a significant difference in sensory scores of the three black rice *kheer* variants as the p-value <0.05.

Physico-chemical analysis of optimized black rice *kheer*

The data given in Table 3 depicts the proximate composition of black rice *kheer*. Black rice *kheer* contains 58.45±2.10% moisture, 2.84±0.05% ash, 5.11±0.26% fat and 13.54±0.36% protein. Chanu & Yenegi (2015) reported similar composition of raw material and product nutrient contents of black rice i.e. moisture content of rice products prepared from black rice. The fat content of *kheer* (5.11±0.26%) prepared from black rice was found higher than the raw black rice (1.90±0.01%). The similar fat content was reported by Chung & Lim, (1999); Sampong et al. (2011) and Saikia et al. (2012). Protein influences the nutritional quality of rice. The protein content of *kheer* prepared from black rice and raw white rice were 13.54±0.36% and 2.70±1.40%. This may be due to the incorporation of protein rich ingredients specially black rice. Sampong et al. (2011) also observed the protein content of black rice varieties in the range of 8.44% to 10.84%. The amount of ash present in food product plays important role while determining the levels of essential minerals. The ash and crude fiber content

Table 1 Comparison of nutritional profile of black rice and white rice

Particulars	Black rice	White rice
Moisture (%)	11.10 ± 0.04	12.85±0.15
Protein (%)	10.80±0.14	2.70 ±1.40
Fat (%)	1.90±0.01	0.67±0.01
Crude fibre (%)	1.40 ±0.01	0.80±0.08
Ash (%)	2.80± 0.09	1.57±0.04
Zinc(mg/100g)	6.20 ± 0.10	1.38±0.24
Iron (mg/100g)	3.50 ± 0.05	1.20 ±1.00
Total carbohydrate (%)	75.30± 0.12	80.45±0.42
Total phenolic content (mg of GAE/g of dry weight)	75.59^{b}±7.28	11.94^{a}±0.97
DPPH (%)	56.23^{d}±3.56	11.67^{c}±1.18

Values are expressed as Mean± Standard deviation of 3 replications.
Values in the same column with different letters are significantly different at P < 0.05

Table 2 Mean sensory score for *kheer* prepared by using different levels of black rice

Level of black rice Incorporation (%)	Color and Appearance	Body and Texture	Taste and Flavor	Overall Acceptability
K0 (Control)	6.55^{a}±0.09	6.14^{a}±0.07	6.55^{a}±0.05	6.41^{a}±0.07
K1	7.88^{b}±0.12	7.21^{b}±0.09	7.22^{b}±0.04	7.43^{b}±0.08
K2	8.34^{c}±0.11	7.38^{b}±0.13	8.54^{c}±0.08	8.08^{c}±0.11
K3	9.68^{d}±0.11	7.76^{b}±0.05	9.78^{d}±0.07	9.07^{d}±0.07

Values are mean ± Standard Deviation (n=20)
Values in the same column with different letters are significantly different at (.05)

of both the type of *kheer* were at par. The values of crude fiber are comparable with those given by Yodmanee et al. (2011) for different black rice products in the range of 1.63% to 2.06%. Zinc and iron of *kheer* prepared from black rice was found to be differ significantly in comparison to white rice.

Total phenolic content & DPPH activity of optimized black rice *kheer*

Based on evaluation, TPC was found to be 54.46±0.56 and 17.65±0.5413mg gallic acid equivalents/g dry weight for optimized black rice *kheer* and control, respectively. Similarly, 2,2-diphenylpicrylhydrazyl DPPH (%) capacity was found to be 47.90±0.52 and 9.70±0.55 for optimized and control *kheer*, respectively. Therefore, it can be concluded that TPC & DPPH activity of *kheer* prepared from black rice is three times higher than that of control *kheer*. These results were comparable with the observations given by Jun et al. *(*2012) i.e. the phenolic content & DPPH activity of different black rice products ranged from 20.87 to 64.13mg gallic acid equivalents/100g and 49.56% to 11.27%, respectively.

Storage studies of black rice *kheer*

During storage, the pH of black rice *kheer* was decreased and acidity was increased significantly (P<0.05) and their interaction effect was found non-significant on pH and acidity of product (Table 4). Similar results were reported by More et al. 2017. The authors reported that during storage of little millet *kheer* at 6±1°C and the pH decreased significantly while acidity and viscosity increased.

Standard plate count (SPC) count of *kheer* were increased significantly during storage. Yeast & mould count and coliform

Table 3 Chemical composition and antioxidant properties of control and optimized black rice *kheer*

Particulars	Control	Optimized black rice *kheer* (K3)
Moisture (%)	63.59± 1.04	58.45±2.10
Ash (%)	1.81±0.09	2.84±0.05
Protein (%)	9.05±0.14	13.54±0.36
Fat (%)	1.90 ± 0.01	5.11±0.26
Crude Fiber (%)	1.4±0.01	1.51±0.18
Total Carbohydrate (%)	75.3±0.12	72.57±0.51
pH	6.71±0.02	6.81±0.44
Zinc (mg/100g)	1.65±0.05	6.20±0.10
Iron (mg/100g)	0.93±0.05	3.44±0.05
TPC (mg of GAE/g of dry weight)	7.65^{a}±0.54	54.46^{b}±0.56
% DPPH	9.70^{c}±0.55	47.90^{d}±0.52

Values are expressed as Mean± Standard deviation of 3 replications
Values in the same column with different letters are significantly different at P < 0.05

Table 4 Changes in pH, acidity and microbial count of black rice *kheer* during storage

Storage duration (days)	pH	Acidity (%)	SPC (log CFU/g)
0	Control : 6.42±0.32	Control : 0.32±0.01	Control : Nil
	Optimized: 6.40±0.34	Optimized: 0.20±0.12	Optimized: Nil
2	Control : 6.40±0.42	Control : 0.35±0.03	Control : Nil
	Optimized: 6.32±0.53	Optimized: 0.23±0.23	Optimized: Nil
4	Control : 6.31±0.72	Control : 0.41±0.12	Control : 1.45±0.54
	Optimized: 6.30 ±1.01	Optimized: 0.25±0.34	Optimized: 1.32±0.32
6	Control : 6.24±0.71	Control : 0.44±0.03	Control : 2.81±0.16
	Optimized: 6.22±0.50	Optimized: 0.28±0.04	Optimized: 4.13±0.14
8	Control : 6.23±0.22	Control : 0.51±0.06	Control : 3.65±0.41
	Optimized: 6.20±0.11	Optimized: 0.29±0.15	Optimized: 3.42±0.25
10	Control : 6.16±0.64	Control : 0.56±0.02	Control : 4.50±0.23
	Optimized: 6.10±0.45	Optimized: 0.41±0.11	Optimized: 4.40±0.43
12	Control : 6.00±0.56	Control : 0.77±0.06	Control : 6.40±0.42
	Optimized: 5.86±0.86	Optimized: 0.50±0.22	Optimized: 6.89±0.23

Values mentioned as mean ± Standard deviation, (n=3) at (p< 0.05)

Table 5 Cost analysis of functional black rice *kheer* (100g)

Ingredients used	Quantity	Cost (₹)
Black rice	20g	6.4
Coconut Sugar	15g	13.5
Toned Milk	500ml	22.0
Total (Quantity of *kheer*)	300g	41.9
	100g	13.96
Packaging cost	1 cup + Aluminium foil	1.0
	Total	14.96
Processing cost	Per cup	1.0
	Total	15.96
Marketing and distribution expenses	Per cup (100g)	3.29
@25% of product	Total	Rs.19.25

count was found nil during the storage period of 12 days. On 13th day of storage the yeast & mould count were visible. Black rice *kheer* was found to be free from coliform.

Cost analysis

In order to determine the feasibility of this study, cost of production was calculated for optimized *kheer* (Table 5). Cost of raw materials was added along with packaging cost and marketing and distribution expenses. 100g (one cup) of functional black rice *kheer* was prepared approximately ₹ 15.96. The profit margin at 25% of cost of product is also applied which took overall price of black rice *kheer* to a very nominal price of ₹ 19.25.

Conclusions

Antioxidant activity of black rice is two fold stronger with respective to antioxidant activities of blueberries. Black rice can be utilized as a functional food with high antioxidant and low-fat sugar, salt, gluten and cholesterol. It can be used in desserts or dressing or condiment because the black color turns to shiny indigo or purple when cooked. There are limited studies reported on the food product development and functional properties. The research findings of the study can be used to know therapeutic value of black rice when used in different food products.

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