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Effect of roasting and cooking on physiochemical properties of black rice soluble extract

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Abstract
Rice (*Oryzae sativa* L.) is a significant cereal consumed widely by over one- half of the world's population. There are numerous unique rice varieties present, some of them known as Traditional or Ancient grains. Black rice or 'Karupu Kauvni' is one such colored rice varieties known for their rich source of dietary fiber, resistant starch, minerals, carotenoids, flavonoids and polyphenols and intake of these pigmented varieties help in improving human health. This study shows the difference in the physicochemical properties of these glutinous rice samples. The physicochemical properties of three samples of Black rice soluble extract (control, roasted and cooked) were analyzed to determine pH, color, enzyme activity, viscosity and pasting properties. The tested samples and their results could serve as the baseline for further research and information on rice-based beverages and products' quality evaluation parameters. The soluble extract from such a plant-based source displays numerous benefits in comparison to other plant-based extracts. Despite its high carbohydrates, significantly lower amount of calcium and no cholesterol, it has been used significantly less in the food industry area.

Keywords: Black rice, physico-chemical properties, enzyme activity, starch

Introduction
Rice (*Oryza sativa* L.) is a vital and most common food staple in many Asian continents and is consumed by a significant sector of the world-wide population. This cereal variety supplies significant nutrients such as carbohydrates, proteins, fatty acids and micronutrients like vitamins and trace minerals, thereby maintaining a daily calorie intake of 50 to 80% (Khush, 2005). Traditional rice varieties in India are reported to accumulate many genetic variations, including medicinal benefits and aroma (Ramanathan *et al*., 2014) [15]. These ancient varieties are more potent sources for several agronomic traits and sources of various bioactive non-nutrient components (Crozier *et al*., 2009) [2]. Rice is usually consumed after boiling or steaming as white rice with the husk, bran, and germ removed.

The widely popular eaten rice is the 'White Rice,' this rice, compared to other varieties of the same, has a lower number of essential micronutrients, which leads to micronutrient deficiency in individuals on regular intake. Consumption of a higher quantity of polished rice grains, i.e., high GI food, has also been reported contributing to higher insulin resistance and dyslipidemia (low-high density lipoprotein) among the Asian population. Therefore, further research on micro-nutrient and vitamin-rich rice with genetic engineering is a matter of great urgency and importance for the welfare of human health and nutrition (Crozier *et al*., 2009) [2].

There are numerous rice present varieties, and some contain colored pigments of red and black, most commonly known as Traditional or Ancient grains. Such traditional colored rice varieties are known for their rich source of dietary fiber, resistant starch, minerals, carotenoids, flavonoids and polyphenols, and intake of these pigmented varieties help in improving human health (Hanhineva *et al*., 2010; Hudson *et al*., 2000; Rao *et al*., 2010) [3, 4, 16]. The bioactive phytochemicals and micronutrient components of these traditional rice varieties serve as dietary supplements that play an essential role in diminishing the chances of non-communicable diseases like cardiovascular diseases, diabetes, cancer and stroke (Vichapong *et al*., 2010) [20]. One of the traditional dark brownish-black rice varieties called "Black Rice," also known as the 'Forbidden Rice' in China, is an ancient and rare rice variety.

Black rice has been a part of the Indian diet for many centuries, mainly cultivated in the North East region, called 'Chak Hao' and in the Southern parts, called Kavuni in Tamil.
Black rice varieties contain shorter chains of starch molecules and low amylose content than white rice (Rice, 2016) [17].
Kavuni rice is glutinous waxy rice majorly known for its anti-diabetic and high anti-oxidant content, mainly anthocyanin (Oikawa *et al*., 2015) [12]. Morphologically, it has a long cultivation duration, low tillering and photosensitive traits, due to which it is not highly cultivated world-wide. Dehusked kavuni is dark brown to black, and the polished grains are light brown. Nutritionally, the recent unraveling of its biochemical properties has revealed that it contains significantly lowers levels of total soluble sugars, higher levels of amylose, dietary fiber, protein, β- carotene, lutein and polyphenols than other popularly consumed varieties of white rice. (Ramanathan *et al*., 2014) [15].
The soluble extract from such a plant-based source displays numerous benefits in comparison to other plant-based extracts. However, it contains more carbohydrates, a significantly lower amount of calcium and no cholesterol.
Kavuni, glutinous type rice, is usually processed and consumed as simple foods like steamed black glutinous rice or boiled black glutinous rice. As glutinous rice is almost entirely dominated by amylopectin and hence has a very sticky feature. Glutinous rice has a high starch content and contains 1-2% amylose and 98-99% amylopectin. Higher amylopectin level contributes to the stickiness of rice.
Starch digestibility means the easiness level with which starch can be hydrolyzed to become a simpler substance. Starch digestibility could be increased with the help of an enzyme. With the hydrolysis process's help, the enzyme can easily break down starch into simple sugars. However, the major drawback of high starch digestibility to easily breakdown the starch into glucose leads to an increased level of blood glucose on consumption. This causes blood glucose levels to elevate, thereby causing an increase in the need for insulin by the body to convert glucose into energy (M. Kang *et al*., 2011)
While being processed to be converted to various types of processed foods, glutinous waxy undergoes a reduction in its nutritional value, starch digestibility, and energy value. The different processes that glutinous rice undergo are roasting, steaming, puffing, and boiling. Roasting is a technique of cooking without oil. Steaming is a method of moist cooking, where the rice is indirectly cooked with the help of boiling liquid or steam. The puffing method uses temperature and pressure to change the structure of the food material, resulting in an expansion in the rice grain volume. Boiling is a technique that uses boiling water as a medium of heat transfer (Rini *et al*., 2019) [18].
This study on the physicochemical properties of black rice extract was conducted to study and further understand its nature. Despite its excellent nutritional properties, due to the lack of product availability world-wide, black rice is rarely used in processing industries.

Materials and Methods

Preliminary preparation of rice

Black ‘kavuni’ paddy was procured from the local farmer market. The rice was then dehusked and polished. 150 g of the dehusked and partially polished rice was then divided into three parts of 500 g each. The first part of 500g of black rice was washed thoroughly to remove all the dirt and unwanted material to prevent the chance of any contamination and soaked for 1 hour with a 1:2 ratio of lukewarm water. The seconds part of 500g rice was roasted at 100-120°C for 2-4 mins until the slight brownish color was obtained. The roasted rice was then washed thoroughly and then soaked for 1 hour with 1:2 of rice is to water ratio. The third part of the rice was washed and cleaned to remove dirt. The rice was then soaked for 1 hour with a 1:2 rice is to water ratio. After the soaking process, the water is drained out from the rice using a filter net/cloth, as this water usually contains harmful toxins released from the rice (Padma *et al*., 2018) [13]. Furthermore, the third part of rice was then cooked with 1:2 rice is to water ratio for 20-25 mins at 140-180°C, until rice turns mushy.

Extract preparation

All three parts of rice, i.e., control, roasted, cooked was then homogenized using a grinder with 1:2 rice to water ratio for 5-7 mins until slurry of medium thick consistency was obtained in case of control and roasted black rice sample and a gelatinized thick sample was obtained in case of cooked rice. The mixture was then filtered using a muslin cloth.
The three samples were then placed heated in a hot water bath until the desired temperature of 65-68°C was obtained for 15 mins. To this mix, the enzyme is added and stirred correctly and allowed to carry the enzymatic reaction for further 15-20 mins. The total time taken by the enzymatic reaction to ensure the liquification and the saccharification step in all three samples provides a milky texture and functionality to the sample. It prevents the occurrence of any undesirable flavors from yielding a non-allergic rice liquid product. (Mitchell *et al*., 2010) [11]. After the enzyme treatment, the three samples were then heated at 85°C for 15 mins and stored in glasses bottles in a refrigerated condition.

Physico-chemical analysis of soluble rice extract prepared from black' Kavuni’ rice

Estimation of Total soluble solids (TSS) before and after enzyme treatment

TSS is the sugar content present in the solution. The TSS of the three sample before and after enzyme addition were determined using a handheld ATAGO refractometer. Before measurement of the TSS of the samples, the refractometer was calibrated using distilled water. A drop of rice soluble extract of each sample was placed in the refractometer sample slot, and the readings were recorded and expressed in ˚Bx.

Estimation of pH

The three samples' pH was measured using a digital pH meter (Innco microprocessor pH-mV- Temp meter). The samples' pH was taken in triplicates, one of the fresh samples (day 1) and one of the samples after 7 days in refrigeration. The pH meter probe was inserted into the samples, and the stable reading attained was considered the final pH value for the respective sample.

Estimation of color

The color parameter of the soluble starch extract was determined using the Hunters lab ColorFlex EZ colorimeter. The mean of the triplicate values of the three samples was taken as the results. The chroma value (C*) and Redness Index (RI) was calculated using equation i and ii, respectively.

$$C^* = \sqrt{(a^*)^2+(b^*)^2} \quad \ldots \text{(i)}$$

$$RI = a^*/b^* \quad \ldots \text{(ii)}$$

Measurement of Pasting Properties and Viscosity
The Rapid Visco Amylograph (RVA) MCR-52 was the preferred instrument for determining the pasting properties and viscosity for the three black (treated and untreated) rice soluble extract samples.

The pasting properties were measured by taking 25 ml of each sample and placing it into the RVA. The measurements were performed under a constant shear velocity of 110 rpm. The temperature profile used was: the sample was held at 50 °C for 2 min; followed by an increase in temperature from 50 °C to 95°C at a rate of 5°C/min; then held at 95 °C for 5 min; later cooled back to 50°C at a rate of 5 °C/min; and finally held at 50°C for 4 min (Yue *et al*., 2009) [23].

For determining the viscosity, the flow properties of the three samples were measured using the RVA. The resulting curves were fitted into the Herschel Bulkley Model using the following equation iii, the n value and the R^2 values were obtained for the respective samples tested.

Herschel-Bulkley model $y = a + b*x$ ... (iii)

Results and Discussion
Three types of the soluble extract of black rice, i.e., control (no heat provided to sample), roasted (120°C for 2 mins) and cooked (160 °C for 20 mins) samples were used to study the variation in different physicochemical properties such as TSS, pH, color, pasting properties and viscosity.

TSS value of the rice extract
TSS obtained for initial and enzyme treated samples (control, roasted and cooked) of black rice soluble extract by using a hand-held refractometer are as shown in Figure no. 1.

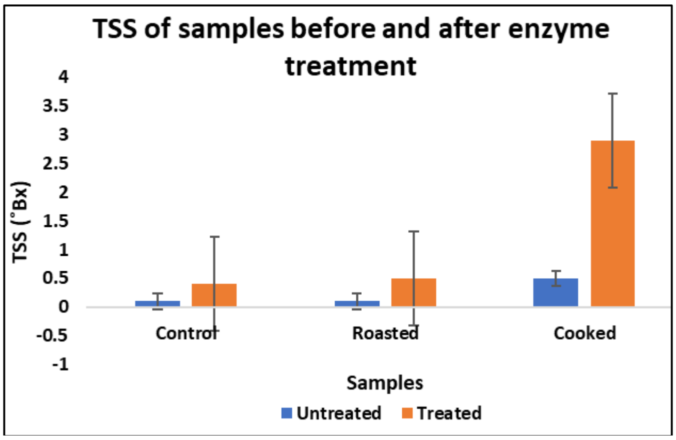

Fig 1: TSS of sample for untreated and treated samples of Black rice extract

In the untreated sample, the initial TSS of the cooked sample was the highest of 0.5°Bx, followed by the control and roasted samples with similar TSS value of 0.1°Bx

In the enzyme treated sample the TSS of the cooked sample was the highest (2.9 °Bx), followed by the roasted and control sample with TSS of 0.5°Bx and 0.4°Bx respectively.

Previous studies conducted on enzyme activity in the time-temperature relationship in falling number in flour have shown that the enzyme had significantly less effect on undamaged starch granules than gelatinized starch, in which the enzyme was able to attack the starch more easily and rapidly. The study also showed that the maximum enzyme activity occurs at 65-80 °C, which is between the interval of gelatinization of starch (50 -65 °C) and the inactivation of the enzyme (75-80°C) (Perten, 1964)

Earlier conducted studies have shown starch digestibility is affected by the amylose content present in the rice starch, and comparatively, black rice has a Meager amount of amylose content to other white rice (Wickramasinghe & Noda, 2008) [22]. Therefore, the starch in such rice is more easily digested by enzyme activity.

Variation of pH on Black rice soluble extract
The three treated samples' pH values were measured in two conditions, i.e., day 1 (freshly prepared) and after day 7 in refrigeration are as shown in Figure 2. The pH values of the three (treated with enzyme) black rice soluble extracts estimated on Day 1 showed very slight variation in pH from the day prepared to Day 7 later in refrigeration. The treated control sample's pH value moderately decreased from 5.97 to 5.52, the pH of the treated roasted sample showed the highest decrease of all three samples from 5.74 to 5.45, and the pH of the treated cooked sample showed the slightest decrease from 6.23 to 6.22. The pH of the black rice soluble extract under all experimental conditions was found to be in the range of 5.45–6.23, with a significant difference ($p > 0.05$)

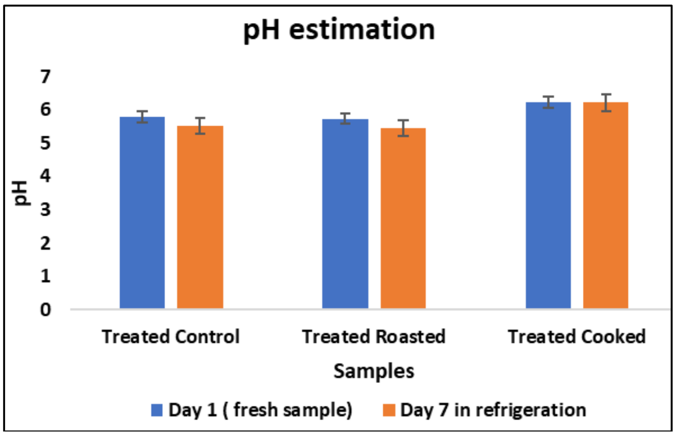

Fig 2: The pH of treated samples on Day 1 and after Day 7 in refrigeration

Studies observed that the cooking and blending parameter significantly affects rice-based beverages and products (Padma *et al*., 2018) [13]. Another two types of study conducted on buckwheat and bean beverages with the addition of different industrial cultures showed no fermentation changes and no changes in pH value from the day of storage in refrigeration until the 21st day of refrigerated storage. After the 28th day, a slight drop in pH was observed, which indicated an active fermentation process in the beverage (Kowalska & Ziarno, 2020) [9].

Colour estimation of Black rice extract
Three samples of black rice extract (control, roasted and cooked) were taken. The roasted and the control sample was tested against the control sample. The samples were pinkish-red in color due to the high levels of anthocyanin content present in the sample. The resultant L*, a*, b* values obtained are shown in Table 1. The RI value obtained from the three samples is as shown in Figure 3.

Table 1: Colorimeter values (L*, a*, b*, Chroma C*)

	Treated Control	Treated Roasted	Treated Cooked
L*	54.38 ±1.2	70.13±1.8	73.01±2.01
a*	4.14±0.03	5.73±0.16	4.92±0.14
b*	1.48±0.01	6.03±0.13	4.76±0.23
C*	4.396	8.318	6.845

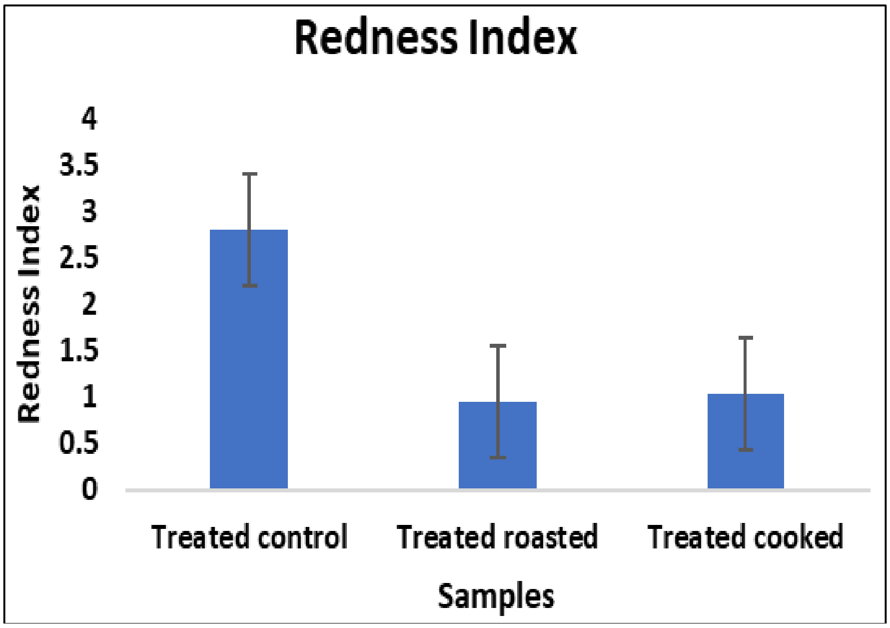

Fig 3: Redness Index of treated samples of black rice soluble extracts

Viscosity and Pasting Properties of Black rice soluble extract

The viscosity of the three samples (treated and untreated with enzyme) was measured using the RVA. The viscosity parameters were determined using the Herschel-Bulkley method using equation iii.

The results are as shown in Table 2. The untreated cooked sample showed a flow index n =1.1657 (shear-thickening properties), where the remaining sample displayed the flow index n = 0.01 (shear- thinning properties)

Table 2: RVA readings - Flow index (n), correlation ratio (R^2) and type of liquid of black rice soluble extract samples

Types of Samples	Correlation Ratio (R^2)	Flow Index (n)	Type
Untreated control	0.89957	0.01	shear-thinning
Treated control	0.90874	0.01	shear-thinning
Untreated roasted	0.84985	0.01	shear-thinning
Treated roasted	0.84965	0.01	shear-thinning
Untreated cooked	0.87852	1.1654	shear-thickening
Treated cooked	0.80475	0.01	shear-thinning

Previous studies showed that depending on the type, amylose content and gelatinization temperature of the rice deeply affects its rheological and cooking properties. Also, the amylose to amylopectin ratio in raw rice has an impact on the functional properties of the starch, which in turn affects the quality of the end product, such as changes in the rheological properties of different rice-based beverages and products (Joshi *et al*., 2015; Lee *et al*., 2019) [5, 10]. The amount of amylose present in rice impacts the pasting and viscosity properties of the rice starch. Studies have shown that rice starches obtained from waxy or low amylose content rice display lower pasting properties than non-waxy type rice starches (H. J. Kang *et al*., 2006) [6]. The starch swells up during heating of the rice starch in water, causing the amylose to leach out. These swollen starch granules increase the viscosity, causing the resultant breakdown of the starch's viscosities from the breakdown of the gelatinized starch granules (Shih *et al*., 2007) [19]. The pasting properties of the three samples, i.e., control, roasted and cooked (with and without enzyme treatment), were tested using the RVA and the results are as shown in Figure 4.

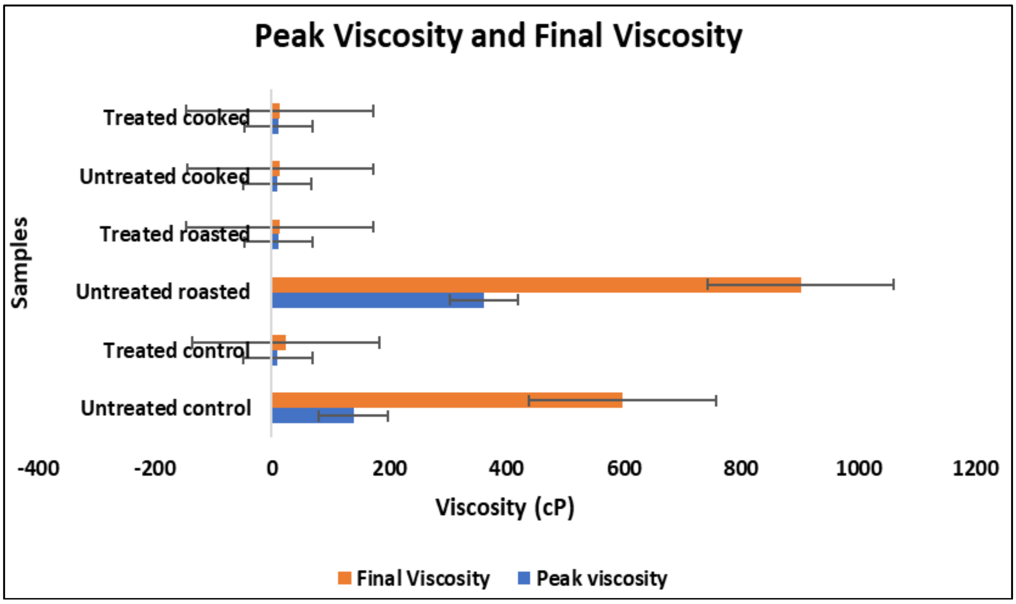

Fig 4: Peak and final viscosity readings of black rice treated and untreated soluble extract samples

The peak viscosity and final viscosity of the three samples, i.e., control, roasted and cooked (with and without enzyme treatment), both had no significant difference (with $p > 0.05$). The high peak and breakdown viscosities of starch granules in control and roasted samples show the ease with which the starch granules can be broken upon heating after the maximum swelling at the peak viscosity. Generally, rice with low amylose content possesses this property, which results in the stickiness of the paste. (Sompong *et al*., 2011) [20]. Samples without the addition of enzyme contain more starch content and hence more viscosity causing the samples to become chewy, sticky, highly adhesive and low in hardness (Chen *et al*., 2019) [1].

Conclusion
Black rice is a nutrient-rich, low-fat source of high carbohydrates. It is tasty and versatile applications in food processing. The present conducted studies illustrated the physicochemical properties of three different samples of black rice soluble extracts. The analysis of the rice extract's parameters is necessary for the quality evaluation, research and future possibilities in food industries. This study's findings might lead to a better understanding of black rice and its unique characteristics for designing specific specialty foods.

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