



Project Report on

AROMA AND NUTRITIONAL PROFILE OF BLACK RICE-BASED RICE ANALOGUE

A Dissertation Submitted By

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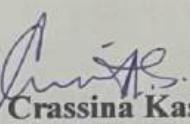


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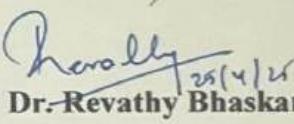
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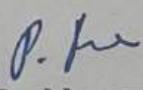

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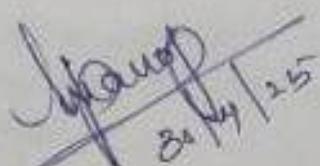
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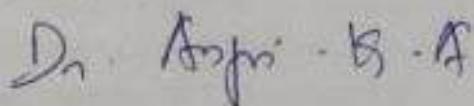
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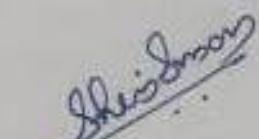


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The results represented in the current thesis have not been presented to any other university or institute for the award of any degree or diploma.

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MINNA MIRIYA CEMARAJH

ABSTRACT

Black rice, often hailed as the "future's superfood" due to its rich anthocyanin content, has yet to realize its full potential in the food sector. This is primarily attributed to its lengthy cooking time and limited applications in food value addition. Considering that rice serves as a staple food for over half of the global population, along with consumer prejudices towards broken rice, this study was undertaken.

The study aimed to develop a Black Rice-based rice analogue blended with wheat flour using extrusion technology while analysing its nutritional, functional, physical, and sensory characteristics.

Black rice flour (BRF) was mixed with wheat flour in the following formulations: 0%, 20%, 30%, 40%, and 50%. Proximate analysis revealed a slight reduction in the nutritional value of the rice analogue compared to raw materials, likely due to the thermal treatments applied. Cooking quality assessments indicated that higher BRF levels increased cooking time, water absorption, cooking loss, and yield, although the cooking time remained shorter than that of black rice.

The physical traits determined were colour, hardness, grain dimensions, grain weight, and bulk density. As the proportion of BRF increased, colour tended to darken generally and grain thickness increased, while grain length remained relatively constant. Both grain weight and bulk density exhibited a slight rise corresponding to higher BRF concentrations.

Water binding capacity (WBC), Oil binding capacity (OBC), Water solubility index (WSI) and Swelling power were analysed as part of evaluating the functional property. WBC, OBC and WSI showed an increase with BRF content increase, while swelling power decreased respectively. 40% BRF showed a slight deviation from this trend in both WBC and swelling power.

Phytochemical tests, including Total Phenolic Content, Total Flavonoid Content, and Total Anthocyanin Content, were conducted to analyse the rice analogues. These tests revealed that increasing the proportion of BRF led to a corresponding rise in phytochemical content. However, the thermal processes involved caused minor degradation compared to the original raw materials.

Aroma and flavour profiles were analysed using advanced tools such as the E-Nose and E-Tongue, which provided insights into the complex sensory attributes. Sensory evaluation done using a sensory panel on kheer prepared using rice analogues also helped in selecting the best formulation.

Ultimately, the 40% BRF formulation was optimized due to its close resemblance to black rice in nutritional, functional, and sensory attributes, making it the most promising candidate for further development.

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CHAPTER – 1

INTRODUCTION

INTRODUCTION

1.1 Rice

Rice is a staple food in more than half of the world (Das et al.2023) . Several types of rice like white, red, purple, brown, black etc. exist around the world. Different names exist due to the physical appearance of the paddy, and different colours, which exist due to the presence of different pigments in different rice varieties.

White rice is the result of processing whole rice, which removes the bran and germ, leaving only the endosperm. This process degrades many of the nutrients found in whole rice, such as fibre, vitamins and minerals, making white rice less nutritious in comparison (Cañizares et al.2024). Pigmented rice (black, red, and purple rice), retains its bran and germ, giving it its characteristic colour and a richer nutritional profile. (Pang et al. 2018). Among the healthy gluten-free raw materials, both black and red rice flours (*Oryza sativa L.*) present higher amounts of protein, fibre, and phytochemicals compared to white rice flour. (Massaretto et al. 2011; Mira et al. 2009; Shen et al. 2009). Pigmented rice possesses unique colour and flavour and are used as major ingredient in many dishes. Despite these benefits, the use of pigmented rice in cooking is limited due to its textural challenges (Chanu, 2015).

Though the origins of different types of pigmented rice are unclear, but many are thought to have originated in Asian countries such as India, China, Bangladesh, Japan, Myanmar, Sri Lanka and Thailand (Sangma and Parameshwari,2024). These varieties have been grown for centuries and are deeply ingrained in the cuisine and culture of their respective regions. Aside from their nutritional value, pigmented rice varieties are important for diversifying agricultural production and encouraging healthier, more balanced diets.

1.2 Black Rice

Black rice, known by various names such as forbidden rice, emperor's rice, or luck rice (Kumar & Murali, 2020), fortune rice, purple rice, king's rice (Ito & Lacerda, 2019) and long-life rice as it is said to prolong the life (Kannayiram et al. 2023). *Oryza sativa L. indica* is the scientific name for the Black Rice variety (Sangma and Parameshwari, 2024).

Black rice has piqued the popularity and interest of many people including researchers in the current era because of its dietetic value and distinctive sensory characteristics (Ito & Lacerda, 2019). According to a report published by NIFTEM, Black Rice is considered the Twenty-first century superfood because of its high nutritional content, in addition to the fact that it's naturally high in anthocyanins particularly Cyanidin 3-glucoside and Peonidin 3-glucoside, which are the antioxidant pigments that give the rice its unique colouring (Abdel-Aal et al., 2006 and Yang et al., 2008). Black pericarp rice is known for its high concentrations of bioactive compounds, particularly flavonoids and phenolic acids (Pang et al. 2018). The anthocyanin content is closely related to the colour of the rice pericarp and its high concentration result in the purplish-black colour (Cañizares et al.2024). Apart from the pigment, anthocyanin, it also contains minerals like iron, calcium, selenium, zinc, Vitamins B

complex, Vitamin E, proteins and fibres (Das et al. 2023). It has a relatively intense flavour, distinctively different from other types of aromatic rice and is considered as the major critical quality trait in rice which affect the consumer preference (Yang et al., 2008).

The bioactive compounds especially Cyanidin 3-glucoside is generally unstable at high pH environment and when undergoing thermal processing (Lasunon et al. 2022). According to studies by Hiemori, Koh, & Mitchell (2009), Walter et al. (2013), Surh & Koh (2014), and Min, McClung, & Chen (2014) showed that thermal cooking processes like boiling, frying, steaming, roasting, and pan-frying, decrease the total anthocyanin and contents of black rice, but increase Protocatechuic acid (PA), a major degradation product of anthocyanins. Study by Bhawamai et al. (2016) showed that the anti-inflammatory activities possessed by the cooked Black Rice is similar as that of raw rice, and suggest that cooking processes do not impair the potential health-promoting effects of black rice.

The inclusion of Black Rice in diet is be proved beneficial for health as its consumption has a positive effect on inflammation reduction, several types of cancer prevention, detoxification, boosting cardiological health by maintaining a balance between LDL and HDL content in the body etc. (Sangma and Parameshwari 2021; Duyi et al., 2017; Mazumdar et al., 2022).

Despite its effectiveness as a functional food, this rice has several drawbacks including a harder texture and longer preparation time compared to white rice. Techniques such as soaking in water an hour before cooking for 20 minutes can reduce its cooking time, but the texture remains firm even after extended cooking (Kushwaha, 2016). Given the preparation challenges associated with black rice, innovations such as parboiled rice and analogue rice can offer similar nutritional benefits with improved convenience.

The development of Black Rice analogues could offer a more convenient option as these analogues can be designed as quick-cooking substitutes, combining the nutritional benefits of Black Rice with the convenience of instant preparation. This approach integrates the two concepts by positioning rice analogues as a practical solution to black rice's preparation challenges.

1.3 Rice Analogue

The analogue rice or artificial rice is an imitation of rice made from ingredients from tubers and cereals that looks like rice grains which may use granulation or extrusion (cold and hot process) methods (Pudjihastuti et al. 2019 and Kusumayanti et al., 2023). It can be developed as a potential product from different grain types with or without added functionalities and nutrients (Sumardiono et al. 2018). Based on the raw material used for analogue rice production, diverse would be its nutritional contents. Therefore, selection of the raw materials must be carefully done as they determine the nutritional content, as well as the physical and chemical characteristics of the resulting analogue rice product (Budijanto et al. 2017). Analog rice is usually made of 50–98% starch or starch derivatives, 2–45% enriching ingredients, and 0.1–10% hydrocolloid (Kurachi, 1995). Analog rice could be made from various raw materials

using hot or cold extrusion technology such that the produced analogue will resemble oval-shaped rice (Putri and Sumardiono, 2020 and Kusumayanti et al., 2023).

1.4 Extrusion Technology

Food extrusion, a process by which a food material is forced to pass, under one or multiple varieties of conditions of mixing, heating and shear, flow through a die which is designed to form and/or puff-dry the ingredients; plays a key role in the production of analogue rice, an artificial product made from starches and other carbohydrate sources, that resembles natural rice in shape and texture (Riaz, 2000 and Sumardiono et al. 2021).

Extrusion can be classified into two categories: cold extrusion and hot extrusion. Hot extrusion involves relatively high temperatures (above 70 °C) while, cold extrusion is primarily a low temperature (below 70 °C) (Mishra et al. 2012) . Both processes pass dough made of principal component (mostly rice flour), an additive mix, and water through a single or twin-screw extruder which uses shear force and pressure leading to destruction and degradation of rice starch, denaturation of proteins, and the inactivation of enzymes, microbes and several anti-nutritional factors present in the food material (Chalermchaiwat et al. 2015). Therefore, this technology has been used for producing ready-to-eat products, such as snacks and breakfast cereals (Leyva-Corral et al. 2016), texturized vegetable proteins (TVP), breading substitutes, etc (Sapariya, Sangani, & Muliya, 2020) etc.

The versatility in ingredients and the efficiency of the extrusion process make analogue rice a practical and convenient option for consumers seeking quick, nutritious alternatives to traditional rice (Sumardiono et al., 2021).

1.5 Objectives of Study

1. To formulate and standardize black rice-based rice analogue using extrusion technology
2. To analyse the chemical characterization of raw materials
3. To determine the nutritional profile of Black Rice-based Rice Analogue
4. To determine the cooking quality, physical and functional properties of Black Rice-based Rice Analogues
5. To evaluate sensory properties of Black Rice-based Rice Analogues kheer
6. To quantify the phytochemical properties of the raw materials and Rice Analogues
7. To determine the aroma profile of Black Rice-Based Rice Analogue
8. To estimate the flavour profile of Black Rice-Based Rice Analogue

CHAPTER – 2

LITERATURE REVIEW

LITERATURE REVIEW

2.1 Black Rice

Black rice (*Oryza sativa L.*) is one of the rice species that is mainly cultivated in Asia, is glutinous in nature and is a storehouse of many nutrients. It has been consumed by the people throughout Asia for many centuries and has a historical reported use in China, India, and Thailand. Due to the natural presence of anthocyanin in high content in the pericarp of the rice kernel, the rice appears black. and the name ‘black rice’ is a description of the grain colour rather than other properties it holds.

Once the rice is cooked, it mutates into variants of purple hue similar to that of blueberries. It is known by various names like Purple Rice, Heaven Rice, Forbidden Rice, Imperial Rice, King’s Rice and Prized Rice etc (Ujjawal Kr. S. Kushwaha, 2016). Around the world, this rice is said to include more than 200 types of Black Rice (Kong et al. 2008). Black Rice is classified based on its shape, size, colour and nutritional profile.

Some of the Black Rice varieties include; (i) Black Japonica Rice is a mixture of mahogany medium-grain rice and black short-grain, cultivated together. It has an earthy flavour with a light, sweet spiciness. (ii) Black Glutinous Rice also referred to as Black Sticky Rice, a sticky, short-grain variety of rice often used in Asian desserts due to its uneven coloration. (iii) Italian Black Rice is a long-grain rice variety that merges Italian and Chinese Black Rice offering a rich and buttery taste. (iv) Thai Black Jasmine Rice, a medium-grain rice from Thailand which is a combination of Chinese Black Rice and jasmine rice. When cooked, it emits a delicate floral fragrance. (Saha soma, 2016).



(a)



(b)

Figure 2.1: (a) Raw Black Rice

(b) Black Rice Flour (BRF)

2.1.1 Nutritional Composition

According to Kushwaha (2016), no other rice with such a higher nutritional spectrum exists near black rice. The rice is a whole grain that is gluten-free, cholesterol-free, and low in fat, sugar, and salt. It is proved to be super nutritious in terms of fibre, anthocyanin, anti-oxidants, vitamins like E and B, and minerals such as iron, magnesium, phosphorus etc.

No.	Components	Amount Per 100g
1	Water	10.5 g
2	Carbohydrates:	
	Fiber, total dietary	4.2 g
	Starch	71.4 g
3	Protein	7.57 g
4	Nitrogen	1.21 g
5	Total lipid (fat)	3.44 g
6	Energy:	
	At water General Factors	370 kcal
	At water Specific Factors	361 kcal
7	Ash	1.34 g
8	Minerals:	
	Phosphorus, P	307 mg
	Potassium, K	256 mg
	Magnesium, Mg	113 mg
	Calcium, Ca	14 mg
	Manganese, Mn	3.91 mg
	Sodium, Na	<2.5 mg
	Zinc, Zn	1.72 mg
	Iron, Fe	1.12 mg
	Copper, Cu	0.21 mg
9	Vitamins and Other Components:	
	Niacin (Vitamin B3)	8.28 mg
	Thiamine (Vitamin B1)	0.319 mg
	Pyridoxine (Vitamin B6)	0.202 mg
	Biotin (Vitamin B7)	4.88 µg

Table 2.1: Proximate composition of unenriched, raw Black Rice
(Source: USDA FoodData Central)

2.1.1.1 Carbohydrate

Starch is one of the main components present in all rice varieties including Black Rice and is responsible for providing energy. The major two types of α -glucans present are (i) linear amylose, and (ii) branched amylopectin. They influence the crystallinity of the grains due to

their arrangement in the chain formation of grain starch (Ito and Lacerda, 2019). According to OECD (2014), non-glutinous rice comprises 10-30% amylose and 70-90% amylopectin. Juliano (2003) stated that for glutinous rice, amylose content is below 2% while the amylose content in common rice can range from as low as 5-12% to as high as 25-33%.

Amylose content is a key factor in determining rice quality, particularly in relation to cooking and pasting properties. Black Rice with low amylose content becomes moist and sticky when cooked. In contrast, Black Rice with an intermediate amylose content turns out dry and fluffy after cooking and maintains its soft texture upon cooling. On the other hand, Black Rice with high amylose content also cooks to a dry and fluffy consistency but tends to harden after cooling due to the retrogradation of amylose molecules (Adu-Kwarteng, Ellis, Oduro, & Manful, 2003).

Starch, based on the rate of glucose release and its absorption in the gastrointestinal tract can be also classified as (i) rapidly digestible starch (RDS), (ii) slowly digestible starch (SDS) and (iii) resistant starch (RS) (Englyst et al, 1992). RDS is the starch fraction that is responsible for the sudden blood glucose level increase after ingestion, and SDS is a starch fraction which is completely digested in the small intestine at a lower rate when compared to RDS. RS is the portion of the starch that can be fermented in the large intestine instead of being digested in the small intestine (Chung et al.2009). RS, along with other polysaccharides like hemicellulose and pectin, are part of the dietary fibre fraction as they are not able to be digested by the enzymes in the gastro-intestinal tract. They help the human digestive system by increasing the food viscosity and delaying the accessibility of the digestive enzymes to the starch granules (Angioloni & Collar, 2011).

2.1.1.2 Protein

Protein in Black Rice is present in the rice kernels. Based on the solubility, OECD, (2014) classified rice proteins as (i) Glutelins, alkaline solutions 60% of total proteins; (ii) Prolamin, which is soluble in alcohol and represents about 25% of total proteins; (iii) Globulin; which is soluble in salt water and represents about 10% of total proteins; and (iv) Albumin, which is soluble in water and represents about 5% of total proteins.

Proteins are made up of amino acids, which can be essential, semi-essential or non-essential in nature. Therefore, the nutritional quality of any protein is dependent on its essential amino acids content, its ability to supply the metabolic requirements of humans, and the bioavailability of these amino acids (Nunes, Seferin, Maciel, Flôres, & Ayub, 2016). Rice (Amino Acid Score (AAS) of 68) is proved to have a more complete and balanced amino acid composition compared to corn (AAS of 35) and wheat (AAS of 43) due to its elevated amounts of lysine and sulphur-containing amino acids (WHO, 2007). Also, Carvalho et al., (2013) stated that rice presents higher digestibility, biological values, and protein efficiency quotient than other cereals.

Frank, Reichardt, Shu, and Engel (2012), reported in their study that the Black Rice when compared to non-pigmented rice, exhibited higher levels of amino acids, organic acids, fatty acid methyl esters, free fatty acids.

2.1.1.3 Fat

Generally, lipid content of the rice is relatively low. Chemically, they are classified as (i) saponifiable lipids, which includes triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids and waxes; and (ii) unsaponifiable lipids which includes phytosterols, triterpene alcohols, γ -oryzanol and vitamin E homologues; tocopherols and tocotrienols (Ito and Lacerda, 2019).

Lipid profile of Black Rice consists of triglycerides, free fatty acids, sterol and diglycerides along with lipid-conjugates like acyl-sterol glycoside and sterol glycoside; glycolipids like cerebroside and phospholipids including phosphatidylcholine and phosphatidylethanolamine (OECD, 2014).

Triglycerides are the main types of fats in black rice. Glycerol and three fatty acids make up a triglyceride molecule. In black rice, the primary fatty acids are Oleic Acid (42.10%), Linoleic Acid (29.30%) and Palmitic Acid (20.30%) (Frei & Becker, 2005).

2.1.1.4 Vitamins

Vitamins are chemical compounds that are required in small amounts with our regular diet in order to carry out certain biological functions and for the maintenance of our growth. Vitamins can be classified as water-soluble or fat-soluble, depending on how they are absorbed and stored in the body. Vitamin A, D, E and K are fat-soluble. They are stored in adipose tissues, which is why they are referred to as fat-soluble vitamins. The Vitamins in B-group and vitamin C are water-soluble and cannot be stored in our bodies as they pass with the water in urine. These vitamins must be supplied to our bodies with regular diets.

Black Rice is a rich source of several essential vitamins, including Vitamin B complex and Vitamin E. Vitamin E is a fat-soluble antioxidant that protects cell membranes from oxidative damage caused by free radicals, and it is crucial for immune function, skin health, and cellular repair processes.

Among the B vitamins, Vitamin B1 (Thiamine) is vital for energy metabolism, converting carbohydrates into glucose for daily activities and supporting nerve function, muscle contraction, and heart health. Vitamin B2 (Riboflavin) plays a role in energy production, red blood cell formation, antioxidant defence, and promotes healthy vision, skin, and hair. Vitamin B3 (Niacin) aids in converting food into energy, helps maintain healthy cholesterol levels, supports cardiovascular health, and is involved in nervous system function and skin health. Collectively, these vitamins contribute to the health benefits offered by Black Rice (Thanuja & Parimalavalli, 2018).

2.1.1.5 Minerals

Minerals are naturally occurring inorganic substances that are essential for various bodily functions and overall health. They play crucial roles in building and maintaining bones and teeth, supporting muscle function, regulating metabolism, and maintaining proper fluid balance (Kuna, A. 2018).

It's also to be noted that Black Rice is rich in iron, which aids in red blood cell production, as well as manganese, which supports reproductive health. Additionally, it contains potassium for muscle growth and phosphorus for maintaining internal water balance (Kushwaha, 2016). Ahmad et al. (2009) also reported that the levels of manganese (Mn) and zinc (Zn) are higher in Black Rice varieties compared to non-pigmented rice samples.

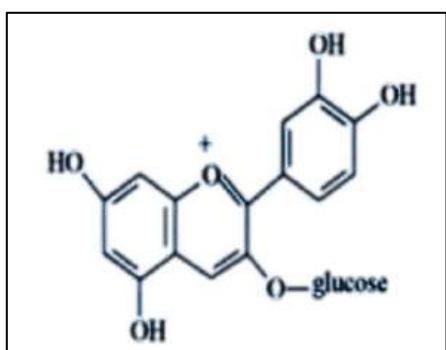
2.1.2 Bioactive Compounds

Bioactive compounds are present in different layers in the bran. The bran comprises of the pericarp, seed coat and aleurone (Callcott et al., 2018). Bioactive compounds in Black Rice can interact with starch during the gelatinization process, altering its pasting, thermal, and digestibility properties (Zhu, 2015).

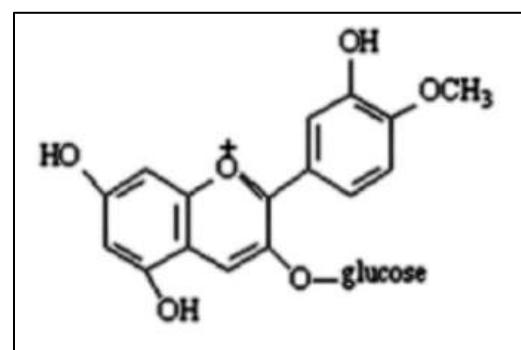
2.1.2.1 Anthocyanins

They are a group of water soluble reddish to purple natural flavonoid pigments of Black Rice and act as a source of antioxidants which have the ability to inhibit or reduce the formation and concentration of reactive free radicals that can damage cells (Adom and Liu, 2002).

Cyanidin-3-glucoside (C3G) is the main anthocyanin which represents 88% of the total anthocyanins (Hou, Qin, Zhang, Cui, & Ren, 2013). Abdel-Aal, Young, and Rabalski (2006) confirmed that Black Rice boasts the highest total anthocyanin content, with 327.60 mg per 100 grams, compared to all the other colored grains studied. Other anthocyanins that have been identified in lower levels are peonidin-3-glucoside, cyanidin-3-rutinoside and malvidin-3-glucoside (Chen, Nagao, Itani, & Irifune, 2012). Only six types of anthocyanins, cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, delphinidin, cyanidin, pelargonidin, and malvidin are said to be present in raw Black Rice bran according to several researches.



(a) *Cyanidin-3-glucoside*



(b) *Peonidin-3-glucoside*

Figure 2.2: Two major anthocyanin pigments present in Black Rice
(Source: Hou et al., 2013)

Total anthocyanin content	Reference
327.60 mg/ 100 grams	Abdel-Aal, Young, and Rabalski (2006)
109.50 - 256.60 mg/ 100 g	Sompong, Siebenhandl-Ehn, Linsberger-Martin, and Berghofer (2011)
32.40 - 50.30 mg/ 100 g	Frank, Reichardt, Shu, and Engel (2012)
4.35 - 7.16mg / g	Somsana et al, (2013)
145 - 441mg / g	Maisuthisakul and Changchub, (2014)
83.31 mg/g	Bhat and Riar, (2017)
340.10 mg/kg	Rajendran et al, (2018)
162.5 - 773.7mg / kg	Wongsa et al, (2018)
49.11 mg/g	Agustin et al, (2021)
1 - 806.17mg / kg	Shozib et al, (2021)

Table 2.2: Total anthocyanin content in Black Rice
(Source: Pedro, Granato, and Rosso (2016) and Dewan et al., (2023))

Type of anthocyanin	Quantity (mg/ kg)	Reference
Cyanidin-3-glucoside	88.6-2013	Seo et al., (2011) Sompong et al., (2010) Abdel aal et al., (2006)
Petunidin-3-glucoside	4-10	Seo et al., (2011)
Peonidin-3-glucoside	135-1275	Seo et al., (2011) Sompong et al., (2010)
Cyanidin-3-rutinoside	19.90	Abdel aal et al., (2006) Huang and Lai, (2016)

Table 2.3: Types of anthocyanin in Black Rice
(Source: Arunima et al. (2021))

2.1.2.2 Phenolic Acids

Black Rice contains a good number of phenolic acids. They can be classified as free or soluble phenolic acids (cinnamic, protocatechuic and gallic acid) and bound phenolic acids (ferulic, coumaric and caffeic acids) (Alves et al., 2016 and Duyi et al., 2017).

The study by Ryu and Koh (2017) on the phenolic acids in Korean Black Rice gave the conclusion that Protocatechuic acid (PA), a metabolite of Cyanidin-3-glucoside, was

predominant (81–93% of the total amount) in the free form, and ferulic acid was predominant (about 60%) in the bound form.

In their study, Amagliani et al. (2016) examined the phenolic acid content in different Black Rice varieties. They found that the free-form phenolic acids in these varieties ranged between 7.4 to 10.5 mg per 100 grams. The most abundant phenolic acids identified in Black Rice varieties were ferulic acid, vanillic acid, and p-coumaric acid (Amagliani et al., 2016).

According to Sirisoontaralak, Keatikasemchai, Mancharoen, and Na Nakornpanom (2020), Black fragrant rice starts with a robust antioxidant profile, boasting a total phenolic content of 186.20 mg GAE/100 g. However, the milling process significantly reduces these beneficial compounds.

Other researchers, including Zhou et al. (2004) and Walter et al. (2011), have reported that the light brown pericarp and black pericarp contain relatively higher amounts of phenolic compounds (70–90% and 92–97%, respectively). The variations in phenolic content are influenced by factors such as the rice cultivar, phenolic compounds in the rice kernels, the degree of milling, and the methods used for extraction.

The individual free phenolics in raw polished rice, cooked for 18–20 min, included ferulic acid (0.0–248.8 mg/100 g), gallic acid (454.5–688.0 mg/100 g), kaempferol (50.7–99.3 mg/100 g), myricetin (30.0–100.8 mg/100 g), PAA (0.0–118.8 mg/100 g), and vanillic acid (44.5–81.5 mg/100 g) (Cañizares et al., 2025).

2.1.2.3 Tocopherols and Tocotrienol

Tocopherols, popularly known as Vitamin E are compounds found within the bran of rice that possess strong antioxidant properties (Goufo & Trindade, 2014). These antioxidants help protect cells from damage caused by free radicals and contribute to overall health and well-being. There are 8 different vitamin E forms: 4 tocopherols and 4 tocotrienols. The 4 types of tocopherols reported are α , β , γ and δ . The key distinction between tocopherols and tocotrienols lies in their tail structures: tocopherols have a saturated tail, while tocotrienols possess an unsaturated tail. Additionally, the various forms within each group are differentiated by the position of the methyl groups on the ring (Lumen Learning).

Tocotrienols, specifically γ -tocotrienol which is also known as γ -oryzanol, are compounds found in rice bran oil. They are part of the Vitamin E family and are known for their strong antioxidant properties (Rogers et al., 1993). Oryzanol is a group of compounds within γ -oryzanol, which include ferulic acid esters of sterols and triterpene alcohols. The concentration of γ -oryzanol in Black Rice varies depending on the variety and cultivation conditions. Huang and Lai (2016) reported that Black Rice bran contains γ -oryzanol levels ranging from 3.95 to 7.72 mg per gram of dry matter. This is significantly higher compared to red rice bran, which has 3.59–3.69 mg per gram, and white rice bran, which has 1.55–3.13 mg per gram of dry matter.

2.1.2.4 Carotenoids

Carotenoids are naturally occurring pigments found in numerous forms throughout the food chain and human diet. High levels of carotenoids in food can help prevent various types of cancer, heart diseases, and the oxidation of lipoproteins. Additionally, dietary carotenoids offer significant benefits for ocular function and overall eye health (Eggersdorfer and Wyss, 2018).

Study by Pereira-Caro et al. 2013 concluded that xanthophylls, lutein and zeaxanth were the major carotenoids in the Japanese black–purple rice and they comprised more than 94% of total carotenoids followed by carotenes, lycopene and β-carotene occurring as minor components.

2.1.3 Aromatic Compounds

Black Rice is a unique and aromatic variety of rice that holds a special place in Asian cuisine. Its distinctive flavour contributes significantly to its reputation and popularity among consumers. The rich taste and aroma of Black Rice make it a preferred choice for various culinary dishes, enhancing its acceptance and demand in the market. Gas chromatography mass spectrometry has identified thirty-five volatile compounds. Among these, aldehydes and aromatics are the most abundant, making up 80.1% of the total relative concentration of volatiles. This significant presence of aldehydes and aromatics likely contributes to the distinctive flavour and aroma of black rice, enhancing its appeal to consumers (Ujjawal Kr. S. Kushwaha, 2016).

The major contributors of the distinct aroma of Black Rice are 2-acetyl-1-pyrroline (2-AP) and guaiacol; which have been identified as based on their odor thresholds, relative concentrations, and analysis through olfactometry (Yang et al. 2008). Buttery, Ling, & Juliano, (1982) stated that 2-AP is present in aromatic nonpigmented rice like Jasmine rice and is absent in nonaromatic nonpigmented rice cultivars. 2-AP is responsible for a characteristic popcorn-like aroma in aromatic rice (Bryant & McClung, 2011).

Study by Choi and Lee, (2021) provided the volatile profiles of cooked Black Rice using GC chromatography and identified 51 volatiles and 19 volatiles' identities were confirmed with authentic standards. The volatiles included 13 aldehydes and ketones, 6 acids and esters, 3 alcohols and 16 additional compounds. 5-Pentyl-2(5H)-furanone, a Maillard-reaction-derived volatile compound, was also present in lower concentration in milled, cooked rice.

Compared to non-pigmented green and red rice, concentrations of ferulic acid and vanillin are relatively higher in Black Rice (Choi, Seo, Lee, Lee, & Lee, 2018a, 2018b; Jun, Song, Yang, Youn, & Kim, 2012; Sumczynski, Kot'askov'a, Druzb'ikov'a, & Ml'cek, 2016; Tian, Nakamura, & Kayahara, 2004)

Vanillin (i.e., 4-hydroxy-3-methoxybenzaldehyde) is an important odor-active volatile compound found in cooked rice (Jezussek, Juliano, & Schieberle, 2002; Maraval, Mestres, Pernin, Ribeyre, Boulanger, Guichard, et al., 2008). During cooking, thermal degradation of ferulic acid and vanillin give rise to 2-methoxy-4-vinylphenol (ie, 4-vinyl guaiacol) (Coghe, Benoot, Delvaux, Vanderhaegen, & Delvaux, 2004; Esatbeyoglu, Ulbrich, Rehberg, Rohn, &

Rimbach, 2015). 4-vinyl guaiacol and guaiacol are the key odorants of the smoky aroma in Black Rice (Ajarayasiri & Chaiseri, 2008; Yang, Lee, Jeong, Kim, & Kays, 2007).

Volatile compound		Odor description
A] Aldehydes:		
1.	Hexanal	Green, tomato, green
2.	Octanal	Citrus
3.	Nonanal	Aldehydic, waxy, citrus, tart, sweet
4.	(2E)-2-Octenal	Sweet, fatty, citrus peel
5.	Furfural	Sweet, woody, almond, bread baked
6.	Decanal	Citrus
7.	Benzaldehyde	Almond
8.	2-Nonenal	Beany, cucumber
9.	2,6,6-Trimethyl-1-cyclohexene-1-carbaldehyde	Tropical, saffron, herbal, clean, rose
10.	Phenylacetaldehyde	Sweet, floral, nutty, fruity
11.	2-Butyl-2-octenal	Fruity, pineapple
12.	Pentadecanal	Fresh, waxy
13.	Vanillin	Vanilla, sweet, creamy
B] Ketones:		
1.	Propan-2-one	Acetone, ethereal, fruity
2.	2-Heptanone	Cheesy
3.	2-Octanone	Parmesan cheese like
4.	6-Methyl-5-hepten-2-one	Fruity, apple, musty
5.	2-Nonanone	Fruity, cheesy
6.	3-Octen-2-one	Creamy, earthy, oily, mushroom
7.	2-Decanone	Fermented, generic cheese notes
8.	6-Methyl-3,5-heptadien-2-one	Cinnamon, coconut, spicy
9.	1-Phenylethanone	Sweet, aromatic, almond, nuts, almond phenolic taste
10.	2-Tridecanone	Waxy, fatty
11.	(5E)-6,10-Dimethyl-5,9-undecadien-2-one	Fresh, green, fruity, waxy
12.	2-Pentadecanone	Fresh, Jasmin, celery
13.	6,10,14-Trimethyl-2-pentadecanone	Oily, herbal, Jasmin
C] Alcohols:		
1.	1-Hexanol	Green, fruity, apple-skin, oily
2.	1-Octen-3-ol	Mushroom
3.	1-Octanol	Waxy, green, citrus
D] Acids and esters:		
1.	Hexanoic acid	Cheesy, fatty

2.	Octanoic acid	Rancid, soapy, cheesy
3.	Nonanoic acid	Cheese, dairy, fatty, waxy
4.	Methyl palmitate	Waxy, fat, candle
5.	Decanoic acid	Fatty, rancid
6.	Methyl (9Z)-9-octadecenoate	Mild, fatty
E] Additional compounds:		
1.	2-Butylfuran	Mild, fruity, wine, sweet, spicy
2.	p-Xylene	Medicinal
3.	m-Xylene	Plastic
4.	o-Xylene	Peanut
5.	Propylbenzene	Solvent, sweet
6.	2-Pentylfuran	Floral, fruit
7.	Styrene	Balsam, styrene
8.	1-Isopropyl-4-methylbenzene	Mild pleasant, carrot
9.	2-Acetyl-1-pyrroline	Pandan, cooked rice, sweet, pleasant, Popcorn-like
10.	(1S,4S)-4-Isopropyl-1,6-dimethyl-1,2,3,4-tetrahydronaphthalene	Weak spicy, weak floral
11.	Guaiacol	Smoky, black rice-like
12.	5-Pentyl-2(5H)-furanone	Minty, fruity
13.	Butylphenol	Aromatic
14.	2-Methoxy-4-vinylphenol	Smoky, amber, cedar, peanut
15.	4-Vinylphenol	Chemical, phenolic, medicinal
16.	1H-Indole	Animal, fecal, naphthyl, with earthy

Table 2.4: Identified volatiles in cooked Black Rice
(Source: Choi & Lee, 2021)

2.1.4 Health Benefits

Black Rice is known for its health benefits due to the presence of its rich nutrient profile. The presence of elevated level of anthocyanins and other bioactive compounds present in it along with the basic nutrients make it a functional food.

2.1.4.1 Anti-Inflammatory Activity

Systemic inflammation plays a significant role in various diseases, such as arthritis, asthma, Alzheimer's, heart disease, and cancer. Black Rice bran can reduce inflammation at a cellular level, improving overall cell health. The anti-inflammatory properties of Black Rice decrease reactive oxygen species and increase anti-inflammatory mediators like superoxide dismutase, which helps prevent aging-related symptoms and certain cancers (Nitenberg & Raynard, 2000)

Additionally, Black Rice reduces inflammatory responses in the body, which is beneficial since chronic inflammation is linked to many serious illnesses. Research shows that pigmented rice, such as black rice, helps reduce inflammation, including inflammatory skin conditions like dermatitis. In contrast, brown rice does not have the same effect. This makes Black Rice a superior choice for its anti-inflammatory and anti-allergic properties (Oki et al., 2005 and Rassarin et al., 2015).

2.1.4.2 Antioxidant Properties

Compounds that can help to prevent the reactive oxygen species (ROS) and the regular oxidation processes in the body are called antioxidants (Dash and Podh, 2019). They are also called free radicle scavengers as they inhibit the free radicle activity which can otherwise lead to both functional and structural cell damage (Jakobs et al. 2006). ROS can interact with cellular components such as lipids, nucleic acids, proteins, and enzymes, potentially causing genetic mutations that can lead to cancer. Antioxidants play a crucial role in reducing oxidative damage to nucleic acids and help in lowering abnormal cell proliferation.

Naturally occurring antioxidants include compounds like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tocopherols, tocotrienols, oryzanol, polyphenols, flavonoids, and vitamin C-like bioactive compounds (Huber & Rupasinghe, 2009). Enzymes such as superoxide dismutase, glutathione peroxidase, and glutathione reductase neutralize free radicals through quenching reactions. Additionally, various metal-binding proteins like ferritin, albumin, and lactoferrin can catalyse oxidative reactions in the body (Thanuja & Parimalavalli, 2018). Black Rice bran oil is particularly noted for its rich antioxidant content (Hu et al., 2003).

2.1.4.3 Anti-Diabetic Properties

Scientists from the Harvard School of Public Health estimated that replacing two servings of white rice per week with Black Rice could reduce diabetes risk by 16% (Archives of Internal Medicine, 2010). Insulin resistance is strongly linked to non-alcoholic fatty liver disease. Multiple studies suggest that natural anthocyanins, potent antioxidants, can help prevent diabetes. Jang et al. (2015) proposed that black rice, containing C3G, may reduce liver fat accumulation and improve insulin resistance. A study in the "American Journal of Clinical Nutrition" found that anthocyanins are the only flavonoid group significantly associated with a lower risk of Type II Diabetes (Wedick 2012). C3G is thus considered to have strong anti-diabetic effects, including alleviating diabetic progression, combating metabolic syndrome, and providing antioxidant and anti-inflammatory benefits.

2.1.4.4 Anticancer Properties

Flavonoids and phenols are major compounds in rice bran, especially in colored rice. These compounds are thought to have anti-cancer properties for several types of cancer cells. Evidence suggests that flavonoids and phenolic compounds in Black Rice bran work through different mechanisms. The compounds in each Black Rice cultivar may affect how they inhibit

cancer cell growth in vitro. Cyanidin 3-glucoside and peonidin 3-glucoside, found in Black Rice anthocyanin, can be combined with doxorubicin to inhibit cancer cell growth. These anthocyanins might be used for cancer prevention. The research also suggests that these active anthocyanin compounds can prevent cancer from spreading to other tissues (Pratiwi & Purwestri, 2017).

2.1.4.5 Protecting Hepato and Renal Health

Anthocyanins, which are potent antioxidants are dense in black rice. It can protect the liver by reducing oxidative stress and inflammation. The high fibre content of Black Rice aids in detoxifying the liver and promoting regular bowel movements. In addition, black rice's anti-inflammatory properties may reduce inflammation in the kidneys, supporting overall renal health. The grain also provides essential nutrients, such as iron and magnesium, that bolster kidney function and help regulate blood sugar levels. Furthermore, the phytonutrients in black rice, including flavonoids and carotenoids, are associated with a reduced risk of chronic diseases, including kidney disease. Incorporating Black Rice into the diet can significantly contribute to maintaining hepato and renal health, making it a valuable addition to a balanced diet (Thanuja & Parimalavalli, 2018).

2.1.5 Effect of Processing Methods

Black rice, often hailed as a superfood, has been celebrated for its remarkable nutritional benefits. However, the processing methods applied to Black Rice can significantly impact its nutritional composition, texture, and overall quality. Understanding the effects of different processing techniques on Black Rice is crucial for optimizing its health benefits and culinary uses.

2.1.5.1 Milling and Grinding

During the processing of rice, like grinding, the aleurone layer is adversely (Ma et al., 2022). When performed moderately, grinding can enhance both the flavor and visual appeal of rice. However, as the milling degree increases, there is a notable reduction in the color of black rice, which correlates with a continuous decline in anthocyanin content (Sapna et al., 2019). At a milling degree of 2%, the loss of anthocyanin content was minimal. The total anthocyanin levels remain elevated at milling degrees between 2% and 4%, which also ensures a favorable taste quality. Conversely, when the milling degree reaches between 4% and 7%, the loss rate of total anthocyanin content escalates to between 57% and 90% (Ma et al., 2020). At a milling degree of 9%, the anthocyanin content loss approaches nearly 100% (Paiva et al., 2014). Consequently, the milling process of rice grains resulted in varying degrees of nutrient loss (Bagchi et al., 2021; Mohidem et al., 2022), particularly affecting colored rice varieties (Hu et al., 2022).

After just 10 seconds of milling, the phenolic content drops sharply to 127.82 mg GAE/100 g ($p<0.05$). Prolonged milling further depletes the phenolic content, reducing it to 61.01 mg

GAE/100 g after 100 seconds. Additionally, a moderate amount of phenolic compounds (58.38 mg GAE/100 g or 31%) is lost after 10 seconds of milling (DOM * 6%). When all bran is removed (calculated as the difference in contents of Black Rice and rice milled for 30 seconds or DOM * 12%), approximately 102.17 mg GAE/100 g or 55% of phenolic compounds are eliminated (Sirisooontaralak et al. 2020).

2.1.5.2 Extrusion

It was agreed that extrusion does not significantly alter the amount of added iron, zinc, vitamin B12, and folic acid in the product (Valencia & Purwanto, 2020; Alavi et al., 2008 and Yogeshwari et al., 2019). Raw material with high starch content is preferred for development of extruded food products because during extrusion cooking, gelatinization of starch takes place (Kumar & Murali, 2020). Total volatiles were significantly lower after milling regardless of the cultivar and milling lowered the concentration of 2-ap in aromatic cultivars (choi and lee, 2021). Similarly, in a previous study done by Bergman et al; 2000, 2-AP concentrations were lower in aromatic nonpigmented rice after milling.

2.1.5.3 Drying

Lang et al., (2019) studied how drying temperature, storage time, and storage conditions affect the phenolic compounds in black rice. They tested drying at 20, 40, 60, 80, and 100°C, with a storage duration of 12 months, under normal atmosphere, nitrogen atmosphere, and vacuum atmosphere conditions. Their research found that higher drying temperatures resulted in fewer total phenolic compounds. Total free flavonoids also decreased at temperatures over 60°C. Protocatechuic acid and quercetin were the only phenolic compounds that did not decline with rising drying temperatures. Conversely, ferulic acid, p-coumaric acid, gallic acid, and caffeoic acid levels decreased. It was also noted, the amount of protocatechuic acid increased with higher drying temperatures, possibly because of the breakdown of anthocyanin cyanidin-3-O-glycoside. This suggests that drying temperature significantly affects the phenolic content in black rice, with some compounds being more resilient than others.

2.1.5.3 Cooking

Cooking is a method in which food is prepared by the application of heat like boiling water to soften the foods, frying, baking etc. and it alters their texture and flavour. Cooking leads to a reduction in free phenolic compounds, with certain anthocyanins decreasing while others increasing (Cañizares et al., 2025). According to the study by Aalim, Wang, and Luo (2021) on the processing of Black Rice, there was a noted reduction in the levels of cyanidin-3-glucoside (156.76 to 116.18, 135.15, 36.72, and 21.64 mg/100 g) and rutin (4.32 to 3.97, 0.75, 4.05, and 2.09 mg/100 g), accompanied by an increase in the levels of kaempferol (1.23 to 2.87, 2.57, 1.73, and 2.28 mg/100 g) and isorhamnetin (2.51 to 3.56, 3.01, 5.58, and 4.52 mg/100 g) across different preparation methods, including raw, cooked, roasted, and oil-fried rice.

2.1.6 Need for Value Addition

Rice has always been a staple food to more than half of the world with its wide range of varieties including the Black Rice variety. Though its health benefits are known to many, the accessibility to obtaining its benefits, its popularity and making it more appealing to the rush-filled urban areas has always been a concern. This problem highlights the need for its value addition where it is processed into convenient and nutritious food like ready-made mixes, noodles etc... Value addition also tackles the problem of losses that incurred during traditional postharvest processing methods and helps to increase its commercial value.

2.1.7 Application in Food Industry

Black Rice is naturally gluten free and has many attributes that can impart a positive health effect; which has captured both the general public and research field attention (Das et al., 2023). Previously, Black Rice was primarily used in China for desserts. However, it is now recognized as suitable for a variety of dishes, including pudding, porridge, cakes, bread, noodles, pasta, and even rice bran oil (Kushwaha, 2016). Its application in creating value added products has been invasive.

2.1.7.1 Extruded Products

Extrusion cooking is a food processing method that encompasses several unit operations, including mixing, cooking, shearing, forming, puffing, and drying, to produce a wide range of snacks, breakfast cereals, and ready-to-eat (RTE) foods (Navale et al., 2015).

Meza et al., (2019) successfully prepared very appealing coloured breakfast cereal with desirable expansion, texture, and colour attributes from Black Rice varieties. Approximately 100 kg of Black Rice was refrigerated and processed in an analytical mill. The rice flour was treated with water 24 hours before extrusion using a co-rotating twin-screw extruder at temperatures of 75°C, 100°C, and 125°C. The process maintained a screw speed of 250 rpm and a feed rate of 15 kg/h. post-extrusion, the material was dried until its moisture content was 7% or less.

Pasta, often referred to as "convenience food," is a cereal-based product widely consumed due to its ease of cooking, availability, digestibility, nutritional quality, and long shelf life. Typically made from durum wheat flour (semolina), pasta is a good source of carbohydrates but lacks minerals and fibres, leading to its classification as refined food, which can contribute to obesity when consumed in large amounts (Laishram & Das, 2017; Sethi et al., 2020) and to improve the nutritional profile of pasta, researchers have investigated incorporating Black Rice extract as a bioactive component (Kumar & Murali, 2020). In a study by Sethi et al. (2020), varying amounts of Black Rice bran (5%, 10%, 15%, 20%, and 25%) were used to supplement semolina flour in pasta preparation. The sensory analysis indicated that pasta with 15% Black Rice bran was acceptable in terms of quality. This variant had higher levels of protein, fibre, and anthocyanin compared to pasta made with 100% wheat semolina. Adding Black Rice bran increased fibre content and decreased gluten content, resulting in reduced hardness. However,

it also decreased cohesiveness, gumminess, and chewiness. Pasta with more than 15% Black Rice bran was less accepted due to its darker colour. Black Rice flour exhibited a high swelling index, which increased water absorption and water solubility in pasta (Laishram & Das, 2017; Kumar & Murali, 2020). Given its high anthocyanin, protein, and fiber content, Black Rice presents a promising option for enhancing pasta's nutritional value. According to Subanmanee et al. (2024), the research demonstrated that substituting wheat flour with black glutinous rice flour (BGRF) significantly enhances the nutritional profile of pasta by increasing levels of phenolic compounds and antioxidants, with consumer acceptance peaking at a 40% substitution. While higher levels of BGRF resulted in notable changes in physical properties, such as colour and moisture content, and impacted cooking quality, the findings highlight BGRF's potential to create healthier pasta options without compromising sensory appeal.

Noodles are one of the extruded food products, primarily composed of wheat flour, salt, and oil. It has been quick in gaining popularity globally due to its ease in the cooking process as well as its availability and transportation. However, it is also considered refined by dieticians because, in comparison with its other counterparts, it has a relatively good carbohydrate level. This is the case of most noodles, which are made using flours that lose protein, as well as possibly other micronutrients, during wheat refining (Kong et al., 2012). There has been an increasing interest in functional food, for this reason researchers explored the possibilities of using healthy ingredients in the traditional noodles aiming to improve the nutritional and functional value (Kumar & Murali, 2020). The use of Black Rice bran (Heugjinjubyeo variety) in noodle production as a primary ingredient was taken up by Kong et al., (2012) and they investigated its chemical and functional properties. In this study, different amounts of Black Rice bran (2 %, 5 %, 10 % and 15 %) were used to make noodles instead of wheat flour. Better results were obtained when Black Rice bran was substituted for wheat flour. It was observed that the supplemented noodles had improved content of protein, fat and mineral content along with higher anthocyanin contents. according to profile analysis, they also concluded that as the percentage of Black Rice bran extract increased, the Noodle cohesiveness decreased while its hardness increased according to profile analysis.

2.1.7.2 Baked Products

Black Rice flour has been used in preparation of baked products such as biscuits, cookies, cakes, bread etc.

Biscuits are characterized by their low moisture content, making them thin and crunchy. In contrast, cookies have a higher moisture content, resulting in a thicker and chewier texture. When preparing cookies, 20% of the wheat flour is substituted with Black Rice flour, and the dish is made following traditional methods (Kim et al., 2006).

Cakes are usually made with wheat; the gluten present in it providing the structure. However, black rice, being rich in protein, can be used as an alternative to wheat flour in cake-making. Different concentrations of Black Rice flour were incorporated into chiffon cakes, replacing wheat flour at levels of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100% (w/w) (Lee et al., 2017). The baked cakes incorporating Black Rice powder were found to have a harder, crumblier crust, with increased color and chewiness. Due to its high nutritional content,

Black Rice can enhance the nutritional profile and serve as a suitable substitute for wheat flour in traditional cakes (Parmeshwari et al., 2021).

Study by Kaori et al., (2019) demonstrates that extruded Black Rice flour can be utilized in the bread industry to produce higher-quality products compared to the raw material. To prepare the bread dough, the following ingredients were used: flour, salt (1.5%), yeast (5.3%), sucrose (6%), shortening (3%), and water (60%) and were kneaded in a 5-speed mixer for 8 minutes. The dough was then placed in a pan and fermented for 90 minutes at 30°C and 85% relative humidity (RH). After fermentation, the dough was baked in an electric oven at 190°C for 40 minutes. The loaf was immediately removed from the toast box and allowed to cool to room temperature before other parameters were measured.

Similarly, Androniou et al., (2018) prepared muffins using coconut butter with 80% fat content, brown sugar, hen eggs, wheat flour, and Black Rice flour with wheat flour and Black Rice flours in ratios 1:0, 1:1 and 0:1 respectively. Compared to the control sample baked with wheat flour, the muffins made with Black Rice flour had higher anthocyanin content and antioxidant activity. The textural study indicated that the addition of Black Rice flour increased stiffness, springiness, and chewiness. These findings suggest that value-added muffins made with Black Rice flour could be a good alternative for individuals who are gluten intolerant, while also providing a significant amount of polyphenolic content, which may have several health benefits.

2.1.7.3 Beverage

Many researchers are exploring the innovative use of Black Rice extract in the creation of functional beverages due to its rich nutritional profile, Black Rice offers numerous health benefits, including high levels of antioxidants, anthocyanins, and essential minerals. Black Rice has the potential to act as a key ingredient in the development of beverages that not only satisfy consumer taste preferences but also provide significant health benefits.

Takeshita et al., (2015) conducted a study aimed to develop new types of alcoholic beverages. They investigated the production and antioxidant activity of alcoholic beverages made from various grains, including Black Rice. It was found that the fermentation of Black Rice proceeded more slowly than wild rice but resulted in a brilliant red-colored beverage when uncooked. The cooked version had a faded colour due to anthocyanin pigment denaturation. The resulting Black Rice beverage also contained significant levels of anthocyanin and showed high DPPH radical scavenging activity, indicating strong antioxidant properties. Additionally, it maintained a high phenolic content, particularly when produced using uncooked grains. This suggests that Black Rice can be effectively used to create antioxidant-rich alcoholic beverages, highlighting its potential for developing new, health-focused alcoholic products.

The study by Zhang et al., (2019), investigated the effect of using extruded Black Rice as an adjunct in beer production with their primary focus on flavour compounds such as polyphenols, amino acids, and proteins. They detected one organic acid, one aromatic, ten alcohols, and 23 esters in the beer made with Black Rice. The protein content was comparable to traditional beer, with higher levels of essential amino acids like valine and threonine. Key ingredients like

polyphenols, nerolidol, geraniol, and geranylgeraniol contributed to the beer's functionality and antioxidant properties. The study concluded that the addition of extruded Black Rice improved the overall quality of beer, enhancing its nutritional value and antioxidant capacity.

Research conducted by Katoh et al. (2010) examined the distinct characteristics of meads made from different varieties of Black Rice and various types of honey, including Chinese milk vetch honey, clover honey, and blends of acacia and clover honey. Mead is an alcoholic drink created through the fermentation of honey, often enhanced with various ingredients such as fruit juices (including apple and grape), spices, herbs, malt, and vinegar to enrich its flavour. The resulting mead contained an ethanol concentration ranging from 12% to 13%. The study found that mead produced from unpolished Black Rice grains demonstrated superior antioxidative activity compared to that made from polished grains, attributed to the high content of anthocyanins and phenolic acids in Black Rice.

Yoghurt is another fermented product made from dairy, created through the action of beneficial bacterial cultures, specifically *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. It serves as an excellent source of probiotics, which promote the growth of gut microbiota and enhance overall health, contributing to its global popularity. Kumar & Murali, (2020) used Anthocyanin, derived from black pigmented rice bran, and added to yoghurt as a natural food colorant to create a flavour-enhanced product. Different concentrations of anthocyanin powder (0.2%, 0.4%, and 0.6% by weight) were tested, with the yoghurt containing 0.6% anthocyanin extract achieving a successful outcome, displaying a purplish-pink hue, excellent colour stability, and enhanced phytochemical content.

2.1.7.4 Other Products

The study by Kanabur & Kamath, (2020) explored the development of fryums, a popular Indian snack, using Burma Black Rice (*Oryza sativa*) as a replacement for white rice. The researchers tested three variations: 50%, 75%, and 100% Black Rice. Sensory evaluation using a 9-point hedonic scale and Food Action Rating Scale (FACT) indicated that the 100% Black Rice variation received the highest sensory scores. This may be due to differences in the amylose and amylopectin ratio between white and Black Rice, which affect cooking properties. The 100% Black Rice fryums were found to be the closest to the basic recipe in sensory properties and provided significant nutritional benefits, including 290.5 kcals of energy, 72.25 g of carbohydrates, and 8.71 g of protein per 100 g. The study concluded that Black Rice fryums are a viable and nutritious alternative to traditional white rice fryums, offering potential health benefits and variety to diets.

Dhingra and Poonia (2023), did an investigation focused on developing and evaluating the sensorial and antioxidant properties of functional Black Rice kheer. Researchers substituted white rice with Black Rice in three proportions: 40g (K1), 30g (K2), and 20g (K3), adding 15g of coconut sugar to each combination. Standardized pasteurized milk with 4.5% fat and 8.5% SNF was used alongside the Black Rice grains. The preparation involved soaking cleaned Black Rice in water (rice to water ratio of 1:2) and cooking it at 93°C until the water was fully absorbed. Sensory evaluation identified the kheer prepared with 20g of Black Rice (K3) as the optimized product. This optimized Black Rice kheer was deemed acceptable for up to 12 days

when stored at refrigerated temperatures. It contained $58.45\pm0.04\%$ moisture, $5.11\pm0.26\%$ fat, $72.57\pm0.51\%$ total carbohydrate, and 54.46 ± 0.56 mg of gallic acid equivalents/g dry weight in total phenolic content.

2.2 Wheat

Wheat (*Triticum spp.*) is one of the most important cereal crops globally, serving as a staple food for over a third of the world's population. Its versatility, adaptability to diverse climatic conditions, and nutritional value have made it a cornerstone of human diets and agriculture (Shewry & Hey, 2015). Wheat is processed into a variety of products, including bread, pasta, noodles, and breakfast cereals, highlighting its indispensable role in global food security and culinary applications. With increasing global demand, wheat remains a vital agricultural commodity, supporting economies and livelihoods worldwide (Curtis & Halford, 2014).

The grain's structure, comprising the bran, germ, and endosperm, contributes to its nutritional and functional properties. The bran is a rich source of dietary fiber and micronutrients, while the germ contains essential vitamins, minerals, and healthy fats. The endosperm, primarily composed of starch and proteins like gluten, is critical in determining wheat's suitability for baking and food processing (Liu, 2019). These characteristics underscore its importance in both health and industry, driving continuous advancements in wheat breeding and processing technologies.

Modern research has focused on improving wheat varieties to enhance yield, resilience, and nutritional quality. Efforts such as genetic modification, precision agriculture, and sustainable farming practices aim to address challenges like climate change, pest outbreaks, and resource constraints (Shiferaw et al., 2013). By understanding its agronomic, nutritional, and functional aspects, wheat continues to play a pivotal role in addressing global food security and supporting sustainable development.



Figure 2.3: Wheat

2.2.1 Nutritional Composition

Wheat holds significant nutritional value and plays a vital role among the few crop species widely cultivated as staple food sources. Its importance lies in its seeds, which can be processed into flour, semolina, and other essential ingredients used in bread, bakery items, and pasta. These products serve as primary sources of nutrition for a large portion of the global population (Šramková, Gregová, & Šturdík, 2009).

No.	Components	Amount Per 100g
1	Water	11.1 g
	Carbohydrates:	
2	Fiber, total dietary	3 g
3	Protein	12 g
4	Nitrogen	1.92 g
5	Total lipid (fat)	1.7 g
	Energy:	
6	At water General Factors	362 kcal
	At water Specific Factors	370 kcal
7	Ash	0.56 g
8	Minerals:	
	Phosphorus, P	134 mg
	Potassium, K	150 mg
	Magnesium, Mg	36.1 mg
	Calcium, Ca	22 mg
	Manganese, Mn	0.819 mg
	Sodium, Na	2 mg
	Zinc, Zn	1.15 mg
	Iron, Fe	1.18 mg
	Copper, Cu	0.212 mg
9	Vitamins and Other Components:	
	Niacin (Vitamin B3)	1.59 mg
	Thiamine (Vitamin B1)	0.298 mg
	Pyridoxine (Vitamin B6)	0.085 mg

Table 2.5: Proximate composition of all-purpose, unenriched, Wheat flour
(Source: USDA FoodData Central, 2020)

2.2.1.1 Carbohydrates

Wheat carbohydrates, predominantly starch, are integral to human nutrition. Starch comprises approximately 60-70% of the wheat kernel and serves as the primary energy source obtained from wheat. It is stored as granules within the endosperm and categorized into two types: amylose and amylopectin. Amylose, a linear polymer, and amylopectin, a branched polymer,

significantly influence the functional characteristics of wheat-based products like bread and pasta. The digestibility of wheat starch and its glycemic index are pivotal factors determining its nutritional profile and associated health outcomes (Khalid, Hameed, and Tahir, 2023).

Non-starch polysaccharides (NSPs), such as arabinoxylans and β -glucans, represent another vital carbohydrate component in wheat, primarily located in the bran and aleurone layers. These dietary fibers are well-recognized for their health-enhancing properties, including promoting gut health, lowering cholesterol levels, and aiding in the management of blood glucose. However, integrating wheat bran into food formulations presents challenges, notably alterations in texture and sensory qualities. To address these issues, various modification techniques have been developed and employed successfully (Sztupecki et al., 2023).

2.2.1.2 Protein

Protein, derived from the Greek word *proteios* meaning “primary,” is considered essential for both humans and animals. In wheat grains, protein typically constitutes 10%-18% of the total dry matter. Wheat proteins are categorized based on their extractability and solubility in various solvents, a classification system introduced by T.D. Osborne. His method involves sequential extraction of ground wheat grain to separate different protein fractions: albumins (which are soluble in water), globulins (insoluble in pure water, but soluble in dilute NaCl solutions, and insoluble at high NaCl concentrations); gliadins (soluble in 70% ethyl alcohol) and glutenins (soluble in dilute acid or sodium hydroxide solutions).

Wheat gluten, a protein complex predominantly composed of gliadin and glutenin, accounts for approximately 75-80% of the total protein in wheat. Gluten proteins play a pivotal role in defining the processing attributes of wheat flour, imparting the cohesiveness and viscoelasticity essential for shaping dough into bread, noodles, and various other food products. From a nutritional perspective, wheat gluten is an excellent source of protein, offering essential amino acids, although it is notably deficient in lysine (Wieser, Koehler, & Scherf, 2023). The health effects of gluten differ among individuals. In susceptible individuals, these proteins are also the primary agents responsible for triggering celiac disease and other gluten-related intolerances (Caio et al., 2019). Additionally, non-celiac gluten sensitivity and wheat allergies are conditions in which gluten may lead to adverse outcomes, including digestive discomfort or allergic responses (Scibilia et al., 2006).

2.2.1.3 Fat

Although lipids make up only a minor portion of cereals, their role is crucial in determining the quality and texture of foods. Due to their amphipathic nature, lipids can interact with proteins and starch, forming inclusion complexes that influence food structure and properties. The germ contains the highest lipid concentration at about 11%, but significant quantities are also found in the bran as well as in the starch and proteins of the endosperm. Wheat lipids contain essential fatty acids like linoleic acid, which are crucial for maintaining human health. Additionally, these lipids include sterols and tocopherols, known for their antioxidant

properties and their role in promoting cardiovascular health. However, the refining process significantly reduces the lipid content in wheat flour, as the germ and bran are removed during milling. This reduction can diminish the overall nutritional value of the final product (Šramková et al, 2009).

2.2.1.4 Vitamins

Vitamins are essential organic compounds derived from plants and microorganisms, as they cannot be synthesized by the human body. These micronutrients are crucial for several physiological processes: Coenzyme activity or precursor roles, Specialized functions (eg Vitamin A is vital for vision, while ascorbate aids in specific hydroxylation reactions), Antioxidative defense (Vitamins C and E, along with certain carotenoids, help protect against oxidative damage), Genetic regulation and stability etc..

Tocols, encompassing tocopherols (T) and tocotrienols (T3), are vitamin E compounds with antioxidant properties vital for biological membranes. Structurally, they consist of a chromanol ring and a phytol side chain, with tocotrienols differing due to their unsaturated side chain containing three double bonds. The distribution of tocopherols and tocotrienols varies across wheat seed components, as observed by Hidalgoa and Brandolini (2008). The germ has the highest levels of α -tocopherol, β -tocopherol, and total tocols, while α -tocotrienol and β -tocotrienol are predominantly found in the bran, with substantial amounts present in the flour.

Carotenoids, precursors to vitamin A (retinol), are present in wheat primarily in the form of β -carotene and other related compounds. While wheat itself is not a significant source of vitamin A, its carotenoid content contributes to its nutritional profile. In the human body, β -carotene and other carotenoids from wheat are oxidatively cleaved in the intestinal mucosal brush border or the liver to produce retinol, the active form of vitamin A. Carotenoids in wheat offer an advantage over direct retinol sources, as they can be converted to vitamin A based on metabolic needs, avoiding the risk of vitamin A toxicity associated with excessive retinol consumption. This makes wheat-derived carotenoids a safe and beneficial addition to human nutrition, particularly in regions where vitamin A deficiencies are prevalent

2.2.1.5 Minerals

The presence of various minerals in the wheat, especially the bran, helps to tackle hidden hunger (micronutrient deficiency) in the world. Wheat is rich in essential minerals like potassium (K), phosphorus (P), magnesium (Mg), and calcium (Ca), alongside with micronutrients such as iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu). These valuable minerals are primarily located in the bran and germ layers of the wheat kernel. As a result, whole wheat products offer higher nutritional density compared to refined wheat flour, which loses much of these mineral-rich layers during processing (Yildiz, 2022). Factors like processing methods such as milling and baking can alter the distribution of minerals, often reducing their content in the final product. Genetic makeup, environmental conditions, and

agronomic practices also adds a role in determining the mineral profile of wheat grains (Wysocka, Cacak-Pietrzak, & Sosulski, 2025).

2.2.2 Types of Wheat Flours and its uses

Kumar et al., (2011) in the review titled Nutritional Contents and Medicinal Properties of Wheat has discussed various types of flour and its uses.

2.2.2.1 All-Purpose Flour

All-purpose flour is basically finely milled wheat endosperm which lacks bran and germ. It can be made by hard wheat or a combination of soft and hard wheat and is used to make a complete range of satisfactory baked products such as yeast breads, cakes, cookies, pastries and noodles. Enriched All-Purpose Flour contains iron and B-vitamins added in equal amounts or exceeding that of whole-wheat flour. Bleached Enriched All-Purpose Flour is chlorine treated to mature the flour, condition the gluten present and improve the baking quality. The chlorine evaporates, leaving the nutrients undestroyed while reducing the risk of spoilage or contamination. Unbleached Enriched All-Purpose Flour is oxygen bleached in the air during an aging process and is off-white colour. Both bleached and unbleached flour are the same nutritionally.

2.2.2.2 Bread Flour

Bread flour is produced from the endosperm of the wheat kernel and is primarily milled for commercial bakers. It is known for its higher gluten strength compared to all-purpose flour and is specifically formulated for yeast-based bread recipes. Its enhanced protein content allows for better structure and elasticity during fermentation, resulting in well-risen, airy loaves with a chewy texture. Beyond its use in yeast breads, bread flour is also favoured for baking artisan-style breads, rolls, pizza doughs, and other baked goods where strong gluten formation is essential.

2.2.2.3 Self-Rising Flour

All-purpose flour with salt and leavening added makes Self-rising flour. Specifically, one cup of self-rising flour includes 1½ teaspoons of baking powder and ½ teaspoon of salt. Self-rising flour act as a substitute for all-purpose flour in a recipe by reducing salt and baking powder according to those proportions.

2.2.2.4 Whole Wheat Flour

It is a course ground flour from the entire wheat kernel and thereby contains the bran, germ and endosperm. The gluten development is reduced due bran presence. Baked products tend to be heavier and denser when made using whole-wheat flour than those made from white flour.

2.2.2.5 Other Flours

(i) Cake flour is milled from soft wheat and is suitable for cakes, cookies, crackers and pastries as it is low in protein and gluten. (ii) Pastry flour is also milled from soft, low gluten wheat and has lower in starch than cake flour. (iii) Gluten flour is used by bakers in combination with

flours having a low protein content as it helps to improve the baking quality and produce gluten bread of high protein content. (iv) Semolina is coarsely ground endosperm of high protein content durum wheat and is used in high quality pasta products. (v) Durum flour is a by-product of semolina production. (vi) Farina is the coarsely ground endosperm of hard wheat and is the prime ingredient in many U.S. Breakfast cereals.

2.3 Rice Analogue

Analog rice can be manufactured by granulation and extrusion. It is prepared by the use of carbohydrate-based materials and its shape is made similar to naturally produced rice (Sumardiono et al., 2018). Usually, analog rice has low glycemic index (GI) and can act as a good alternative for the ones that suffer from obesity and diabetes. Analog rice may contain functional ingredients which can provide several health benefits when consumed given that the bioactive compounds existed in raw material used. Some of health benefits are included to modulation of diabetes, lowering the blood pressure and cholesterol, antioxidant activity, and anti-cancer feature (Purwaningsih et al., 2020 and Mulyono et al., 2013).

2.3.1 Ingredients Used in Production of Analog Rice

Based on the selection of primary ingredients to produce the rice analogue, the chemical and physical characteristics of the final product will be affected. The primary mixture consisting of flour or broken rice and water is necessary for production of rice, but other additives can be used occasionally (Valencia & Purwanto, 2020; Ridwansyah et al. 2020 and Putri & Sumardiono, 2020).

2.3.1.1 Starch Source

It is one of the primary ingredients in rice analogue production. Starchy materials like corn, potato, sorghum, cassava including broken rice particles can be used (Nateghi et al., 2021). Rice grains are much more preferred as it can achieve the appropriate amylose-amylopectin ratio (Zhiyuan & Yanyan, 2011 and Valencia & Purwanto, 2020). In extrusion processes, starches with high amylopectin content tend to melt rather than gelatinize. Conversely, starches that are rich in amylose gelatinize effectively at high temperatures because water molecules become trapped between the polymer chains (Zhiyuan & Yanyan, 2011). Currently, sorghum Flour, soybean Flour, modified cassava Flour as well as corn flour are used to prepare rice analogues.

2.3.1.2 Water

A limited quantity of water leads to elevated shear stress within the extrusion chamber and a significant level of gelatinization. For the production of analogue rice, the dough should consist of 12-25% water by weight. If the water content surpasses 25%, the mixture adheres to the extruder during the cooking process; conversely, if the water content falls below 12%, additional mechanical energy is necessary for extrusion, resulting in complete gelatinization of

the mixture. Consequently, an optimal water content of 20% is essential for dough preparation (Mishra et al., 2012).

2.3.1.3 Additives

The incorporation of a binder into flour results in the formation of a pliable dough that maintains its integrity despite fluctuations in moisture and temperature. Sodium alginate serves as one example of a binder utilized in this process. Other binders include chitin, pectin, casein, gelatine, and methylcellulose. Various gums, such as xanthan, guar gum, and carrageenan, are also being used as binders (Yogeshwari et al., 2019). Additives like calcium lactate and calcium chloride are incorporated at concentrations ranging from 0.01% to 20% w/w of the binder to ensure the stability of the mixture and to improve the efficacy of certain binders (Budijanto, 2013). Micronutrients like vitamins are introduced into the final mixture at levels between 0.1% and 5% w/w. Commonly added vitamins are A, B1, B2, B5, B12, C, E, K, folic acid, niacin, and biotin, along with minerals such as iron, zinc, calcium, and selenium. Oil-soluble vitamins are incorporated into the formulation using an oily matrix (Noviasari, Widara, & Budijanto, 2017).

2.3.2 Recent Studies

The study conducted by Dhanang Puspita, Sarlina Palimbong, and Erika Immanuela (2023) explored the formulation of analogue rice from Gadung tuber (*Dioscorea hispida*) with the addition of seaweed extract as a potential dietary option for individuals with diabetes mellitus. The research aimed to create analogue rice from Gadung tuber to develop a functional food suitable for diabetic patients. The results showed that the analogue rice contained 19.85% amylose, 8.75% fibre, 27.08% fat, and 44.50% protein. Sensory evaluation, performed using the hedonic test method, indicated no significant differences in taste, texture, aroma, and colour compared to IR 64 rice. The study concludes that developing analogue rice from Gadung tuber, supplemented with seaweed extract, presents a promising functional food, contributing to food diversification and providing a nutritious option for individuals with diabetes mellitus due to its low glycaemic index and high fibre content.

Damat et al., 2021 created analogue rice from arrowroot starch, enriched with red seaweed and spices, to address the bland taste and low functional value often associated with such products. The inclusion of seaweed significantly boosted the fiber and resistant starch content, categorizing it as a high-fiber food, while spices like onion, garlic, ginger, turmeric, and lemongrass enhanced antioxidant activity and sensory properties, including flavour and aroma. All formulations met the Indonesian National Standard No. 6128-2008 for paddy rice in terms of water content and carbohydrate levels. Additionally, the final product displayed vibrant colours, improved taste, and high consumer acceptance. This advancement underscores the potential of combining seaweed and spices with arrowroot starch to produce functional, appealing rice analogues tailored to the needs of rice-consuming populations.

Study by Nugraheni, Purwanti, & Ekawatiningsih, (2022), focuses on the development of analogue rice made from composite tubers, germinated legumes, and cereal flours, with three

different formulations. The ingredients included native and modified tuber flours (e.g., cassava, sweet potato), germinated legume flours (e.g., soybean, cowpea, mung bean), germinated cereal flours (e.g., sorghum, corn), and sago starch. All formulations—Analogue Rice I, II, and III—were high in dietary fiber, resistant starch, and protein, while being low in fat and carbohydrates. They were classified as low glycaemic index foods and demonstrated antidiabetic properties by lowering glucose levels and improving food efficiency ratio in diabetic mice. Additionally, these formulations reduced triglycerides, total cholesterol, LDL, and atherogenic index, while increasing HDL levels. The study concludes that these analogue rice formulations could serve as functional foods for individuals managing glucose and lipid profiles.

Another notable study is the creation of rice-shaped kernels made from broken rice flour (60-90%) combined with cowpea flour (10-40%), along with salt and water (Yogeshwari et al., 2019). The extrusion process, conducted under controlled conditions (20 kg/h feed rate, 40% feed moisture, and a barrel temperature of 60°C), produced kernels that were then dried for 4 hours. The resulting analogues exhibited favorable cooking properties, such as a 5-minute cooking time, high rehydration capacity, and minimal cooking loss, while also showcasing enhanced nutritional profiles. Protein, fiber, iron, and calcium contents were significantly higher compared to natural rice, making these analogues a valuable source of essential nutrients. Sensory evaluations further highlighted the acceptability of these products, particularly the formulation with 30% cowpea flour, which received the highest score.

2.3.3 Benefits

2.3.3.1 Nutritional Enhancement

Traditional rice may lack certain vitamins, minerals and other nutrients. It can be compensated by fortifying the rice analogue, which elevates the overall nutrient content. This makes rice analogue a viable option in regions where rice is the major staple food (Yogeshwari et al., 2018).

2.3.3.2 Dietary Diversification and customization

Rice analogues can be tailored to meet the specific dietary needs and preferences of different populations and can also promote dietary diversification by using alternative ingredients like legumes, grains or tubers. This flexibility allows for the development of specialized products that cater to various health conditions and cultural preferences while providing a wider variety of nutrients as required. For example, those with diabetes or gluten intolerance (Sumardiono et al., 2021).

2.3.3.3 Functional Foods

Rice analogues can be formulated to have specific health benefits, such as a low glycemic index for managing blood sugar levels, high fiber content for digestive health, or enhanced protein content for muscle maintenance.

2.3.3.4 Food Security

Developing rice analogues using locally available ingredients can reduce dependency on traditional rice cultivation, which can be affected by climate change, pests, and other factors. This can contribute to greater food security and sustainability.

2.3.3.5 Economic Opportunities

The production of rice analogues can create new economic opportunities for farmers and food manufacturers, particularly in regions where traditional rice farming is not viable. It can also stimulate innovation in the food industry.

2.4 Extrusion Technology

Extrusion technology has been applied in cereal grain processing. The grains are extruded into various products. The flour form of the food ingredients is mostly used to produce extruded products. Extrusion is a highly adaptable cooking process that integrates several unit operations into a single system. In the extrusion process, the food material is subjected to compaction, shearing, particle size reduction, phase transition, and molecular breakdown (Della Valle et al., 1995; Lai and Kokini, 1991). All of this happens in a fairly short time duration, typically, in less than a minute of residence time in the extruder. Within the extruder, materials face high temperatures and pressures generated by mechanical shear and die restriction. Upon exiting the die, they encounter atmospheric conditions, leading to further changes. As water vaporizes, it helps expand the material. Given these complex phenomena, extrusion is characterized as a "high shear, high temperature, high pressure, and short time cooking system."

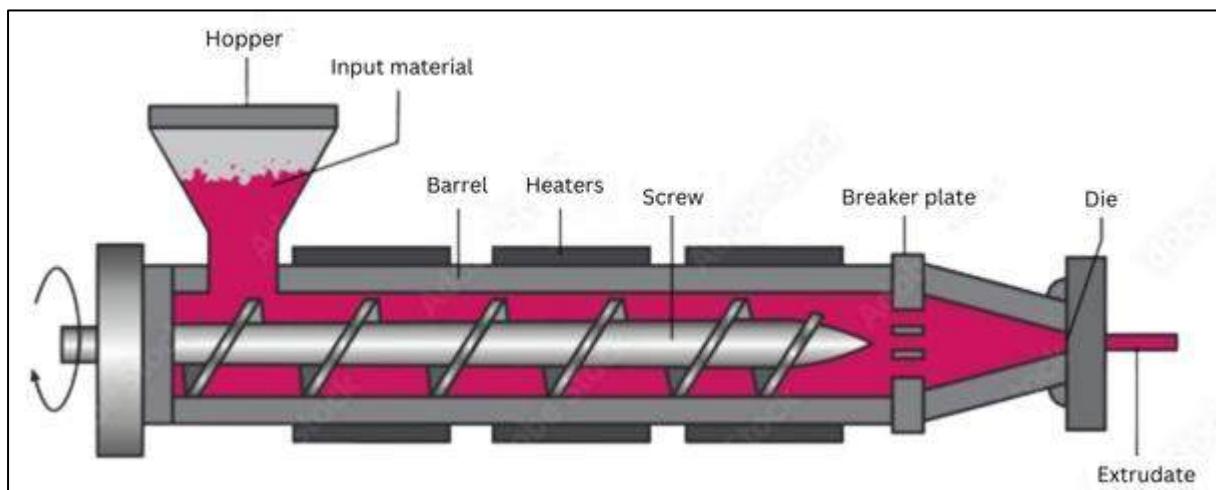


Figure 2.4: Illustration of an extruder

2.4.1 Types of Extruders

Extruders are classified into two main types: single-screw and twin-screw (co-rotating and counter-rotating). They vary in screw diameter, length, and design.

Single-screw extruders are said to be Cost-effective and widely used for low shear applications. They are common in pet food, snacks, and general extrusion cooking. It is enhanced by variable screw speed drive, instrumentation, and control. It's limited in handling difficult formulations and ensuring consistent product quality.

Twin-screw extruders are more expensive but offer better economic performance and can handle both common and challenging formulations well. The Co-rotating twin-screw extruders excel in difficult applications where single-screw extruders fall short. The Counter-rotating twin-screw extruders are rare in the food industry, used mainly for low melt viscosity applications requiring high pressure, like candy manufacturing, and typically operate at lower speeds to reduce wear. Their output is lower than co-rotating twin-screw extruders (Ganjal 2020).

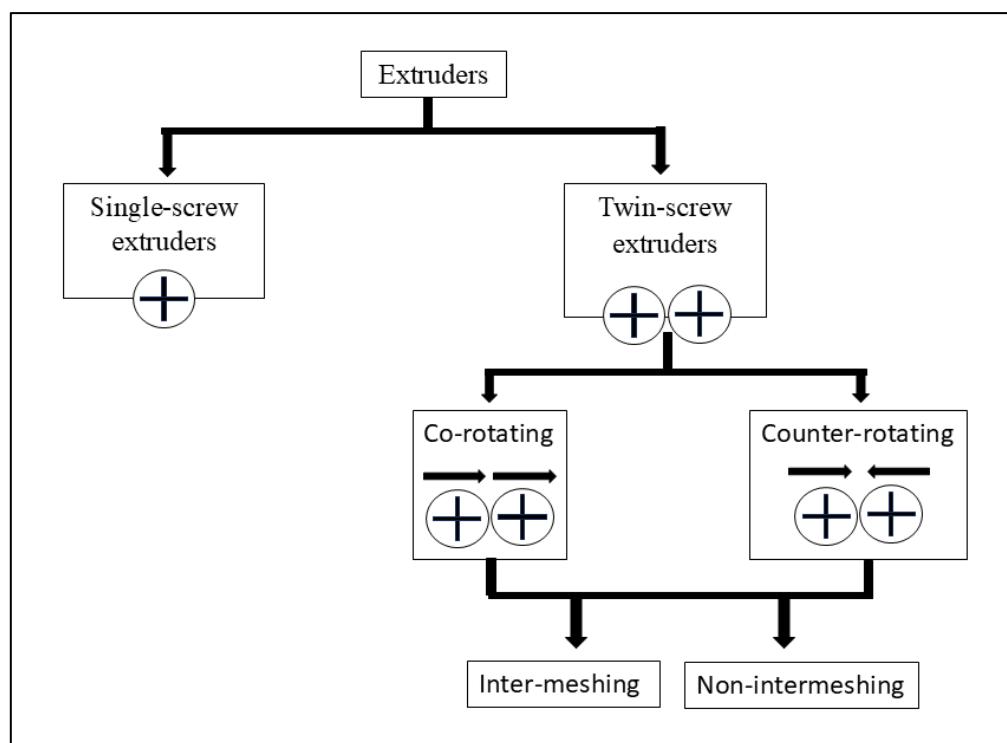


Figure 2.5: Classification of extruders

2.4.2 Role in Rice Analogue Production

Analog rice production involves two main methods: granulation and extrusion. Granulation yields round, pearl-shaped rice, while extrusion produces rice that is oval and resembles common rice. Extrusion is the more commonly used method in analogue rice processing (Maskan & Altan, 2012; Budi et al., 2016 & Sumardiono et al., 2018).

Research by Mishra et al. (2012) highlights that extrusion allows for the incorporation of broken rice kernels, which are typically considered waste, into reconstituted rice analogues fortified with essential nutrients such as proteins, vitamins, and minerals. This process not only addresses food waste but also provides a means to combat nutritional deficiencies in populations reliant on rice as a staple. Additionally, extrusion facilitates the modification of physical properties like texture, shape, and cooking characteristics, making rice analogues more appealing to consumers.

The impact of extrusion parameters on the quality of rice analogues has been extensively studied. According to various researches, factors such as screw speed, feed rate, and die temperature significantly influence the texture, bulk density, and water absorption capacity of the final product. For instance, optimizing these parameters can enhance the sensory attributes and cooking performance of rice analogues, ensuring they meet consumer expectations. Studies have also shown that extrusion-induced starch gelatinization and protein denaturation improve the digestibility and functional properties of rice analogues. These findings underscore the importance of precise control over extrusion conditions to achieve high-quality products (Kishore and Dwivedi, 2025).

Recent advancements in extrusion technology have expanded its applications in rice analogue production. Kusumayanti et al. (2023) explored the use of alternative raw materials, such as cassava flour and fermented soybean cake flour, in the extrusion process to produce rice analogues with improved nutritional and physical properties. Their study demonstrated that extrusion could yield rice analogues with high carbohydrate and protein content, along with desirable cooking characteristics. This versatility makes extrusion a promising approach for developing sustainable and nutritious rice analogues, catering to the growing demand for alternative food sources.

CHAPTER – 3

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Materials

The raw materials used were Black Rice, wheat flour and water. The raw, dehusked Black Rice was sourced from Manipur while the wheat flour was bought in bulk quantity from the stores. The water used was potable water.

The equipment used for the experiments were: Aluminium 36 R Cylindrical Probe, Balance, Boiling Flask, Buchner Flask, Centrifuge, Cuvette, Desiccator, Digital Vernier Calliper, Dough Mixer, Dryer, Extruder, Falling Number Apparatus, Filtered Glass Crucible, Graduated Measuring Cylinders, Grinder, Hot Air Oven (105°C and 130°C), HunterLab Color Measuring System, Kjeldahl Apparatus, Kjeldahl Flask, Laboratory Mill, Mettler Balance, Muffle Furnace, Petri Plate, Rice-shaped Die, Sedimentation Shaker, Soxhlet Apparatus, Steamer, Analyzer (TAHDi), UV-Vis Spectrophotometer, Vacuum Pump, Viscometer Stirrer, Vortex Mixer, Water Bath, Weighing Machine, Whatman Filter (1 and 40) etc..

Chemicals used for the experiments were: Acetone, Aluminium Chloride, Amyl Glucosidase, Ascorbic Acid, Boric Acid, Bromophenol Blue, Copper Sulphate, Ethanol, Folin-Ciocalteu's Reagent (10%), H₂SO₄, HCl, Isopropyl Alcohol (99-100%), KCl Buffer, Lactic Acid Reagent, MES-TRIS Buffer, Methanol, Mixed Indicator, NaOH, Petroleum Benzene, Potassium Sulphate, Protease, Quercetin, Sodium Acetate Buffer, Sodium Carbonate (7.5%), Sodium Hydroxide, Sodium Nitrite, α -Amylase etc..

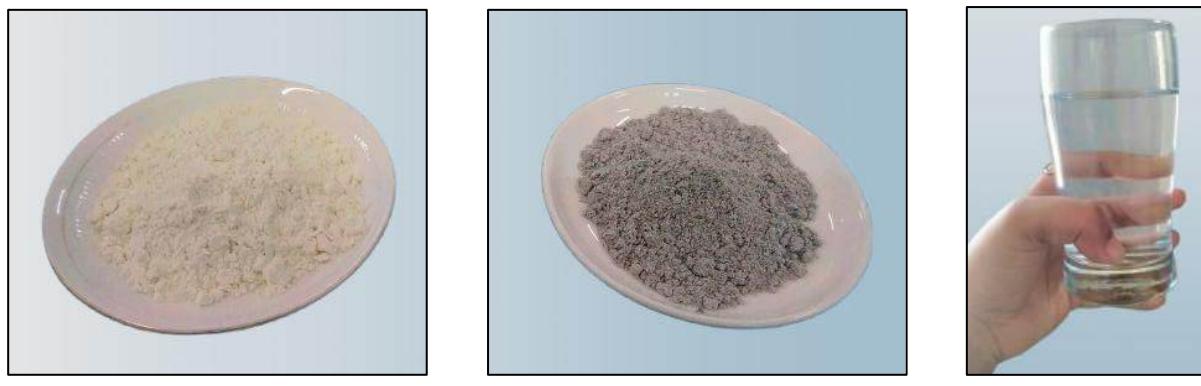
3.2 Method of Preparation

3.2.1 Ingredients

The basic ingredients used for the Black Rice-based rice analogue are wheat flour and Black Rice flour in various ratios along with water.

The raw, dehusked Black Rice was manually cleaned and winnowed to remove any unnecessary substances present in the bulk quantity of Black Rice, it was then milled in the small-scale laboratory mill with the capacity of 200-300 gm. The ground Black Rice was then transferred to plastic kits and kept in the desiccator to cool it off for 10-15 minutes and was immediately stored in the refrigerator to prevent the deterioration of the flour quality. The flour was kept in the desiccator for thawing prior to the product preparation in small quantities.

Since ground wheat flour was used, the flour was usually kept in the refrigerator and was kept in the desiccator for thawing prior to product preparation. Potable drinking water from water cans and water purifiers were in the product preparation.



(a) Wheat Flour (WF)

(b) Black Rice Flour (BRF)

(c) Water

Figure 3.1: Ingredients used in rice analogue preparation

3.2.2 General Steps

Wheat flour, Black Rice flour, and water were blended in a dough mixer to create a well-mixed, loosely held dough. The dough was then rested for 20 minutes before being extruded into rice shapes using a single-screw extruder fitted with a rice-shaped die. Extrusion was performed quickly to preserve the moisture content of the product, while ensuring the rice analogue maintained a consistent shape similar to real rice grains. The extruded rice was evenly spread on trays in thin layers and steamed at 95°C with 95% relative humidity for 10 minutes using a cabinet steamer, which had been pre-steamed for 10 minutes beforehand. After steaming, the trays were moved to a cabinet dryer and dried at 50°C for 3-4 hours until the product became brittle. Once dried, the product was cooled to room temperature for 5 minutes before being packaged in plastic covers and stored in a refrigerator for future use.

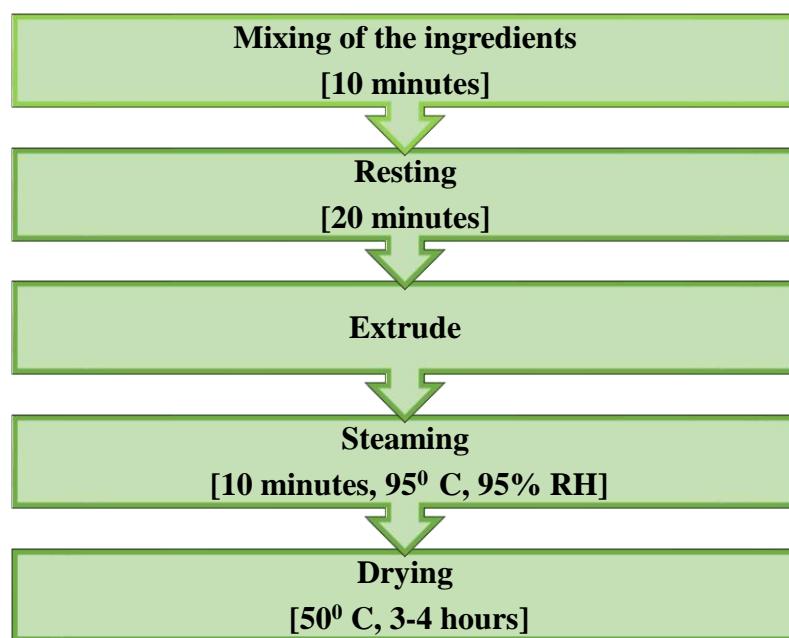
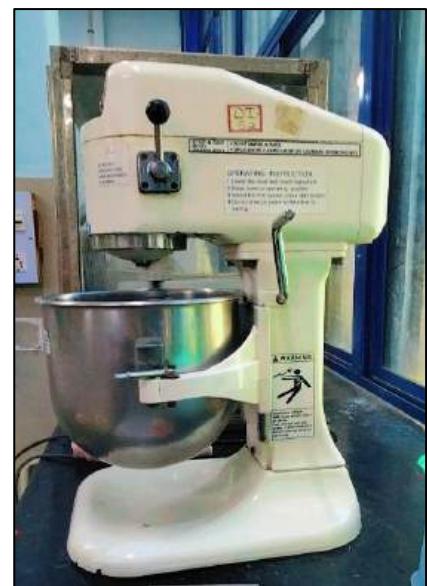


Figure 3.2: General flow chart for product preparation



(a) Laboratory Mill



(b) Mixer



(c) Steamer



(d) Dryer



(e) Extruder



(d) Die

Figure 3.3: Equipment used for rice analogue preparation

3.2.3 Product Formulation and Sample Preparation

The base rice analogue formulation (100% wheat flour) comprised of wheat flour and water. Wheat flour was then substituted with Black Rice flour with varying proportion of: 20%, 30%, 40% and 50%. The amount of water was kept a constant (30%) through all the formulations. The different formulations for ingredients are as shown in the *Figure 3.4*.

The ingredients were individually weighed as per the different formulations and was mixed and the mixture formed was kept for resting. It was later extruded and was immediately kept for steaming followed by drying. The dried product was transferred to a plastic cover and stored in the refrigerator.

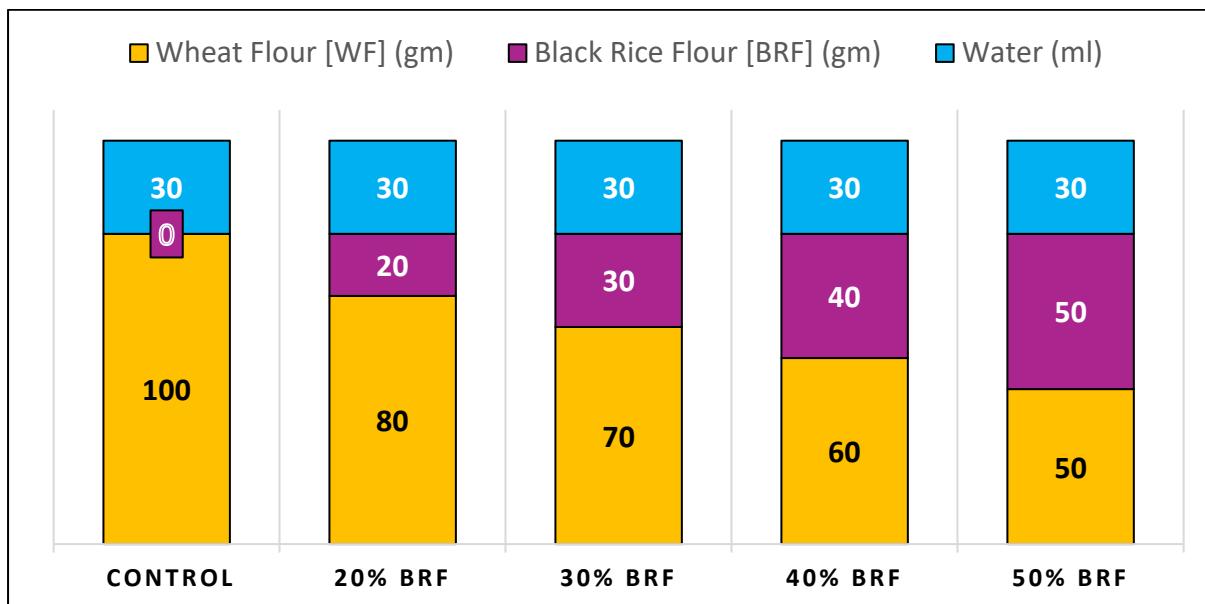


Figure 3.4: Sample formulations

3.3 Characterization of Wheat Flour

3.3.1 Falling Number

Principle: The time in seconds required to stir and allow a viscometer stirrer to fall a fixed distance through a hot aqueous flour suspension being liquefied by the enzyme in a standardized apparatus.

Apparatus: Grinder, balance and falling number apparatus (water bath, test tube, stopper and stirrer)

Procedure: Grind 100g wheat in the lab grinder. Put on the healer of falling number apparatus and allow the water to come to boiling. Weight 7 grams of sample and place in falling number tube. Insert rubber stopper and shake tube in upright position 10 times and make sure all flour is suspended in water. Scrape down upper part of tube with viscometer stirrer. Place the falling number water bath and start the timer. The flour suspension is automatically stirred by the

stirrer for 60 seconds. The apparatus gives a liquefied gel. Calculate the falling number on 14% moisture basis.

$$\text{Falling number} = \frac{\text{Falling number uncorrected} * [100 - 14]}{100 - \text{moisture in the flour}}$$



Figure 3.5: Falling number system

3.3.2 Determination of Zeleny Sedimentation

Principle: The volume of sediment, formed when flour is suspended in water and treated with Lactic acid, consisting of swollen gluten and occluded starch is the sedimentation value.

Apparatus: Grinder, sedimentation shaker.

Reagents: Isopropyl alcohol (99-100%), bromophenol blue, lactic acid reagents

Procedure: 3-2 grams of flour is placed in 100ml stoppered measuring cylinder containing 50ml of water containing bromophenol blue. Mixture is thoroughly mixed by shaking the cylinder horizontally 12 times. Cylinder is placed on the shaker and shaken for 5 minutes. At the end of 5 minutes, 25ml of isopropyl alcohol is added and then again kept on shaker for 5 minutes, after which the cylinder is kept in upright position and at the end of exactly 5 minutes, volume of sediment in ml is read. This gives directly sedimentation value in ml, which is then expressed on 14% moisture basis.

$$\text{Sedimentation value} = \frac{\text{Sedimentation uncorrected} * [100 - 14]}{100 - \text{moisture in the flour}}$$



Figure 3.6: Sedimentation shaker

3.3.3. Estimation of Gluten

Principle: Gluten in a sample of flour could be estimated by washing the dough free of starchy sugars, water soluble proteins and other minor components. The wet cohesive mass obtained is referred to as wet gluten while the dried product obtained from it is referred to as dry gluten.

Procedure: Exactly 25g flour is kneaded with about 15 ml water to get a dough ball. The dough ball is allowed to remain in immersed in water for one hour to ensure proper hydration after which, the starch is washed out by kneading gently in a gentle stream of water over a fine sieve or silk till the washed liquid is clear. The gluten which is cohesive is pressed as dry as possible; and weighed. The wet gluten so obtained is dried at 105°C for 24 hr. And weighed again to get the value for dry gluten.

$$\text{Wet gluten (\%)} = \frac{A \times 100}{C}$$

$$\text{Dry gluten (\%)} = \frac{B \times 100}{C}$$

Where, A = Weight of wet gluten

B = Weight of dry gluten

C = Weight of flour



(a) Wet



(b) Dry

Figure 3.7: Gluten

3.4 Proximate Analysis

3.4.1 Analysis of Moisture Content

Principle: The loss in weight after the drying the sample for 1 hour at 130°C expressed in percentage is the moisture content.

Apparatus: Hot air oven, lab grinder, aluminium dishes, Mettler balance and desiccator.

Procedure: The moisture content was determined using a hot air oven. Approximately 5-10g sample was weighed, ground and transferred into a clean glass petri-dish which was previously weighed. The weight of the sample with Petri-dish was recorded and it was then heated in a hot air oven at 105°C for 3 hours. Later, the dish was weighed after cooling in a desiccator. The process was repeated to obtain a constant weight.

Moisture content was estimated using the formula,

$$\text{Moisture \%} = \frac{W_2 - W_3}{W_2 - W_1} * 100$$

Where, W_1 = Weight of empty petri dish

W_2 = Weight of dish + sample

W_3 = Weight of dish + sample after drying



Figure 3.8: Hot air oven

3.4.2 Analysis of Ash

Principle: Total ash is the inorganic residue remaining on incineration in the open under atmospheric pressure.

Apparatus: Muffle furnace, crucible, tongs, Mettler balance and desiccator.

Procedure: Take fresh sample for the determination, rather than left over after determination of moisture. Ignite the dried material in the dish left after the determination of moisture with the flame of a burner till charred. Transfer to a muffle furnace maintained at $550\text{-}600^{\circ}\text{C}$ and

continue ignition till grey ash is obtained. Cool in a desiccator and weigh. Repeat the process of heating, cooling and weighing at half hour intervals till the difference in weight in two consecutive weighing is less than 1 mg. Note the lowest weight. If ash still contains black particles add 2-3 drops of pre- heated water at 60°C. Break the ash and evaporate to dryness at 100-110°C. Re-Ash at 550°C until ash is white or slightly grey.

The percentage ash was calculated as follows,

$$\% \text{ of ash} = \frac{(B - C) * 100}{A}$$

Where, A = sample weight prior to drying

B = weight of dish and contents after ashing

C = weight of empty dish



Figure 3.9: Muffle furnace

3.4.3 Analysis of Fat

Principle: For semi continuous solvent extraction, the solvent builds up in the extraction chamber for 5-10mins and surrounds the sample, and then siphons back to the boiling flask. Fat content is measured by the weight loss of the sample or by the weight of fat removed.

Procedure: Accurately weigh 5g of the substance in a suitable thimble, then let it dry for two hours at 100°C. The thimble should be placed in the Soxhlet extraction device and extracted with Petroleum Benzene at 40–60 °C for eight hours. Dry the extract in the Soxhlet flask, whose empty mass was earlier measured by tarring at 95 to 100 °C for an hour. Desiccate and weigh after cooling. Continue drying, cooling, and weighing at intervals of 30 minutes until there is a mass change of no more than 2 milligrams between two subsequent weighing. Record the lowest mass obtained.

The percentage fat was calculated as follows,

$$\text{Fat \% by mass} = 100 * (M1 - M2) / M$$

Where, M₁ = mass in g of Soxhlet flask with the extracted fat

M₂ = mass in g of empty Soxhlet flask

M = mass in g of the material taken for test



Figure 3.10: Soxhlet apparatus

3.4.4 Analysis of Protein

Principle: The protein content is determined from the organic Nitrogen content by Kjeldahl method. The various nitrogenous compounds are converted into ammonium sulphate by boiling with concentrated sulphuric acid. The ammonium sulphate formed is decomposed with an alkali (NaOH) and the ammonia liberated is absorbed in excess of standard solution of acid and then back titrated with standard alkali.

Reagents:

Digestion mixture: Powdered potassium sulphate and copper sulphate were mixed thoroughly in the ratio 9:1.

Concentrated sulphuric acid 10ml for digestion.

Sodium hydroxide solution 20%: 200 gm of sodium hydroxide pellet was dissolved in 1000 ml of distilled water for digestion.

Sodium hydroxide solution 40%: 40 gm of sodium hydroxide pellet was dissolved in 100 ml of distilled water for distillation.

Boric Acid 4%: 4 gm of boric acid was dissolved in 100 ml of boiling distilled water. After cooling transfer the solution into a glass stoppered bottle.

Mixed indicator: Dissolve 0.1% bromocresol green with 2 ml of methyl red solution in a bottle provided with a dropper which will deliver about 0.05 ml / 4 drops

0.1N HCl: approximately 1.43 ml of anhydrous HCl was dissolved in 1L of distilled water.

Apparatus: Micro kjeldahl apparatus

Procedure: Weigh about 0.5 gm of the sample and transfer it to a 500 or 800 mL Kjeldahl flask, taking care to see that no portion of the sample clings to the neck of the flask. Add 3gm of digestion mixture and 10 mL of concentrated sulphuric acid. Place the flask on the stand in the digestion chamber and digest. The digested sample was made up to 100ml using distilled water, turning it pale blue. For distillation of the digested sample, a manually operated kjeldahl apparatus was used. 5ml of digested sample along with 20ml NaOH (40%) was poured together at one end and steam was applied to boil the mixture. The evaporated component was collected at another end in a conical flask containing 20ml boric acid (4%) and 3 drops of mixed indicator with the help of the condenser. The distillate was then titrated against 0.1N HCl till the end point of green to pale pink. A reagent blank was also run to subtract reagent nitrogen from the sample nitrogen.

Calculation,

$$\text{Moles of HCl} = \text{moles of NH}_3 = \text{moles N in the sample}$$

$$\text{Nitrogen (N) \%} = \frac{14.01 \times 0.1\text{N} \times (\text{TV} - \text{BV})}{\text{W} \times 1000} \times 100$$

$$\text{Protein (P) \%} = \text{N\%} \times 6.25$$

Where, BV = blank value

TV = titre value

W = weight of the sample

$$\text{Protein on dry wt. basis} = [\text{Protein content} / (100 - \text{Moisture content})] * 100$$

Ideally the protein content of food stuff is calculated by multiplying its total nitrogen content by a factor 6.25. This factor is used whenever the nature of the protein is unknown or when the product to be analysed is a mixture of different proteins with different factors. However, use of different Nitrogen conversion factors for different matrices may lead to better accuracy of results.



(a) Digestion unit



(b) Distillation unit

Figure 3.11: Kjeldahl apparatus



(a) Distillate for titration



(b) Titrated distillate

Figure 3.12: Protein content determination (end point)

3.4.5 Analysis of Carbohydrates

The carbohydrate content was determined by difference, that is, addition of all percentages, moisture, fat, protein, Ash was subtracted from 100. This gave the amount of nitrogen – tree extract otherwise known as carbohydrate.

Total carbohydrates are calculated as follows after determining the percentage of moisture, total protein, fat and total ash:

$$\text{Total carbohydrates} = 100 - (A+B+C+D)$$

Where, A = percent by mass of moisture

B = percent by mass of total protein

C = percent by mass of fat and

D = percent by mass of total ash

3.4.6 Analysis of Dietary Fibre

Principle: The petroleum benzene ether-defatted samples are treated with enzymes that are similar to the digestive process in the human digestive system. The digestible carbohydrates are broken down into simpler sugars and then removed from the sample by precipitation and filtration. The non digestible precipitates contain dietary fiber as well as protein and inorganic material.

Method: The sample was defatted with hexane prior to the fiber analysis. In a conical flask, 0.5gm of the defatted sample is suspended in 40ml of MES-TRIS buffer treated with 50 μ L of α -amylase (heat stable) and then kept in a boiling water bath for 15 minutes at 95°C with continuous agitation and then cooled down to 60°C. 10ml of distilled water is added to the conical flask. After that, 100 μ L of Protease was added and incubated for 30 minutes at 60°C in

the water bath with agitation. After this, the flask was removed from the water bath, and 5ml of 0.561M HCl solution was dispensed, and the pH was adjusted to 4.0-4.7 with 1M NaOH, or 1M HCl. About 300 μ L Amyl Glucosidase was added, and the samples were incubated for another 30 minutes at 60°C. Then, the solution is filtered through a washed and dried, weighed, and marked glass crucible with 0.5g Celite bed in a Buchner flask.

3.4.6.1 Residue (Insoluble Fiber)

The prepared digested solution is filtered, and the crucible residue is the insoluble fiber. It is then washed with 15ml of 78% ethanol, 15ml of 95% ethanol, and 15ml of acetone, then dried in a hot air oven at 105°C for 4 hrs. After cooling to room temperature in a desiccator, the crucible is weighed and placed in the muffle furnace for incineration at 550°C for 5 hours, cooled down in a desiccator, and weighed again.

3.4.6.2 Filtrate (Soluble Fiber)

The filtrate collected from the insoluble fiber was transferred to a beaker, and four times the quantity of preheated 60°C ethanol was added and kept overnight for precipitation. It was then filtered in a dried and weighed crucible with celite bed. It is washed with 15ml of 78% ethanol, 95% ethanol, and 15ml of acetone and then dried in the hot air oven for 4 hrs at 105°C weighed at room temperature, and then kept for incineration in the muffle furnace at 550°C for 5 hours and cooled in a desiccator and weighed.

The dietary fibre can be calculated as follows,

$$\% \text{ of dietary fiber} = \frac{(W_1 - W_2) - (W_3 - W_2) \times 100}{W}$$

Where, W = weight of sample,

W1 = weight after oven drying,

W2 = weight of empty crucible,

W3 = weight after muffle furnace drying



(a) Vacuum pump



(b) Filtered glass crucible

Figure 3.13: Instruments used in dietary fibre analysis

3.4.7 Analysis of Energy

Use the following conversion factors for calculating the energy values corresponding to the carbohydrate, protein, and fat contents.

Carbohydrates - 4kcal/g

Protein - 4kcal/g

Fat - 9kcal/g

Calculation of the energy value (total calories) per 100g of the food as follows:

$$\text{Energy (kcal/100g)} = (\text{Fat} * 9) + (\text{Carbohydrates} * 4) + (\text{Protein} * 4)$$

Where, Carbohydrate, Protein and Fat content in g/100g

3.5 Cooking Quality Characteristics

3.5.1 Optimal Cooking Time (OCT in min)

The cooking time for extruded product is analysed by the standard method AACC Method 66-50. Sample (5 g) were immersed in boiling water (60 ml), one piece of sample was taken out every 25s and squeezed to visually observe the time of disappearance of the white core (ungelatinized starch). The OCT was achieved when the sample was fully hydrated.

3.5.2 Cooking Loss (CL)

10g sample were simmered (2-5 min based on OCT) in a beaker with 100 ml of boiled water. The cooked sample were collected in a strainer and allowed to cool for 5 min, whereas the cooking water was collected in a glass beaker and evaporated in a hot air oven at 105°C until constant weight.

For the cooking loss, the final weight (W3) was recorded as the weight of the beaker after drying.

The water absorption (WA) and CL were calculated in triplicate using the following equations.

$$\text{Water Absorption (g/g)} = (W2 - W1) / W1$$

Where, W2 = Weight of cooked rice analogue

W1 = Weight of raw rice analogue

$$\text{Cooking Loss (\%)} = (W3 - W4) / W1 * 100$$

Where, W4 = Weight of Petri plate

W3 = Weight of Petri plate after drying

W1 = Weight of raw rice analogue

$$\text{Cooking yield} = W2 / W1 \text{ (g/g)} \text{ OR } W2 / W1 * 100 \text{ (%)}$$

Where, W2 = Weight of cooked rice analogue

W1 = Weight of raw rice analogue

3.6 Physical Characteristics

3.6.1 Instrumental Colour Analysis

The value of the surface color of Black Rice-based rice analogue was measured using the HunterLab color measuring system (color measuring Labscan XE system, USA). The L*, a*, b*, and dE color scales were used for the measurement. The L* values indicated the level of lightness or darkness, where a lower number indicates darker, and a higher number indicates the lighter color of the sample. The a* value indicates redness or greenness, where the positive value indicates red, and the negative value indicates green. The b* value indicates yellowness or blueness, with positive values signifying yellow and negative values signifying blue. The dE value indicated the color difference. All these values are required to describe the color of the sample. A standard whiteboard made from barium sulfate (100% reflectance) was used as a perfectly white object to set the instrument with illuminant. The product was placed in a sample container, and reflectance was auto-recorded for the wavelength ranging from 360 – 800nm.



Figure 3.14: Hunter L* a* b* colour analyzer

3.6.2 Texture Analysis

The instrumental texture measurement of uncooked rice analogues was determined using a TAHDi (Stable Microsystems, Surrey, UK) equipped with 50 kg load cell. The measurement

of hardness for uncooked samples was performed. The probe used was Aluminium 36 R cylindrical 2-inch diameter with speed of about 5mm/s, and compression was set at 50%. The reported values are the average of 4 different determination. Readings are taken in grams and are converted to Newton using the following equation,

$$N = \frac{G * 981}{1000}$$

Where, N = Newtons (N)

G = Total mass in grams (g)

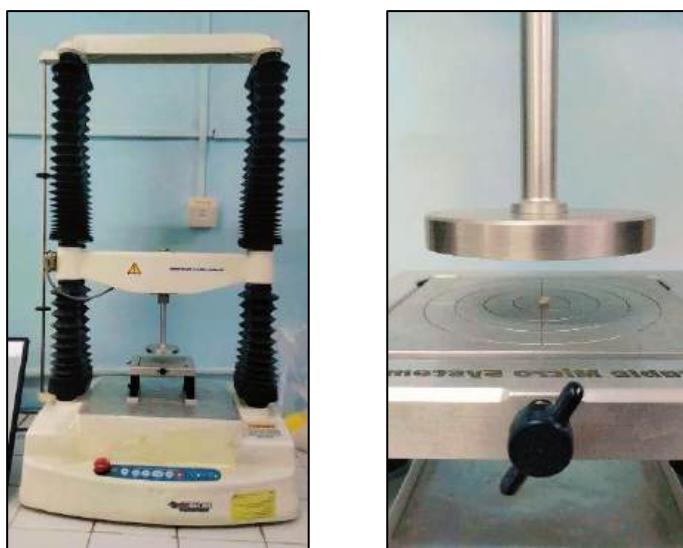


Figure 3.15: Texture analyzer

3.6.3 Grain Dimensions

A total of ten uncooked rice analogue grains were randomly selected from each formulation (ie, control- 0% BRF, 20% BRF, 30% BRF, 40% BRF and 50% BRF). Two different dimensional properties (mm) were determined by measuring the length and thickness of the grains using a Venier digital calliper.

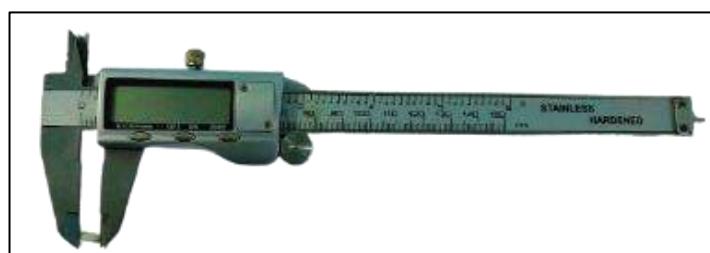


Figure 3.16: Digital vernier calliper

3.6.4 Grain Weight

A total of ten uncooked rice analogue grains were randomly selected from each formulation (ie, control– 0% BRF, 20% BRF, 30% BRF, 40% BRF and 50% BRF) and each grain was individually weighted to obtain the average weight of each rice analogue formulation.



Figure 3.17: Weighing machine

3.6.5 Bulk density

Bulk density (g/ml) is described as the ratio of the mass of the sample to its total volume (Vanrnamkhasti et al., 2008). It was determined by filling a 100 mL cylinder with grains using method of Mariotti, Alamprese, Pagani, and Lucisano (2006). Bulk density (g/ml) was calculated as a ratio between the sample weight and the volume of the cylinder.

$$\text{Bulk density} = \frac{\text{Sample weight}}{\text{Volume}}$$



Figure 3.18: Bulk density

3.7 Functional Characteristics

3.7.1 Water Binding Capacity (WBC)

1 g of the sample was vortexed with 10 ml distilled water for 30 seconds in centrifuge tube. The mixture was allowed to stand at room temperature (28 ± 2 C) for 30 min, centrifuged (5000 g, 30 min). Carefully decant or remove the supernatant (unbound water) without disturbing the sediment (bound water). Weigh the sediment containing the bound water while the volume of supernatant was measured in a 10 ml graduated cylinder. Calculate the Water Binding Capacity using the weight difference before and after water binding. The result is often expressed as grams of water bound per gram of the dry sample.

$$WBC = \frac{\text{Weight of sediment} - \text{Weight of dry sample}}{\text{Weight of dry sample}}$$



Figure 3.19: Water binding capacity of different rice analogue formulations

3.7.2 Oil Binding Capacity (OBC)

1 g of the sample was vortexed with 10 ml refined vegetable oil for 30 seconds in centrifuge tube. The mixture was allowed to stand at room temperature (28 ± 2 C) for 30 min, centrifuged (5000 g, 30 min) and the volume of supernatant was measured in a 10 ml graduated cylinder. Weigh the sediment containing the bound oil while the volume of supernatant was measured in a 10 ml graduated cylinder. Calculate the Oil Binding Capacity using the weight difference before and after oil binding. The result is often expressed as grams of oil bound per gram of the dry sample.

$$OBC = \frac{\text{Weight of oil-bound material} - \text{Weight of dry sample}}{\text{Weight of dry sample}}$$

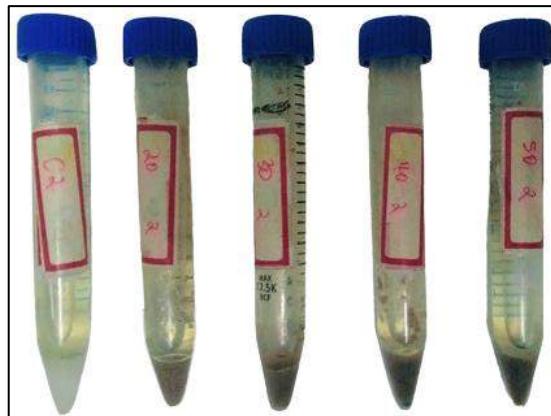


Figure 3.20: Oil binding capacity of different rice analogue formulations

3.7.3 Water Solubility Index (WSI)

1 g sample was suspended in 5 mL distilled water at 30°C in a tarred centrifuged tube for 30 min with gentle intermittent stirring. The content was then centrifuged at 3000g for 10 min. The supernatant was taken into a tarred dish and the amount of dried solids recovered by evaporating the water was expressed as percentage of dry solids in the 1 g sample.

The WSI was calculated as follows:

$$\% \text{ WSI} = \frac{\text{Weight of dissolved solid in supernatant}}{\text{Original weight of sample in dry basis}} * 100$$

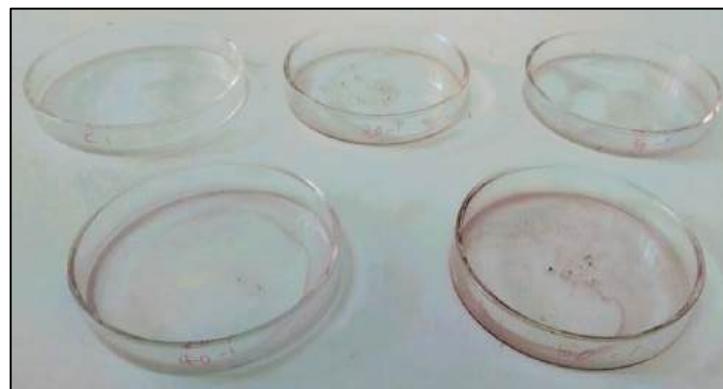


Figure 3.21: Water solubility index of different rice analogue formulations

3.7.4 Swelling Power

The sample (100 mg) was heated in 10 ml distilled water in a water bath at 60°C for 30 min with constant mixing. The samples were centrifuged at 1600 rpm for 15 min. The precipitated part was weighted and calculated as follows:

$$\text{Swelling power} = \frac{\text{Weight of sediment paste (g)}}{\text{Weight of the sample in dry basis (g)}}$$



Figure 3.22: Swelling power of different rice analogue formulations

3.8 Sensory Evaluation

A sensory test was conducted to assess the general preferences for analogue rice. The attributes evaluated in the test were appearance, aroma and flavour, colour, consistency, taste and mouthfeel. A 9-scale hedonic score card was provided to the sensory panel members for rating with 9 being excellent and 1 being very poor.

The rice analogues were used to prepare kheer by boiling in milk in addition to sugar, as they cannot be assessed as plain cooked rice. The ratio of rice to milk powder to sugar was 1:0.75:0.75 with 100 ± 10 ml water.

SENSORY EVALUATION							
PRODUCTS: <i>rice</i>							
Date: 14/02/2025							
<ul style="list-style-type: none"> You receive set of coded samples Score them for various characteristics given below Rate from a score of 1 to 9 on the given characteristics 							
Sample	Appearance (9)	Color (9)	Aroma & Flavour (9)	Consistency (9)	Desirability (9)	Taste (9)	Mouthfeel (9)
Any comments							
Rating: Excellent- 9; V. good- 8; Good- 7; Fair-5; Poor-3; V. poor-1 Desirable characteristics: Appearance: good appeal Color: light to dark purple color Aroma/ flavour: typical black rice aroma (close to basmati flavour) Consistency: not too thick not too thin, absence of lumps Desirability: grains not clogged together, visible separate grains Taste: no off taste, likable taste Mouthfeel: Smooth texture with slight chewy to bite, easy to swallow							

Figure 3.23: 9-Scale hedonic score card



Figure 3.24: Kheer from Black Rice, control, 20% BRF, 30% BRF, 40% BRF and 50% BRF [from left to right, top to bottom]

3.9 Phytochemical analysis

Preparation of extract

To extract the free compounds, 25ml of methanol was added individually to 10g of each sample, and the liquid was agitated at 40 °C overnight before filtering through a Whatman filter-40. The filtrate was centrifuged for 10 minutes at 5000 rpm at 25 °C. The supernatant was collected and stored at -20 °C in the dark until TPC and TFC were determined. Extraction was done in triplicate.



(a) Water bath



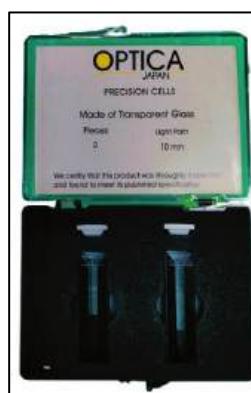
(b) Centrifuge



(c) Vortex Mixer



(d) Spectrophotometer



(e) Cuvette



(f) pH meter

Figure 3.25: Instruments used for phytochemical analysis

3.9.1. Total Phenolic Content

Preparation of reagents:

Folin-Ciocalteu's reagent (10%) - 10 ml of Folin-Ciocalteu's reagent in 100 ml of distilled water.

Sodium carbonate (7.5%) - dissolve 7.5 g of sodium carbonate in 100 ml of distilled water.

Gallic acid was used as the standard in the TPC assay.

The Folin-Ciocalteu method was used to calculate the total free polyphenol content of extracts (Singleton & Rossi, 1965). Test tubes containing samples (80 μ l) with 1.0 ml of Folin-Ciocalteu's reagent (10%) and 0.8 ml of sodium carbonate (7.5%) were made up to a volume of 5.5 ml with distilled water. The mixture was incubated at 25 °C for 45 minutes in complete darkness. Shimadzu, Japan's UV-VIS-1800 spectrophotometer was used to measure the absorbance at 765 nm. TPC was computed using a common gallic acid curve and displayed as mg GAE/100g dry weight of Gallic acid. Every analysis was carried out twice.



(a) After Addition of Folin-Ciocalteu's Reagent (10%)



(b) Followed by Sodium Carbonate (7.5%) addition

Figure 3.26: Changes in the sample colour during various stages of total phenol content test

3.9.2. Total Flavonoid Content

Aluminium chloride colorimetric assay (Woisky and Salatino, 1998)

Principle: A colorimetric assay using aluminium chloride was reported by Woisky and Salatino (1998) to detect flavonoids. In the test, aluminium chloride reacts with flavonoids to create a stable colour complex. The reactions of both the C-4 keto and the C-3 or C-5 hydroxyl groups initiated a stable complex of acid, while a few acid-labile complex complexes could be caused by the reaction of Ortho-dihydroxyl in the A and B ring of a flavonoid. These flavanols complexes offer maximum absorption at 510 nm. The content of the complex is proportional to its colour intensity.

Reagents and apparatus: Aluminium chloride, sodium nitrite, quercetin, reagent bottle, conical glass test tubes, centrifuge, UV-Vis spectrophotometer, sample extracts

Preparation of reagents:

Aluminium chloride (1:10) - dissolve 10 g of aluminium chloride in 100 ml of distilled water.

Sodium nitrite (1:20) - dissolve 5 g of sodium nitrite in 100 ml of distilled water.

Sodium hydroxide (IM) – dissolve 4 g of sodium hydroxide in 100 ml of distilled water.

Quercetin was used as the standard in the TFC assay.

Preparation of extract: To extract the free compounds, 24 ml of acidified methanol (methanol: 1N HCl is 85 : 15 (v/v)) was added individually to 3g of each sample, and the liquid was agitated at 35 °C for 40 minutes at 100 rpm before filtering through a Whatman filter-40. The filtrate was centrifuged for 10 minutes at 5000 rpm at 35 °C. The supernatant was collected and stored at -20 °C in the dark. Extraction was done in triplicate.

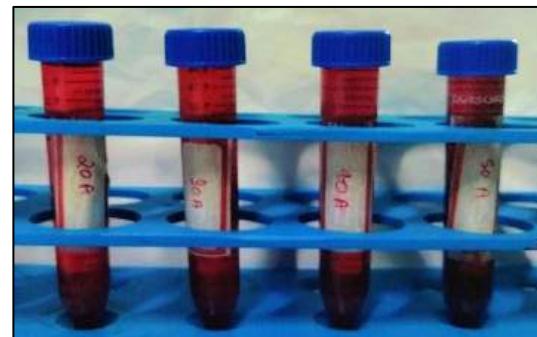
Procedure:

To generate a standard curve, 3 mg of quercetin was dissolved in deionized water and the volume was adjusted to 6 ml.

250 µl of extract with 1.25 ml distilled water was mixed well. 75 µl of 5% sodium nitrite was added; the solution mixture was incubated at room temperature (RT) for 6 min, following which 150 µl of 10% aluminium chloride was added and incubated at RT for 5 min; 0.5 ml of 4% sodium hydroxide was added and the volume was adjusted to 3 ml using distilled water. The solution mixture was then appropriately mixed and was centrifuged at 30°C for 5 minutes at 5000 rpm to obtain clear solution. TFC was determined by measuring the absorbance at 510 nm using a UV spectrophotometer.

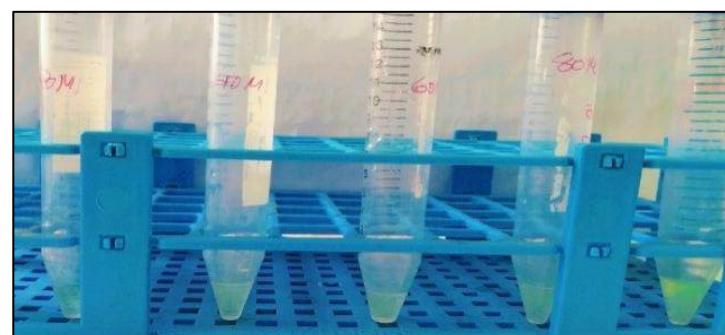


(a) After filtration

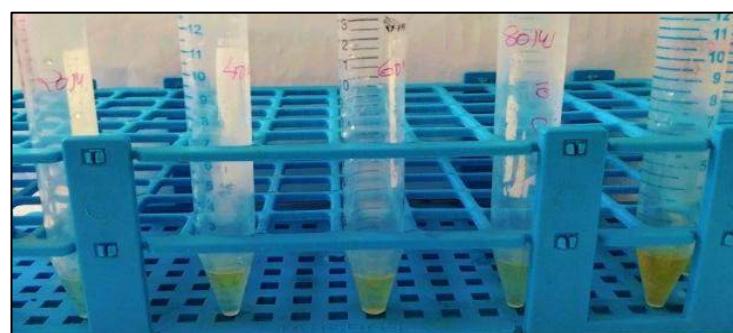


(b) After centrifugation

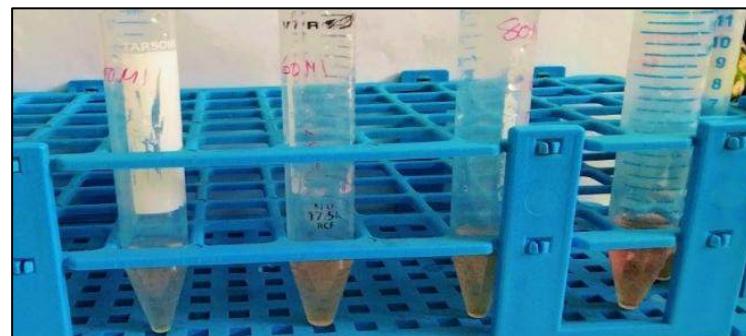
Figure 3.27: Acidified methanolic sample extracts



(a) Addition of sodium nitrite (5%)



(b) Addition of aluminium chloride (10%)



(c) Addition of sodium hydroxide (4%)

Figure 3.28: Changes in the sample colour during various stages of total flavonoid content test

3.9.3. Total Anthocyanin Content

Reagents and apparatus: KCl buffer (pH 1.0) and sodium acetate buffer (pH 4.5), Test tubes, centrifuge, UV-Vis spectrophotometer.

Principle: A colour change in the pH of the monomeric anthocyanin pigments is seen pH 1.0 which is the colourful oxonium form and the colourless hemiketal at pH 4.5. The difference in pigment absorption is proportional to the concentration of the pigment at 520 nm. Cyanidin-3-glucoside equivalent values are expressed. Degraded anthocyanins do not change colour irrespective of pH and are not included as they absorb at both pH.

Preparation of buffer:

KCL buffer – To prepare 0.03M KCl, weigh 0.2236 g of KCl into a beaker and add distilled water 100ml. Measure the pH, and adjust pH to 1.0 with HCL. Transfer to a 100ml, volumetric flask, and dilute to volume with distilled water

Sodium acetate buffer - Weigh 3.28 g sodium acetate in a beaker, and a distilled water 100 ml. Measure the pH, and adjust pH to 4.5 with HC1. Transfer to a 100mL volumetric flask and dilute to volume with distilled water.

Estimation of total anthocyanin content:

Anthocyanin content was estimated by a pH differential method. Two dilutions of extracts were prepared: one with 0.03 M potassium chloride buffer (pH 1.0) and the other extract with 0.4 M sodium acetate buffer (pH 4.5) diluting each with previously determined dilution factor (extract: buffer is 1: 4 (v/v)). The mixture was centrifuged at 40°C for 10 minutes at 1600 rpm to obtain clear solutions. Diluted extracts were measured at 520 nm. The content of total anthocyanin was calculated using the following formula,

$$\text{Absorbance} = \text{pH 1} - \text{pH 4.5}$$

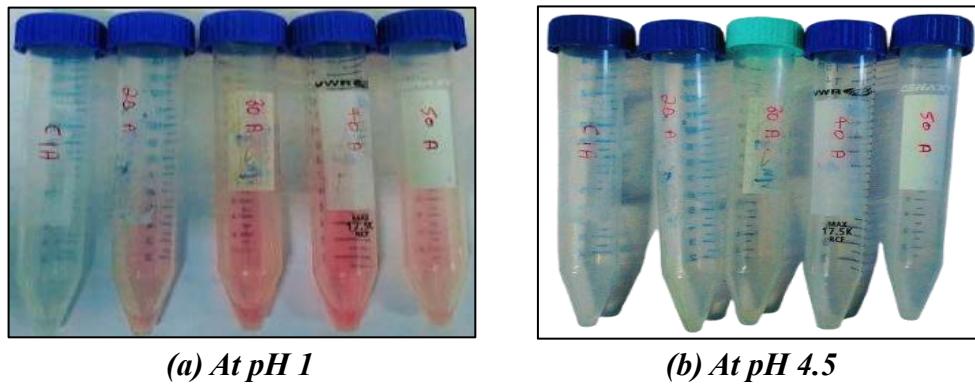
$$\text{Concentration (mg/g)} = \frac{\text{Absorbance} * \text{DF} * \text{MW} * 1000}{\epsilon * 1}$$

where, MW = molecular weight,

DF = dilution factor

ϵ = molar absorptivity

Anthocyanin concentration was calculated and expressed as cyanidin-3-glycoside equivalent (mg/g). MW is 449.2 g/mol for cyanidin-3-glucoside, ϵ is the 26,900-molar extinction coefficient, in L/mol cm, for cyd-3-glu, 1 is the path length in cm, and 1000 is the factor for conversion from g to mg. It was converted to mg of total anthocyanin content/g sample.



(a) At pH 1

(b) At pH 4.5

Figure 3.29: Changes in the sample colour during various stages of total anthocyanin content test

3.10 Aroma Profile Using Electronic Nose Technology

2g of cooked samples from various formulations along with the Black Rice was weighed and transferred into glass vials and capped. It was done in triplicates. Each triplicate sample was placed onto the trays with a blank vial in-between different sample. It was then loaded onto the E-Nose machine – Alpha MOS Heracles Neo E-Nose for analysis. The analysis takes about 3-4 hours after which the output is obtained.



(a) Vials containing 2g of cooked samples



(b) E-Nose machine with loaded samples



Figure 3.30: Aroma profiling using E-Nose

3.11 Flavour Profile Using Electronic Tongue Technology

5g of cooked samples from various formulations along with the Black Rice was weighed and smashed into paste form. To this, 50ml distilled water was added and vortexed. The mixture was then centrifuged at 1600 rpm for 10 minutes at 30°C. The supernatant was then carefully collected after filtering through a Whatman-40 filter paper to obtain about 25ml clear Supernatant. The extraction was done in duplicates.

For the analysis, 25ml extract was transferred into E-tongue glass vials and were loaded into the E-Tongue machine (Astree, Alpha MOS, France). Standard was also run to obtain the output data.

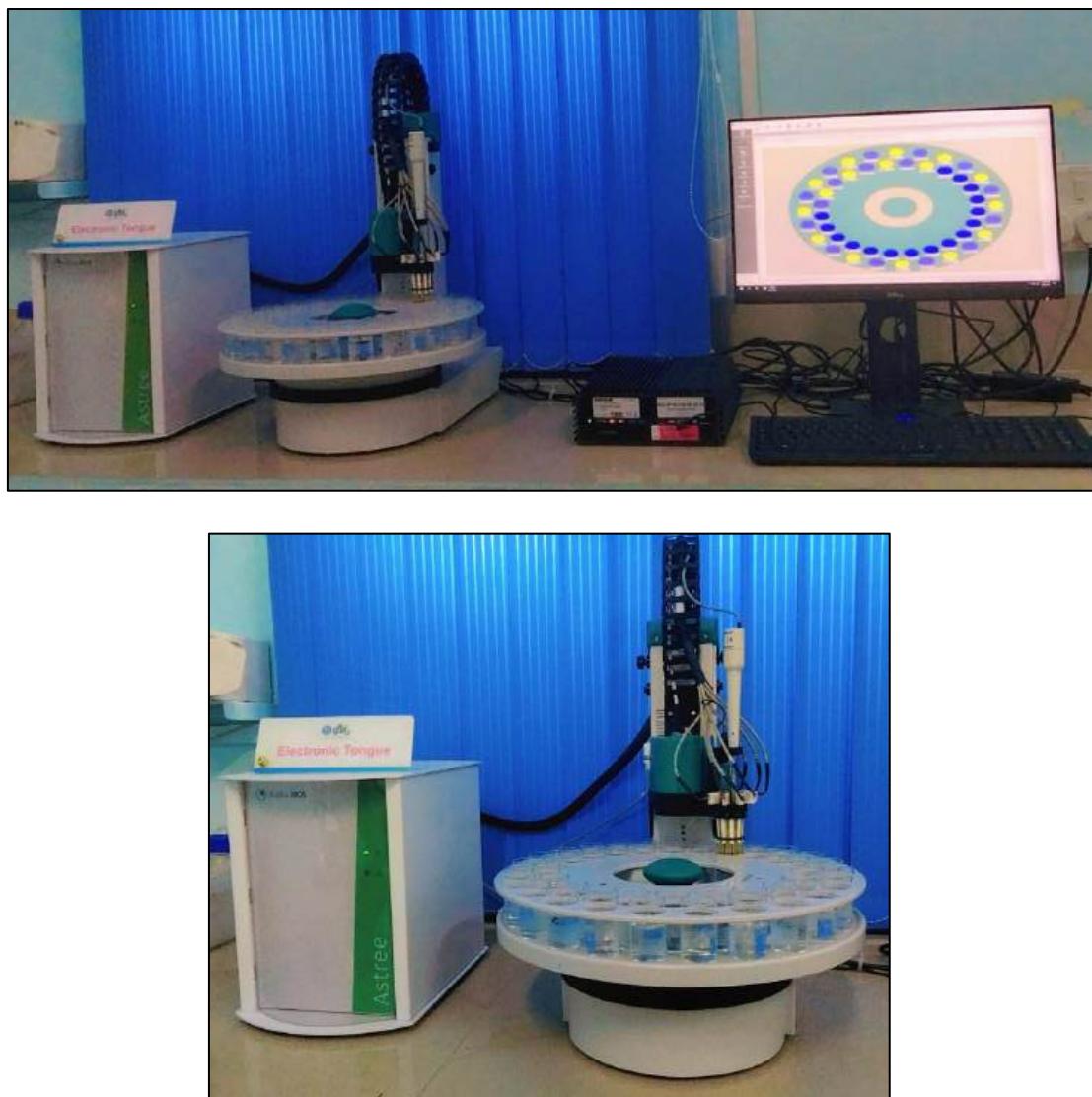


Figure 3.31: Flavour profiling using E-Tongue

CHAPTER – 4

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

4.1 Rice Analogue Formulation and Standardization

The water content was adjusted for the different formulations based on the requirements of texture and water absorption of the raw materials. The general formulation was changed on this requirement and is as shown in the *Figure 4.1*.

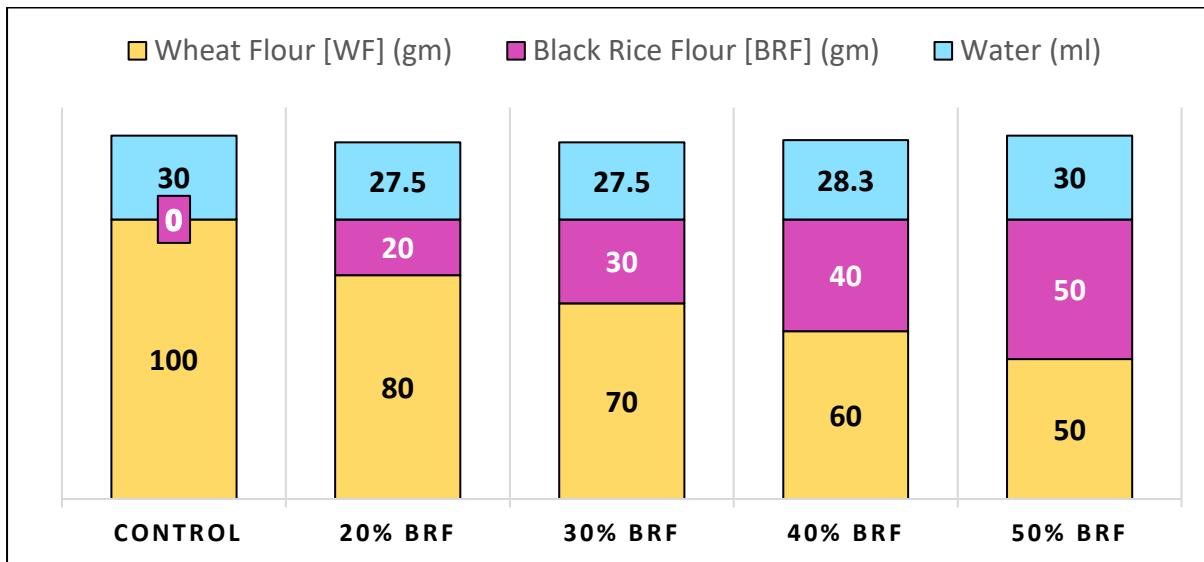


Figure 4.1: Rice analogue formulation and standardization

Standardization was done thrice with utmost care to ensure the proper mixing of the ingredients and the proper resting of the mixture so that it will be properly hydrated. It was also noted that the number of times the raw material underwent extrusion, the colour, texture and cooking parameters of the product was severely affected. It can be probably due to the loss of moisture during the extrusion process.

As the amount of Black Rice flour increased in the formulation, the bulkier the extruded rice became and harder it was to ensure proper and consistent shape of the rice analogue. The freshly extruded rice analogues were allowed to air dry for 3-5 minutes before they underwent steaming.

Proper steaming helped the rice analogue to hold its shape due to the partial gelatinization of the starch and it also helped to impart proper colour to the rice analogue.

The different formulated rice analogues were dried till they were brittle. The dried rice analogues were allowed to cool to room temperatures before packed.

Based on the cooking properties, physical parameters, functional properties and sensory analysis, formulation 40% BRF was selected as the product.

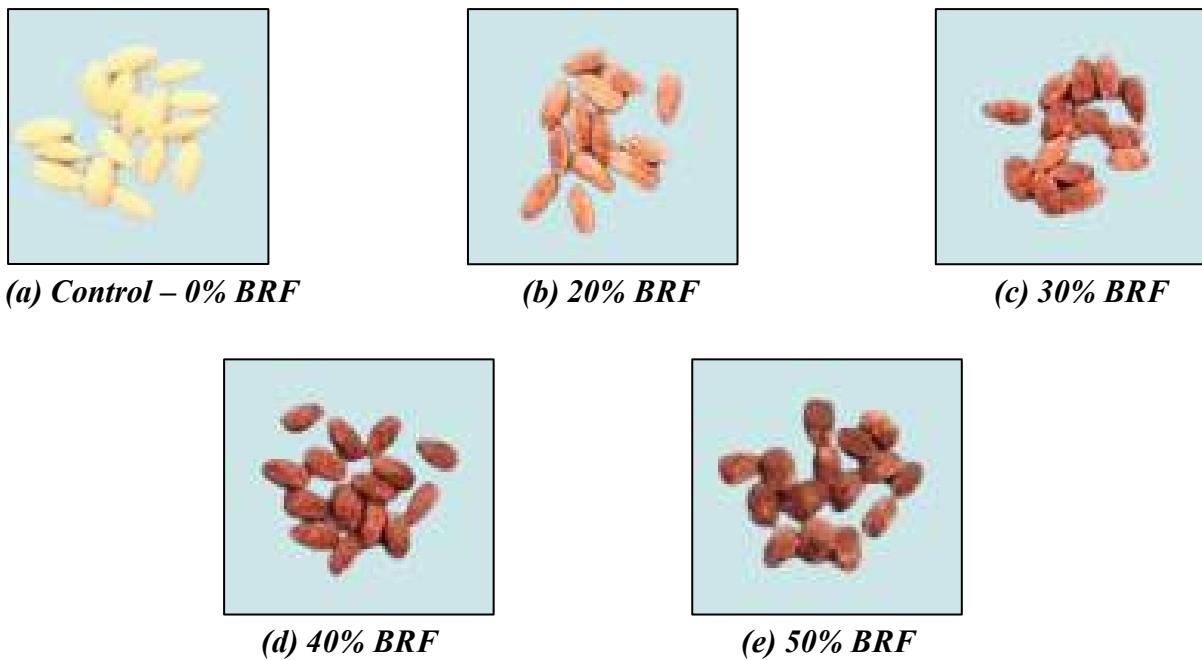


Figure 4.2: Standardized rice analogues

4.2 Characterization of Wheat

The Characterization of Wheat flour used for rice analogue preparation was as given in the *Table 4.1*.

Parameter	Value
Falling Number (sec)	515 ± 5
Sedimentation Value (ml)	29.703 ± 0.471
Dry Gluten (%)	9.021 ± 0.002

Table 4.1: Characterization of Wheat flour

4.2.1 Falling Number

The enzyme activity in flour, particularly that of α -amylase, is evaluated using the falling number (FN) test, a vital measure in assessing flour quality. α -Amylase is a cereal enzyme present in wheat after harvest, and its quantity and activity are influenced by the moisture content of wheat kernels. High moisture levels in the kernels typically lead to increased α -amylase activity, often seen in grains subjected to wet conditions or improper storage.

A higher falling number indicates that the plunger takes longer to pass through the slurry; this indicates that the grain has little enzymatic activity and has not started to germinate. The falling number of wheat flour was observed to be 515 ± 5 seconds.

4.2.2 Sedimentation Value

The quantity and quality (strength) of gluten in the flour sample impact the sedimentation value, measured as the volume in millilitres of settled gluten. Due to their lower density, gluten particles become less compact with increased water absorption in the presence of lactic acid. Consequently, they sediment or sink more slowly. The Zeleny's sedimentation value for the wheat flour was 29.703 ± 0.471 mL, indicating that the wheat flour used in this study was of medium-strong quality.

4.2.3 Gluten

Wheat contains gluten, a protein which is the key indicator of its quality. It affects the texture, elasticity, and chewiness of wheat-based products, such as bread and pasta etc. The percentage of wet gluten and dry gluten of wheat flour was found to be $26.159 \pm 0.057\%$ and $9.021 \pm 0.002\%$.

4.3 Proximate Analysis

The proximate compositions of raw materials (wheat flour and Black Rice flour), control – 0% BRF and 40% BRF are listed out in *Table 4.2*.

Parameter	Wheat Flour [WF]	Black Rice Flour [BRF]	Control	40% BRF
Moisture (%)	8.79 ± 0.13	8.21 ± 0.019	6.67 ± 0.03	8.00 ± 0.06
Ash (%)	0.83 ± 0.00	1.08 ± 0.00	0.67 ± 0.05	1.00 ± 0.01
Fat (%)	1.60 ± 0.03	3.36 ± 0.22	0.54 ± 0.04	0.39 ± 0.04
Protein (%)	10.1 ± 0.24	8.12 ± 0.03	4.26 ± 0.02	3.95 ± 0.04
Total Dietary fibre (%)	4.55 ± 0.28	4.88 ± 0.78	3.83 ± 0.28	3.46 ± 0.37
Carbohydrates (%)	78.66 ± 0.07	79.21 ± 1.03	87.84 ± 0.52	86.64 ± 0.72
Energy (%)	369.51 ± 1.51	379.63 ± 6.27	373 ± 2.56	365.96 ± 3.46

Table 4.2: Proximate composition of raw materials, control and 40% BRF

4.3.1 Moisture Content

Moisture content is directly linked to the shelf-life stability of food materials as higher moisture levels can increase the risk of spoilage by microorganisms. In this study, the moisture content of Black Rice flour was not significantly different from that of wheat flour. The wheat flour had a slightly higher moisture content of $8.797 \pm 0.132\%$ compared to $8.215 \pm 0.0195\%$ moisture content of Black Rice flour. This signifies that wheat flour has a higher chance of being prone to spoilage compared to Black Rice flour. The control sample exhibits the lowest moisture content at 6.67 ± 0.03 . In contrast, 40% BRF shows a slightly higher moisture content,

though it remains within the range of the raw materials used. The moisture-retaining nature of Black Rice Flour, attributed to its composition of starches and dietary fibers, plays a role in elevating the moisture level of the 40% BRF sample, resulting in a higher content compared to the control.

4.3.2 Ash

Ash content serves as an indicator of mineral presence in food. Black Rice demonstrated a higher ash content of $1.085 \pm 0.001\%$ compared to wheat flour, which had $0.830 \pm 0.000\%$. This finding highlights the superior nutritional value of Black Rice, known for its rich mineral composition. Similar results were reported in studies by Anitha Kumari and Kassegn, indicating that colored flour generally has a higher ash content and serves as a better mineral source than white wheat flour. The ash content of the control sample was measured at $0.67 \pm 0.05\%$, while 40% BRF showed $1.00 \pm 0.01\%$. However, extrusion processing slightly reduced the ash content of the rice analogue samples.

4.3.3 Fat

The fat content in flours plays a part in enhancing the taste and overall appeal of the final product. A significant difference in fat content was observed between wheat flour and Black Rice flour. The Black Rice flour showed a higher fat content of $3.36 \pm 0.22\%$ compared to wheat flour ($1.60 \pm 0.03\%$). The fat content of control and 40% BRF was found to be $0.54 \pm 0.04\%$ and $0.39 \pm 0.04\%$ respectively. The fat content of flour is greater than that of extruded samples, indicating fat loss during the extrusion process due to exposure to high temperature, pressure, and shear. This loss is influenced by fat characteristics such as melting point and solubility. Additionally, higher fat content increases the likelihood of rancidity over time, making extruded products more stable against rancidity.

4.3.4 Protein

The protein content of Black Rice flour was almost comparable with that of wheat flour. However, wheat flour was found to have a higher protein content ($10.1 \pm 0.24\%$) compared to Black Rice flour ($8.12 \pm 0.03\%$). This outcome can be linked to the abundant gluten content in wheat flour. The high protein content in wheat is advantageous as it supports the recommended daily protein intake and significantly influences the economic worth of wheat. Additionally, protein content plays a crucial role in shaping the quality and characteristics of its final products. In contrast, Black Rice is inherently gluten-free, which contributes to its slightly lower protein content when compared to wheat flour. This distinction makes Black Rice suitable for individuals with gluten intolerance or those following a gluten-free diet, while wheat flour remains a preferred choice for higher protein needs. The control and 40% BRF showed a protein content of $4.62 \pm 0.02\%$ and $3.95 \pm 0.04\%$ respectively. Starch and protein components can undergo minor changes during extrusion. This loss of protein can be due to the protein denaturation which occurs at high temperature, shear, and pressure.

4.3.5 Dietary Fibre

Dietary fiber (DF) is the edible parts of plants containing carbohydrate polymers, which are resistant to hydrolysis by endogenous enzymes in the human body. They are highly valued for their health benefits as they aid in relieving constipation, support weight management, and reduce the risk of conditions like diabetes and heart diseases. DF can be divided as soluble dietary fiber (SDF) and insoluble dietary (IDF) based on its hot water solubility (He et al., 2022). Black Rice is a rich source of dietary fibre. The insoluble, soluble and total dietary fibre content of Black Rice flour (3.10 ± 0.76 , 1.78 ± 0.01 and $4.88 \pm 0.78\%$ respectively) was significantly higher than the common wheat flour (3.05 ± 0.13 , 1.50 ± 0.15 and $4.55 \pm 0.28\%$ respectively). The insoluble, soluble and total dietary fibre content 3.28 ± 0.17 , 0.54 ± 0.18 and $3.83 \pm 0.28\%$ respectively for control as well as $3.31 \pm 0.41\%$, $0.15 \pm 0.03\%$ and $3.46 \pm 0.37\%$ respectively for 40% BRF.

4.3.6 Carbohydrate

Carbohydrates are organic molecules made up of carbon, hydrogen, and oxygen atoms. They act as a source of energy for living organisms and are one of the main types of nutrients. Carbohydrate content of raw materials and rice analogues were calculated based on their moisture content, fat, ash and protein content. $78.66 \pm 0.07\%$, $79.21 \pm 1.03\%$, $87.84 \pm 0.52\%$ and $86.64 \pm 0.72\%$ are the carbohydrate content of wheat flour, Black Rice flour, control and 40% BRF respectively.

4.3.7 Energy

The energy of food refers to the amount of energy which is released when the food is metabolized by the body. Energy is measured in units called calories or kilojoules and is used by the body to perform various functions like movement, growth, repair, and maintaining essential processes like breathing and digestion. Carbohydrates, Proteins and Fats are the three macronutrients in food that provides energy. The energy provided by wheat flour, Black Rice flour, control and 40% BRF respectively are $369.51 \pm 1.51\%$, $379.63 \pm 6.27\%$, $373 \pm 2.56\%$ and $365.96 \pm 3.46\%$.

4.4 Cooking Quality Characteristics

Consumer acceptance and satisfaction are directly influenced by the cooking quality factors of the rice analogues, thus emphasising its importance. Water absorption, cooking duration, yield, and elongation during cooking are all designed to replicate traditional rice (Naeem et al., 2019). These characteristics ensure that rice substitutes deliver a comparable appetite experience while potentially offering nutritional or environmental benefits (M, John, & Raman, 2023). The cooking quality of the rice analogues are tabulated in *Table 4.3*.

Sample	Optimal cooking time (min)	Water absorption (g/g)	Cooking loss (%)	Cooking yield	
				(g/g)	(%)
Control	6.25 \pm 0.34	1.86 \pm 0.09	5.63 \pm 0.05	2.68 \pm 0.09	286.27 \pm 9.77
20% BRF	6.53 \pm 0.19	2.08 \pm 0.04	7.26 \pm 0.16	3.08 \pm 0.04	308.80 \pm 4.94
30% BRF	6.66 \pm 0.33	2.08 \pm 0.13	8.38 \pm 0.26	3.08 \pm 0.13	308.76 \pm 13.60
40% BRF	6.80 \pm 0.19	2.13 \pm 0.34	9.07 \pm 0.60	3.13 \pm 0.34	313.81 \pm 34.26
50% BRF	6.94 \pm 0.19	2.22 \pm 0.31	9.73 \pm 0.29	3.22 \pm 0.31	322.25 \pm 31.12

Table 4.3: Cooking characteristics of the rice analogues

4.4.1 Optimal Cooking Time (OCT)

The optimal cooking time was found to be the highest for 50% BRF (6.94 min) and lowest for control – 0% BRF (6.25 min). It can be observed that increase in concentration of Black Rice in the rice analogue resulted in the increase of the cooking time. Still, the cooking time of the analogues are less compared to that of the Black Rice which can vary from 20-25 minutes based on pre-treatments like soaking and parboiling while with these, it might take 30-40 minutes (Pangi, Thirunavookarasu, & Athmaselvi, 2023 & Widyasaputra, Syamsir, & Budijanto, , 2019).

4.4.2 Water Absorption

The water absorption of the rice analogues varied between 1.86 to 2.22g per gram of sample. The control showed the lowest water absorption, which increased with the increase in concentration of Black Rice in the product. It led to 50% BRF to have the highest water absorption. The variation in water absorption can be attributed to the composition and properties of Black Rice flour (BRF). Black Rice contains higher levels of dietary fiber, starch, and protein compared to typical white rice or other flours. These components have a greater capacity to bind and retain water, contributing to increased water absorption. As the concentration of Black Rice in the product increases, these hydrophilic (water-attracting) components become more prevalent, enhancing the ability of the mixture to absorb water (Zhang et al., 2022). At 50% BRF, the high concentration of these water-binding components likely maximized the water absorption capacity.

4.4.3 Cooking Yield

The cooking yield of the rice analogues increased with the increase in concentration of Black Rice flour in the product. The cooking yield was observed to be lowest for control (2.68g per gram of sample) and highest for the 50BF (3.22g per gram of sample). The increase in quantity of water absorbed during cooking resulted in the increased cooking yield of rice analogues.

4.4.4 Cooking Loss

The cooking loss of rice analogues increased with increase in concentration of Black Rice flour. The cooking loss was highest for 50BF (9.73%) and lowest for control (5.63%). This might be due to the dilution of gluten protein that helps to exhibit the binding property.

4.5 Physical Characteristics

Understanding the physical properties of grains is vital for ensuring their quality and functionality. Key physical characteristics of grains include grain size and weight, bulk density, texture, colour etc.. These characteristics, however, are largely influenced by factors such as moisture content, temperature, and the inherent density of the grains. When it comes to rice analogues, studying their physical properties is crucial for assessing their quality, performance, and consumer acceptance. These properties also play a key role in evaluating their suitability as an alternative to conventional rice, providing valuable insights into their handling, processing, and overall utility. By analysing these traits, we can work towards the development of higher-quality and more effective rice analogues.

4.5.1 Instrumental Colour Analysis

Colour is an important parameter in assessing food quality and it indirectly assess other attributes like flavour, palatability and builds up a correlation with other physicochemical properties. Colour measurement of food products focuses on both instrumental (objective) and visual (subjective) measurements (Pathare, Opara, & Al-Said, 2013).

Instrumental colour analysis is vital for evaluating Black Rice analogues as it provides objective and precise measurements of their appearance, which is a key quality attribute. The colour of Black Rice analogues, often assessed using parameters like L^* , a^* and b^* values, influences consumer perception and acceptance (D'cruz et al., 2022). This analysis helps in monitoring the effects of processing methods, like extrusion, steaming, drying and cooking on the visual appeal of the product. Additionally, it aids in standardizing production processes and ensuring consistency in product quality.

L^* represents lightness, with values ranging from 0 (black) to 100 (white). It indicates how light or dark the sample appears. a^* measures the colour axis from red (+ a) to green (- a), describing the red-green component of the sample while b^* measures the colour axis from yellow (+ b) to blue (- b), describing the yellow-blue component of the sample. dE denotes the overall colour difference between two samples. It is calculated based on the differences in L^* , a^* , and b^* values.

Sample		Colour			
		L*	a*	b*	dE
Control	After extrusion	64.22 \pm 1.26	1.64 \pm 0.24	10.57 \pm 0.51	29.42 \pm 1.09
	After steaming	60.84 \pm 1.16	1.30 \pm 0.18	11.70 \pm 0.44	33.08 \pm 1.02
	After drying	59.97 \pm 1.43	1.21 \pm 0.21	11.37 \pm 0.51	33.95 \pm 1.30
	After cooking	58.64 \pm 0.46	- 0.13 \pm 0.23	8.31 \pm 0.63	34.36 \pm 0.45
20% BRF	After extrusion	43.75 \pm 0.63	2.29 \pm 0.02	1.15 \pm 0.04	48.00 \pm 2.69
	After steaming	34.67 \pm 0.51	3.05 \pm 0.08	2.15 \pm 0.02	57.87 \pm 0.52
	After drying	36.28 \pm 0.94	2.87 \pm 0.05	1.95 \pm 0.04	56.49 \pm 0.94
	After cooking	27.64 \pm 1.25	3.52 \pm 0.16	0.79 \pm 0.12	65.07 \pm 1.24
30% BRF	After extrusion	37.13 \pm 0.68	2.52 \pm 0.07	0.27 \pm 0.06	55.65 \pm 0.67
	After steaming	28.63 \pm 0.36	3.13 \pm 0.04	1.34 \pm 0.03	63.91 \pm 0.36
	After drying	29.18 \pm 0.82	2.94 \pm 0.05	1.00 \pm 0.04	63.6 \pm 0.82
	After cooking	25.36 \pm 1.06	3.76 \pm 0.16	0.68 \pm 0.05	67.45 \pm 1.05
40% BRF	After extrusion	34.63 \pm 0.88	2.55 \pm 0.04	0.19 \pm 0.04	58.09 \pm 0.88
	After steaming	19.39 \pm 0.51	2.71 \pm 0.08	0.66 \pm 0.07	73.30 \pm 0.51
	After drying	19.77 \pm 0.22	2.68 \pm 0.04	0.54 \pm 0.03	73.14 \pm 0.22
	After cooking	22.39 \pm 0.61	3.80 \pm 0.13	0.67 \pm 0.07	71.94 \pm 3.59
50% BRF	After extrusion	33.62 \pm 0.61	2.44 \pm 0.04	0.03 \pm 0.04	59.12 \pm 0.61
	After steaming	22.59 \pm 0.44	2.44 \pm 0.09	0.58 \pm 0.03	73.42 \pm 7.54
	After drying	23.54 \pm 0.71	2.48 \pm 0.06	0.48 \pm 0.04	23.58 \pm 0.71
	After cooking	20.50 \pm 0.66	4.45 \pm 0.25	0.73 \pm 0.07	72.46 \pm 0.65

Table 4.4: Colour analysis of rice analogue formulations at different stages of production

Table 4.4 presents the results of the instrumental colour analysis for rice analogues after undergoing various processing steps, including extrusion, steaming, drying, and cooking. Each formulation's colour characteristics were assessed at every stage to evaluate the impact of processing methods on the final product.

Based on the data, it can be concluded that as the concentration of BRF increased, the darker the colour of the rice analogue turned out to be. Fresh extrudate of the rice analogues were lighter while cooked ones were darker in colour compared to all the other processing it underwent. The rice analogues tend to get darker after steaming and turned a bit lighter after drying. The rice analogues showed positive a* and b* values indicating its affirmation towards redness and yellowness in most cases except for cooked control which showed to tend towards greenness.



Figure 4.3: Colour variations of rice analogues at various stages

Sample	Hardness (N)	Grain Dimensions		Weight (mg)	Bulk density (g/ml)
		Length (mm)	Thickness (mm)		
Control	12.07 \pm 0.66	6.55 \pm 0.21	2.63 \pm 0.27	27.64 \pm 3.86	0.671 \pm 0.004
20% BRF	27.04 \pm 10.70	6.55 \pm 0.22	2.87 \pm 0.25	29.69 \pm 3.81	0.675 \pm 0.000
30% BRF	39.23 \pm 8.84	6.40 \pm 0.23	3.24 \pm 0.33	31.57 \pm 3.66	0.675 \pm 0.000
40% BRF	46.75 \pm 28.49	6.63 \pm 0.12	3.72 \pm 0.04	34.27 \pm 4.56	0.686 \pm 0.000
50% BRF	53.27 \pm 68.88	6.42 \pm 0.13	3.84 \pm 0.28	39.55 \pm 5.02	0.694 \pm 0.000

Table 4.5: Physical characteristics of different rice analogue formulations

4.5.2 Texture Analysis

The texture profile is stated in *Table 4.5* for all the rice analogues. The textural property, hardness of the samples was calculated as the measure of the force required to break the rice analogue grains. It was noted that as the Black Rice flour content increased, the hardness of the grains also increased. 50% BRF showed the higher hardness value (53.27 \pm 68.88) while control showed the lowest (12.07 \pm 0.66). Black Rice has good amount of starch content and its increase in the rice analogue might have led to starch gelatinization that formed strong bonds (Samosir, 2022).

4.5.3 Grain Dimensions

It can be concluded from *Table 4.5* that as the concentration of Black Rice flour increased, the length though not much difference was seen, the thickness of the rice increased. It can be due to the dilution of the gluten content present in the wheat flour which provides the binding property that holds the contents together. Increase in the starch content as Black Rice flour increased can also contribute to this. The starch can swell up and expand under high moisture content and temperature leading to the increase in the thickness ((Lee, Lim, Lim, & Lim, 2000). Control had the length and thickness of 6.55 \pm 0.21 mm and 2.63 \pm 0.27 mm respectively while 50% BRF showed 6.42 \pm 0.13 mm and 3.84 \pm 0.28 mm respectively.

4.5.4 Grain Weight

The grain weight of different rice analogue formulations showed an increase with increase in Black Rice flour with control having the lowest weight of 27.64 \pm 3.86 mg and 39.55 \pm 5.02 mg for 50% BRF as the highest. 20%, 30% and 40% BRF showed 29.69 \pm 3.81 mg, 31.57 \pm 3.66 mg and 34.27 \pm 4.56 mg grain weight respectively.

4.5.5 Bulk Density

The bulk density of grains refers to the weight of grains per unit of volume and it includes the spaces between the grains. It's a key parameter that affects food storage, and processing etc.. Bulk density can depend on factors such as grain type, moisture content, and compaction level.

The bulk density of rice analogues remained consistent without significant variations with 0.671 ± 0.004 g/ml, 0.675 ± 0.000 g/ml, 0.675 ± 0.000 g/ml, 0.686 ± 0.000 g/ml and 0.694 ± 0.00 g/ml respectively for Control, 20% BRF, 30% BRF, 40% BRF and 50% BRF.

4.6 Functional Characteristics

The functional characteristics of different rice analogue formulations are stated in the *Table 4.6* below.

Sample	Water Binding Capacity (g/g)	Oil Binding Capacity (g/g)	Water Solubility Index (%)	Swelling Power (mg/mg)
Control	1.04 ± 0.01	1.095 ± 0.00	4.54 ± 0.82	8.81 ± 0.25
20% BRF	1.28 ± 0.03	1.131 ± 0.00	6.61 ± 0.00	8.21 ± 0.36
30% BRF	1.29 ± 0.00	1.151 ± 0.00	6.82 ± 0.35	7.92 ± 0.09
40% BRF	2.13 ± 0.01	1.157 ± 0.00	6.97 ± 0.52	8.34 ± 0.08
50% BRF	1.37 ± 0.03	1.175 ± 0.00	7.95 ± 0.65	7.43 ± 0.00

Table 4.6: Functional characteristics of different rice analogue formulations

4.6.1 Water Binding Capacity

Water Binding Capacity (WBC) refers to the ability of a material to retain water within its structure. This characteristic is influenced by factors like the composition, particle size, and extrusion conditions. WBC is crucial for determining the texture, hydration properties, and overall quality of food products (Yousf et al., 2017).

The WBC capacity of rice analogues was showing an increase with increase in the addition of Black Rice flour. Surprisingly, 40% BRF showed a deviation by having the highest WBC of 2.13 ± 0.01 g/g; higher than that of 50% BRF (1.37 ± 0.03 g/g). control showed the lowest WBC (1.04 ± 0.01 g/g).

Processes like extrusion, steaming and drying can often modify WBC by altering the structural and chemical properties of the material. Moisture content and temperature adjustment along with screw configuration during extrusion can significantly impact WBC (Yousf et al., 2017).

4.6.2 Oil Binding Capacity

Oil Binding Capacity (OBC) like WBC, refers to the ability of a material to retain oil within its structure; and is influenced by factors like the composition, particle size, and extrusion conditions. The OBC of the rice analogues decreased from 50% BRF (1.175 ± 0.00 g/g) to control (1.095 ± 0.00 g/g). Decrease in Black Rice flour led to a decrease of OBC.

4.6.3 Water Solubility Index

The Water Solubility Index (WSI) measures the quantity of material, typically polysaccharides, that is released from granules and dissolved when an excess amount of water is added (Yousf et al., 2017).

The WSI of rice analogue ranged from 7.95 ± 0.65 % being the highest (50% BRF) to 4.54 ± 0.82 % being the lowest (control). It can be deduced that, increases in Black Rice concentration led to an increase in the starch content which in turn was released and dissolved in the water. In the control sample, the elevated gluten content resulted in starch binding tightly to the product, thereby reducing the release of starch content.

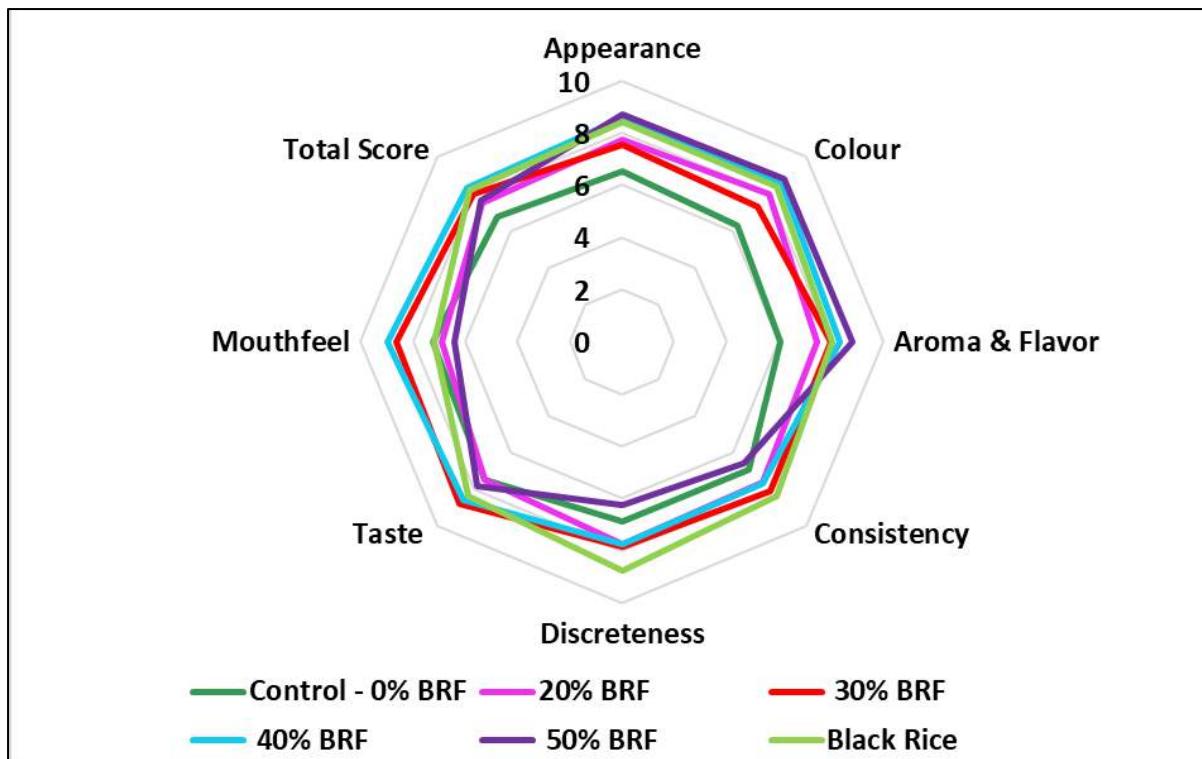
4.6.4 Swelling Power

Swelling power is the capacity of a product to absorb water and swell upon hydration. It reflects the extent of water uptake and the behaviour of starch granules during cooking or processing. The swelling power range was from 7.43 ± 0.00 mg/mg (50% BRF) to 8.81 ± 0.25 mg/mg (control), decreasing with increase of Black Rice flour. 40% BRF showed a swelling power of 8.34 ± 0.08 mg/mg, showing a deviation from the trend.

4.7 Sensory Evaluation

The sensory evaluation was done for the rice analogues by preparing and presenting Black Rice kheer using control – 0% BRF, 20% BRF, 30% BRF, 40% BRF, 50% BRF and Black Rice. The desirable characteristics that were analyzed for the product were good appeal/appearance, light to dark purple color, aroma and flavour similar to typical Black Rice, not too thick not too thin consistency with absence of lumps, Discreteness of the grains, absence of off-taste and Smooth texture with slight chewy to bite, easy to swallow mouthfeel.

Based on the product evaluation, the results are as depicted in the *Graph 4.1*. Both 40% and 50% BRF are well perceived by the consumers. 50% BRF was highly appreciated for its taste and mouth-melting quality. However, it was noted to be sticky, which might impact its texture preference. It was strongly liked overall, especially for taste. 40% BRF was praised for its good mouthfeel. It had the best appearance and color among the samples. 30% BRF met its baseline expectations. 20% BRF was rated as having the best consistency. It was also to be noted that as the duration for sensory evaluation prolonged, the consistency of the different samples were affected; they being to thicken and started to clump up together. Black Rice kheer as such was considered the best in terms of taste though its chewy texture might not appeal to everyone.



Graph 4.1: Sensory evaluation of Black Rice kheer

Hence, 40% BRF was considered as the top seller among the formulations with its features similar to that of the original Black Rice kheer and palatability better preferred by the consumers.



Figure 4.4: Kheer prepared for sensory evaluation

4.8 Phytochemical Analysis

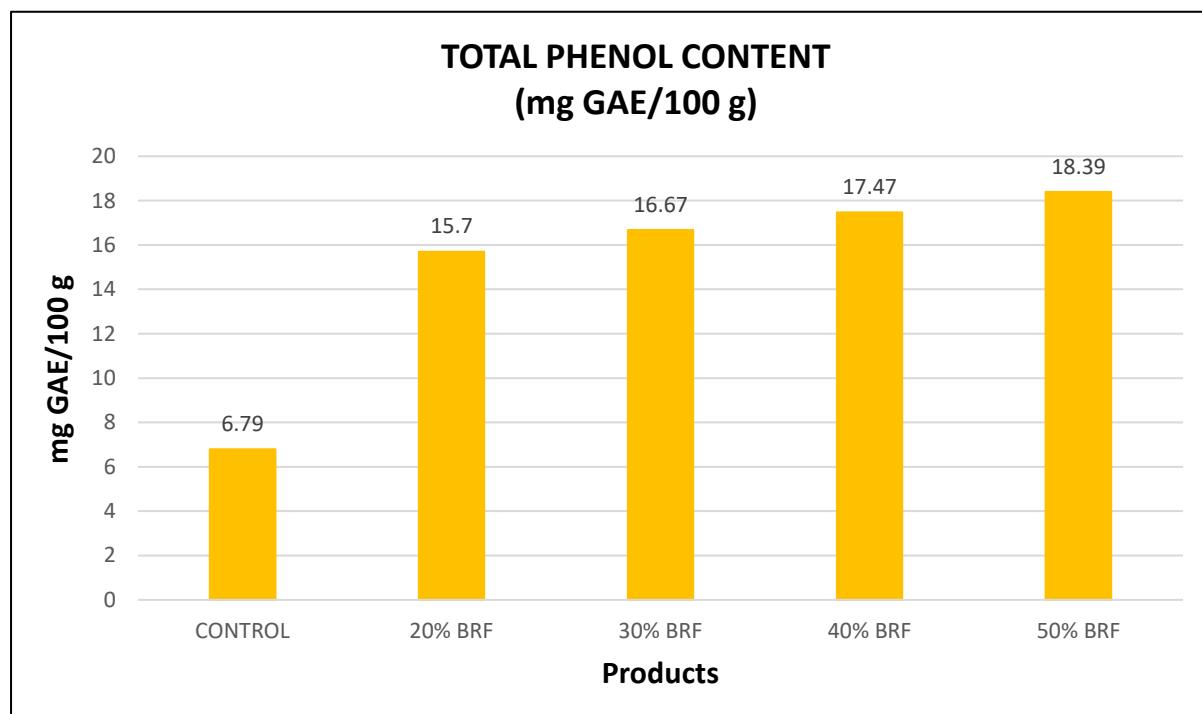
4.8.1 Total Phenolic Content

Phenolic compounds safeguards body tissues from oxidative damage by acting as antioxidants. They also help to delay food spoilage and retain its nutritional value. Total phenolic content (TPC) of rice analogues were measured by the Folin-Ciocalteu reagent method using gallic acid as the standard. A linear calibration curve of gallic acid was used with a linear regression equation for the calibration curve ($y = 0.0108 x - 0.0063$, $R^2 = 0.9812$). The average quantity of the total phenolic compounds found in the raw materials and rice analogues are shown in *Table 4.7* and *Graph 4.2* respectively.

Sample	TPC (mg GAE/100 g)
Wheat flour	11.7 ± 0.02
Black Rice flour	86.99 ± 0.70

Table 4.7: Total phenolic content of raw materials

The amount of total phenolic contents of the rice analogues were in the range of 6.79 ± 0.04 to 18.39 ± 0.29 mg GAE/100g of sample. Among the rice analogues, the highest amount of TPC was exhibited by 50BF and least amount by control. 20%, 30% and 40% BRF showed 15.7 ± 0.66 , 16.67 ± 1.01 , 17.47 ± 0.64 mg GAE/100g sample respectively. Control showed a reduction in total phenolic content when compared to that of wheat flour (11.7 ± 0.02 mg GA/100 g).



Graph 4.2: Total phenolic content of rice analogues

According to Leenhardt et al. (2006), the reduction in total phenolic content might result from the damage or degradation of antioxidant-active compounds in flours caused by the thermal process. Heating alters the molecular structure of phenolic compounds, which can either lower their chemical reactivity or reduce their extractability due to a certain extent of polymerization (Altan et al., 2009).

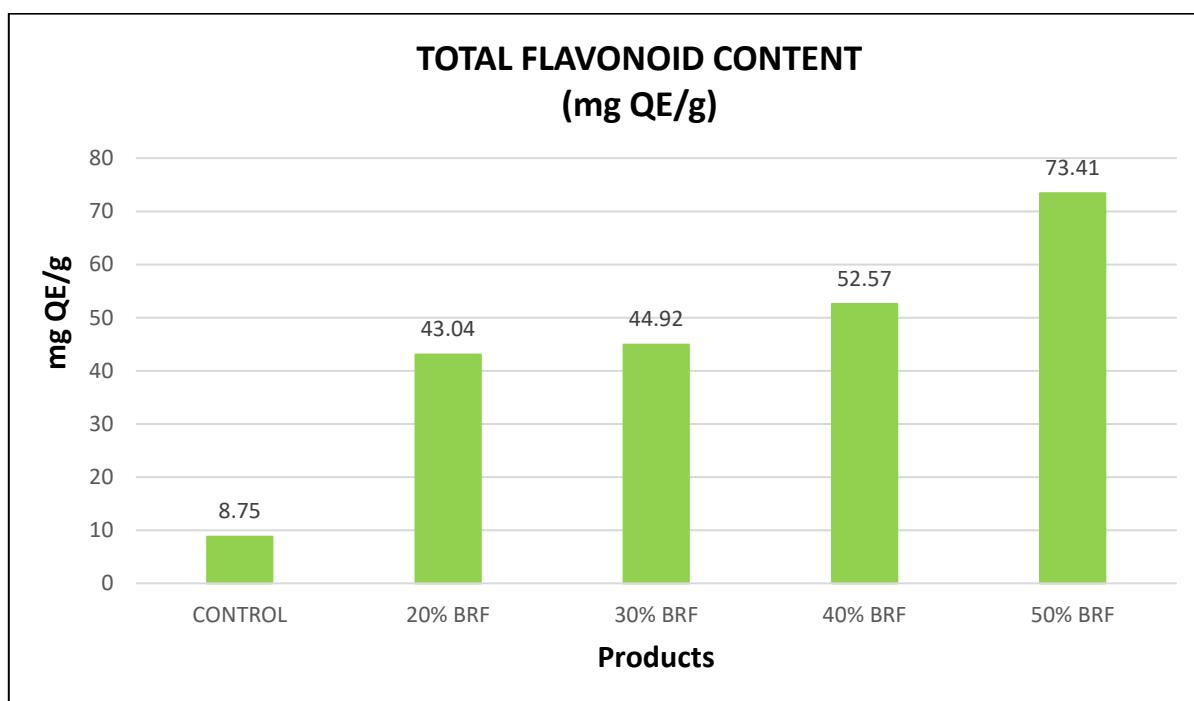
4.8.2 Total Flavonoid Content

A linear calibration curve for quercetin was employed, defined by the linear regression equation $y = 0.0016 x + 0.0047$, $R^2 = 0.9835$. The mean values of total flavonoid content in the raw materials and rice analogues are presented in *Table 4.8* and *Graph 4.3* respectively.

Sample	TFC (mg Quercetin/g)
Wheat flour	15.91 ± 1.49
Black Rice flour	152.47 ± 1.94

Table 4.8: Total flavonoid content of raw materials

The amount of total flavonoids in the raw materials, wheat flour and Black Rice flour were 15.91 ± 1.49 and 152.47 ± 1.94 mg quercetin/g respectively. The rice analogues had total flavonoids in the range of 8.75 ± 2.65 to 73.41 ± 8.27 mg quercetin/g. The highest amount of Flavonoid content was exhibited by 50% BRF. 20%, 30% and 40% BRF showcased 43.04 ± 0.85 , 44.92 ± 0.07 , 52.57 ± 3.28 mg quercetin/g sample respectively. Control showed a reduction in total flavonoid content when compared to that of wheat flour. The processes it underwent can have caused the loss of flavonoids.



Graph 4.3: Total flavonoid content of rice analogues

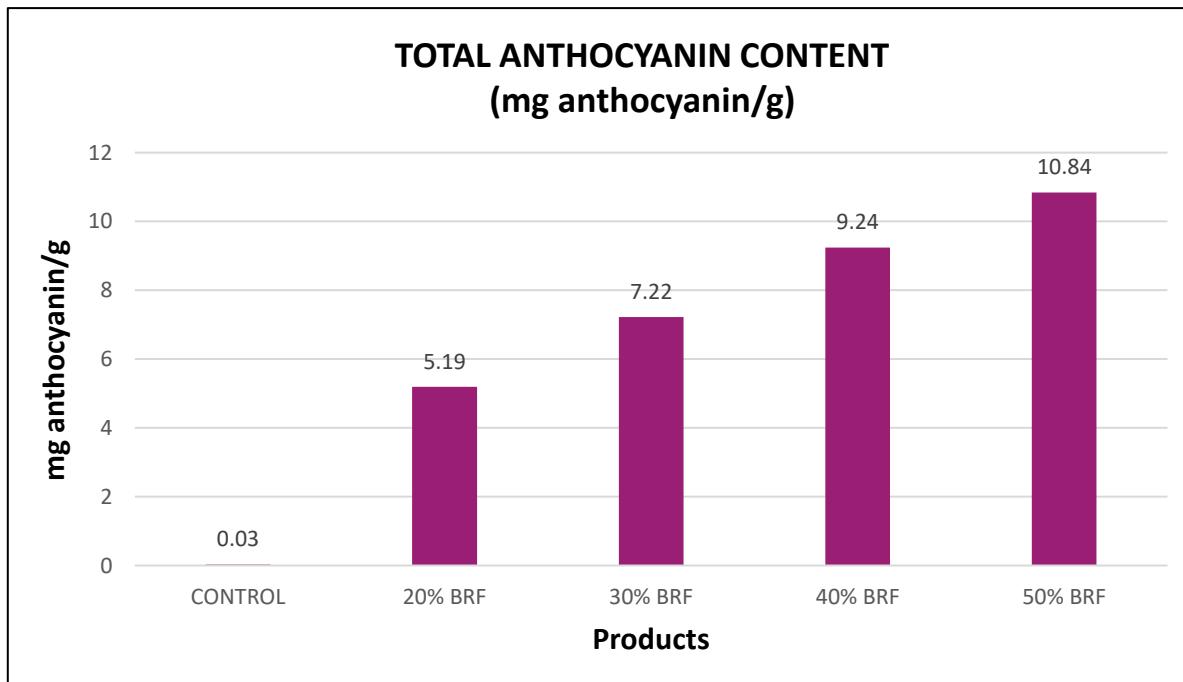
4.8.3 Total Anthocyanin Content

Anthocyanins are a group of water-soluble pigments that are present in plant parts like leaves, flowers, fruits etc. Their colour can range from deep red to purple to blue pigments based on pH due to their chemical structure's sensitivity to acidity or alkalinity. Anthocyanins appear red at low pH, while in neutral pH they take on a purple hue, and in alkaline conditions, they become blue (Ibrahim et al., 2011).

Sample	TAC (mg Anthocyanin/g)
Wheat flour	0.006 \pm 0.08
Black Rice flour	53.87 \pm 0.49

Table 4.9: Total anthocyanin content of raw materials

The total anthocyanin content of rice analogues (*Graph 4.4*) and raw materials (*Table 4.9*) are listed. Wheat flour doesn't contain significant amount of anthocyanin. Refined wheat flour unlike certain varieties of wheat has significantly low content or lacks anthocyanin. Black Rice is rich in anthocyanin which is present in its bran. The Black Rice flour had 53.87 ± 0.49 mg/g anthocyanin. 50% BRF exhibited maximum amount of anthocyanin content (10.84 ± 0.69 mg/g) while the content decreased as the amount of Black Rice flour decreased. Control showed very insignificant value of anthocyanin which is similar to that of wheat flour. 5.19 ± 0.065 mg/g, 7.22 ± 0.21 mg/g and 9.24 ± 0.41 mg/g sample for 20% BRF, 30% BRF and 40% BRF respectively.



Graph 4.4: Total anthocyanin content of rice analogues

According to Ibrahim et al. (2011), increase in the pH and temperature, during processing and storage would increase the degradation rates of anthocyanins. Xue et al., (2024) also stated that the anthocyanins' chemical properties are unstable and can be easily influenced by external factors such as temperature, pH value, oxygen, light, ascorbic acid, and metal ions; agreeing to the statement made by Ibrahim et al. (2011).

4.9 Aroma Profile Using Electronic Nose Technology

An electronic nose was used to analyse the aromatic characteristics of the rice analogues and Black Rice. The principle of E-nose is based on the detection of volatile organic compounds (VOCs) in the air using an array of chemical sensors. These sensors are designed to respond to different types of VOCs, and the pattern of responses from the sensors is analysed using pattern cognition algorithms to identify and classify the odour. E-noses can be used to analyse various products, such as wine, coffee, and spices, to determine their aroma profile.

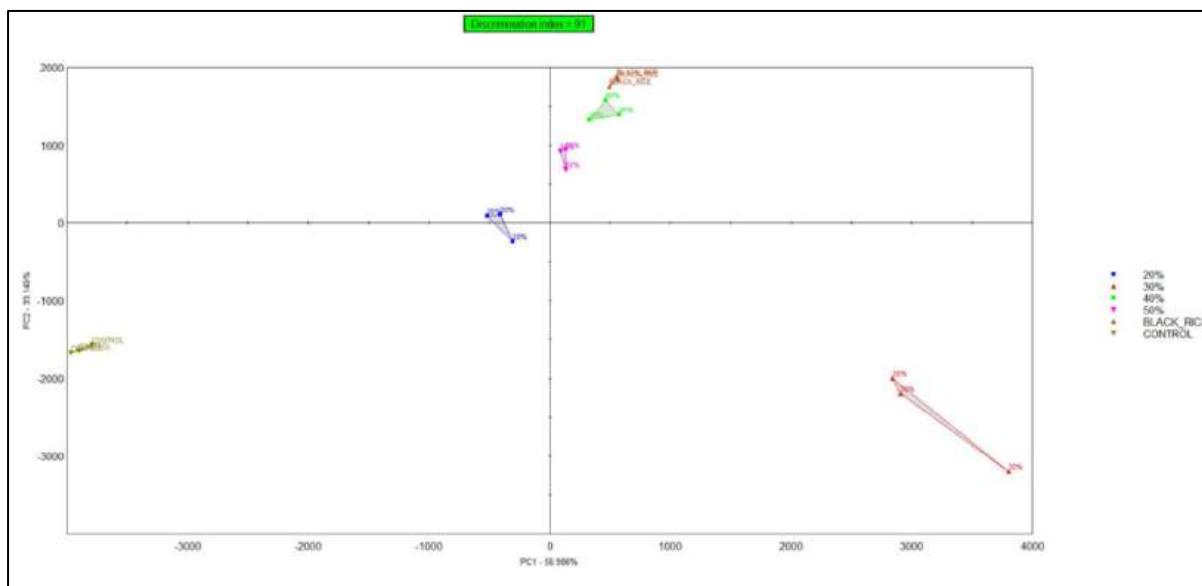
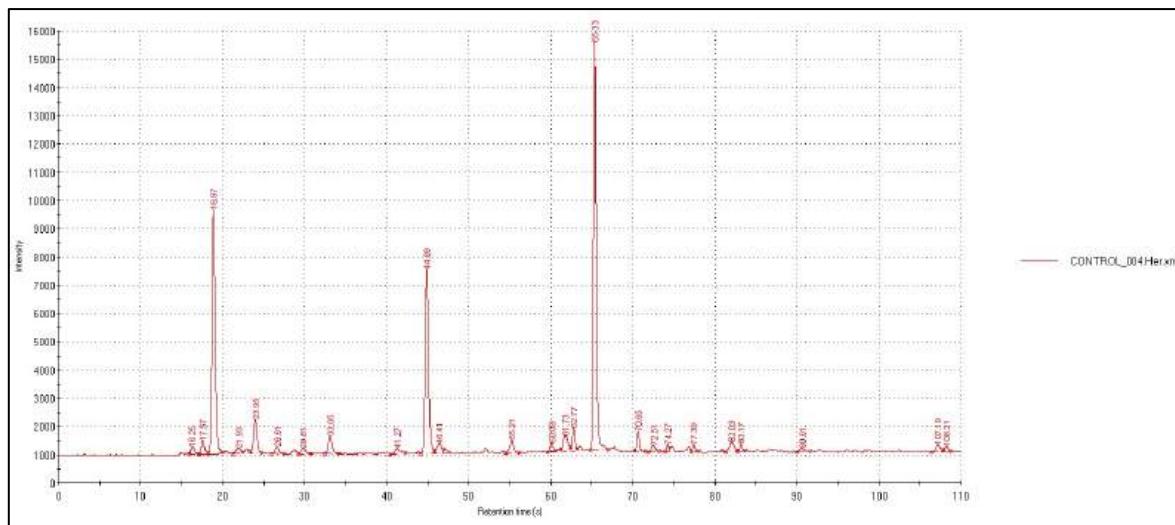


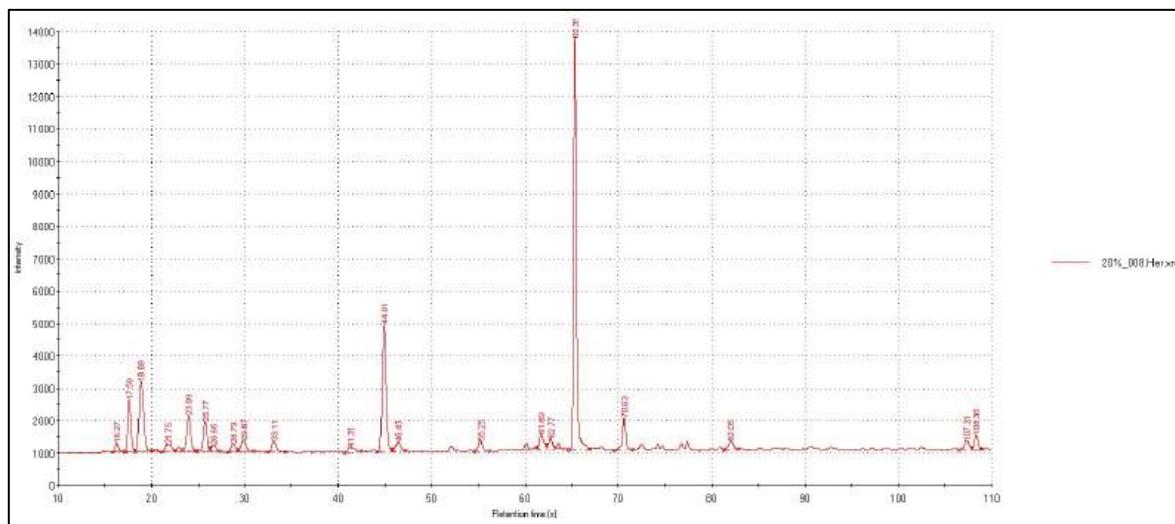
Figure 4.5: PCA of E-Nose data for cooked samples

The volatile components of rice analogues were analysed using E-Nose coupled with PCA (Principal Component Analysis) to identify patterns (*Figure 4.5*). The resulting PCA plot visually presented the relationships and differences among the samples based on their E-Nose data.

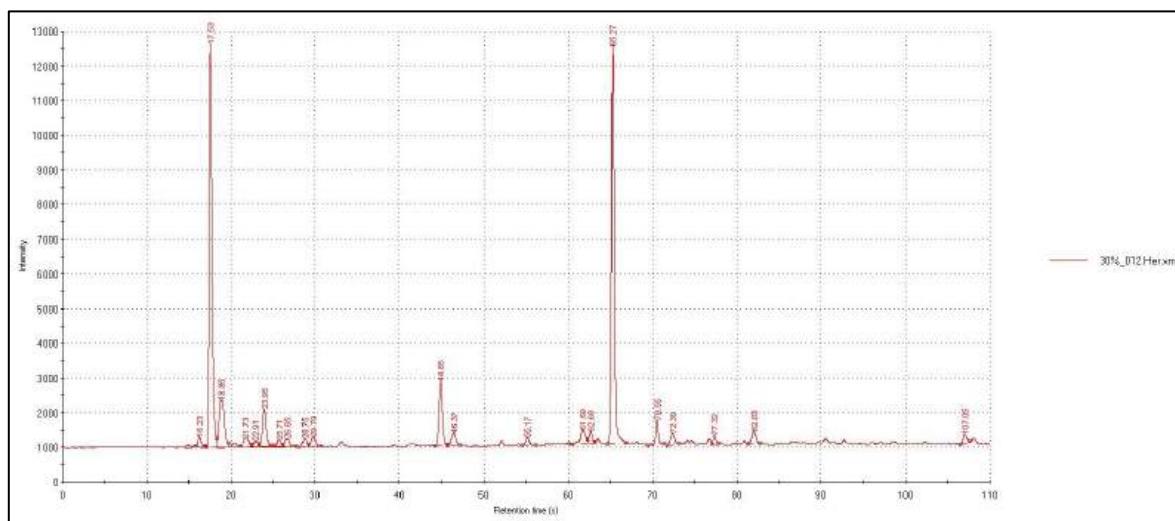
The positioning of the samples in different coordinates reveals distinct clusters. 20% BRF and control are located in the same quadrant, suggesting 20% BRF to have a similar aroma profile as that of control. Similarly, 40% BRF and 50% BRF, with higher amount concentration of Black Rice flour, exhibit a close relationship with Black Rice with 40% BRF sharing more similarity. This suggests that 40% BRF is the closest to Black Rice while 20% BRF is closest to control in terms of aromatic profile. 30% BRF is far from both control and Black Rice and can be interpreted to have the combination of all flavours of both control and Black Rice.



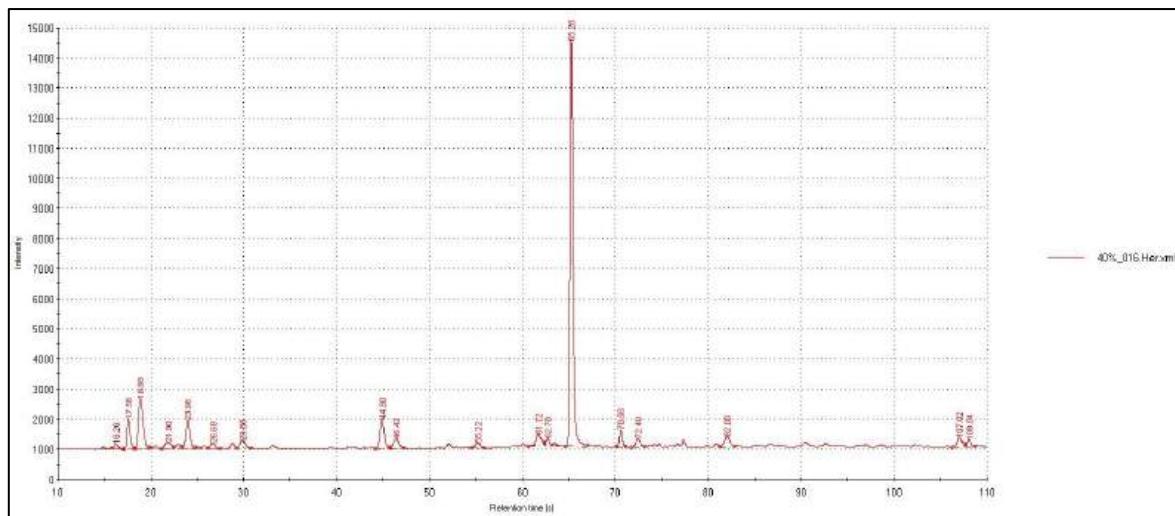
(a) control



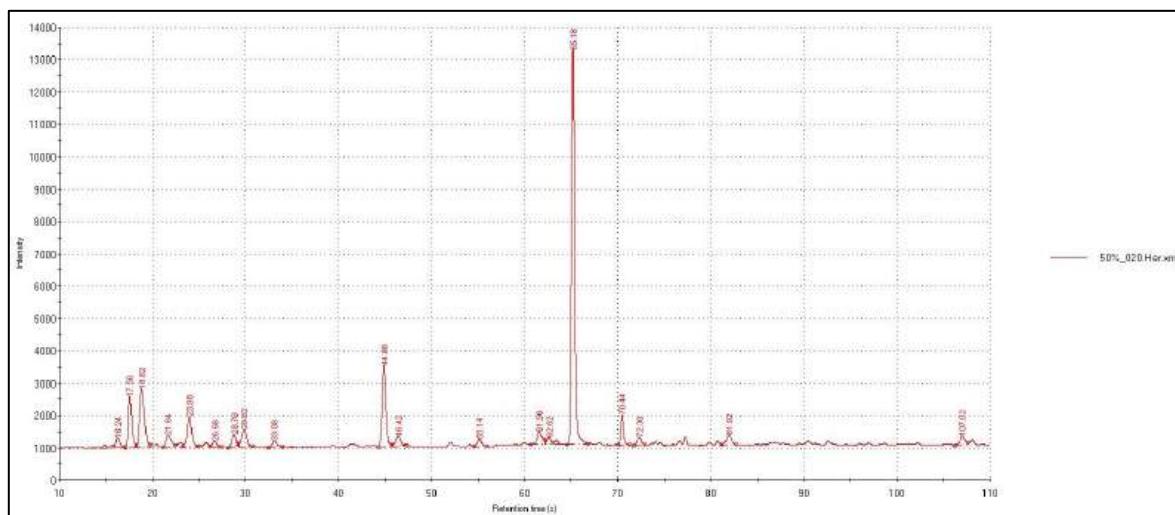
(b) 20% BRF



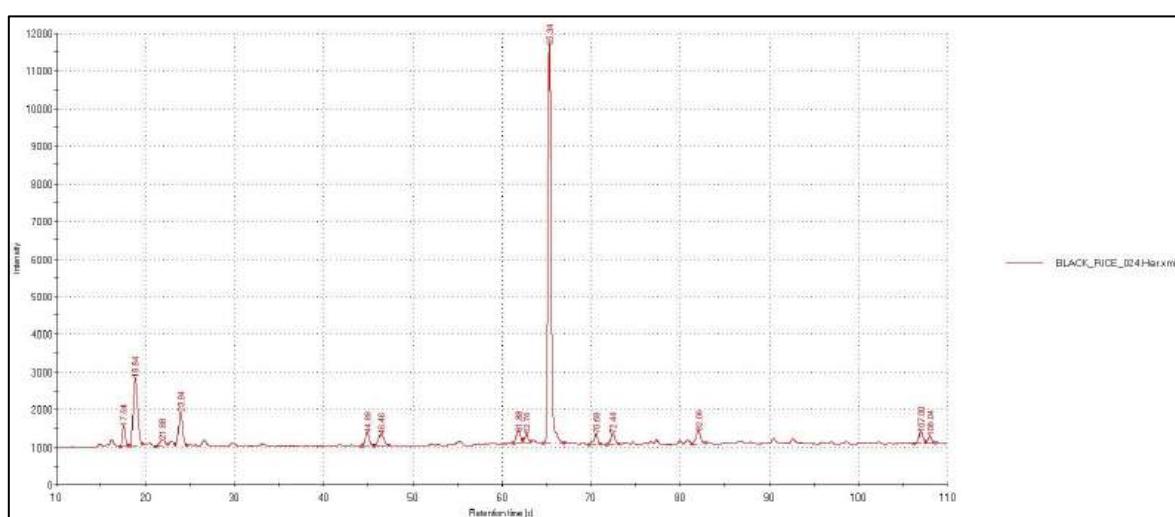
(c) 30% BRF



(d) 40% BRF



(e) 50% BRF



(f) Black Rice

Figure 4.6: Graphs obtained for the E-Nose for the cooked samples

The Kovat indices of the primary volatile chemicals identified in cooked samples' headspace were investigated. The E-nose gas chamber collects volatile gases from the headspace, which are then breathed and monitored by the sensor's functional components. The electrical characteristics of the material change due to the absorption of functional materials for specific gases. As a result, gas concentrations can be characterized using electrically generated signals (Lihuan et al., 2017).

The identified compounds belonged to various functional groups such as aldehydes, ketones, alcohols, acids, esters, sulphur compounds, hydrocarbons, nitrogen compounds and others. The compounds found at various retention times reveal the varied aromatic profile of the cooked samples by contributing a variety of sensory qualities, ranging from fruity and citrusy to nutty and earthy.

The primary components identified in cooked samples with their retention time is as stated in *Table 4.10*.

Retention Time (approx. value)	Component Name	Sensory Description
18 (17.53 – 17.59)	Propanal	Cocoa; Earthy; Nutty
	Dimethyl Sulfide	Fruity
19 (18.82 – 18.89)	2-Propanol	Musty; Pleasant; Woody
	2-Methylpropanal	Baked Potato; Burnt; Green; Malty; Pungent; Toasted
45 (44.85 – 44.91)	Hexanal	Acorn; Fishy; Sweaty; Tallowy
	Butyl Acetate	Fruity
	Ethyl Butyrate	Caramelized, Fruity, Bubble Gum
	Propyl Propanoate	Fruity; Winey
	Methyl 2-Methylbutanoate	Chewing Gum, Fruity
	Butanoic Acid	Butter; Cheese; Rancid; Sweaty
66 (65.18 – 65.35)	1, 8-Cineole	Liquorice; Minty; Pine
	Acetylpyrazine	Bread; Nutty; Coffee; Chocolaty; Roast
	Octanal	Citrus
	Alpha-Terpinene	Citrus; Woody
	Trans-Hex-2-Enyl Acetate	Green; Sweet; Waxy

Table 4.10: Volatile compounds detected in the cooked samples using Kovats retention indices

Based on *Figure 4.6 (a)*, control has shown highest peaks at retention time (RT) around 18 (Propanal & Dimethyl Sulfide), 19 (2-Propanol & 2-Methylpropanal), 45 (Hexanal, Butyl Acetate, Ethyl Butyrate, Propyl Propanoate, Methyl 2-Methylbutanoate & Butanoic Acid) and 66 (1,8-Cineole, Acetylpyrazine, Octanal, Alpha-Terpinene & Trans-Hex-2-Enyl Acetate).

When compared to control, 20% BRF (*Figure 4.6 (b)*) has a decrease in peaks with RT 18 and 19 but increase at 45 and 66; 30% BRF (*Figure 4.6 (c)*) with increase in RT at 18 while 45 and

66 decreased. 40 % BRF (*Figure 4.6 (d)*) showed decrease in peaks at RT 18, 19, 45 and 66. 50% BRF (*Figure 4.6 (e)*) had an increase at RT 45 and 66 with decrease in peaks at RT 18 and 19. RT at 18, 45 and 66 had a decrease with RT at 19 having an increase in case of Black Rice (*Figure 4.6 (f)*).

It can be hence deduced that, the relative proportion of the main classes of volatiles in 100% black rice was significantly different from that in control. Inclusion of black rice flour to wheat flour has caused a shift in the aroma profile with 40% BRF showed an overall decrease across all major retention times, implying a potential loss in intensity of key volatile compounds.

Increased RT 18 corresponds to cocoa, nutty, and fruity notes, while RT 19 introduces more complex, roasted, and pungent scents. The shift at RT 45 highlights a mix of fishy, tallowy, and fruity elements, whereas RT 66, which consistently shows the highest intensity, reinforces the dominance of Acetylpyrazine, contributing roasted, nutty, and chocolaty tones. Any notable shift in these RT will lead to the alteration of overall aromatic profile.

4.10 Flavour Profile Using Electronic Tongue Technology

An electronic tongue with seven sensors and one reference electrode (Ag/AgCl) was used to analyse the taste characteristics of the rice analogues and Black Rice. The principle of e tongue is based on the detection of different chemical compounds and sample using an array of sensors that respond to different types of ions or molecules. These sensors can detect various tastes, such as sour, sweet, salty, and bitter. The working of e tongue involves two main stages: sensing and data analysis.

During sensing, the liquid sample is analysed by the sensors, and the signals are converted into digital data. In data analysis, the data is processed using chemometric techniques to identify and classify the taste based on the pattern responses from the sensors. E-tongue can be used to analyse various products, such as wine, juices and dairy products, to determine their taste profile (Rodríguez-Méndez ML et al, 2008).

The Principal Component Analysis (PCA) plot in *Figure 4.7* illustrates the relationship between 5 rice analogue samples and Black Rice, providing insights into the variations and similarities among the samples based on their E-Tongue data. The discrimination index of 83 indicates a good level of discrimination between the samples, signifying that the E-Tongue analysis successfully distinguishes the samples based on their sensory characteristics.

The positioning of the samples in different coordinates reveals distinct clusters. Samples, 20% BRF and 30% BRF are located closer in the same quadrant, suggesting similar sensory profiles as that of control. Similarly, samples 40% BRF and 50% BRF, with higher amount concentration of Black Rice flour, exhibit a close relationship with Black Rice with 40 % BRF sharing more similarity. This suggests that 40% BRF is the closest to Black Rice while 20% BRF is closest to control in terms of taste profile.

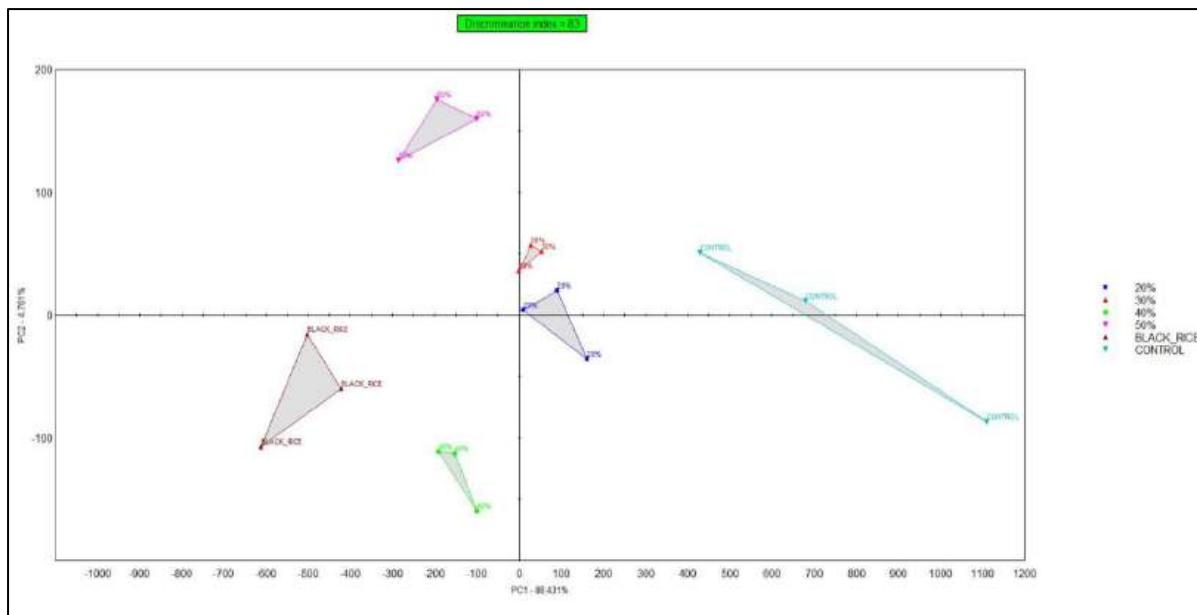
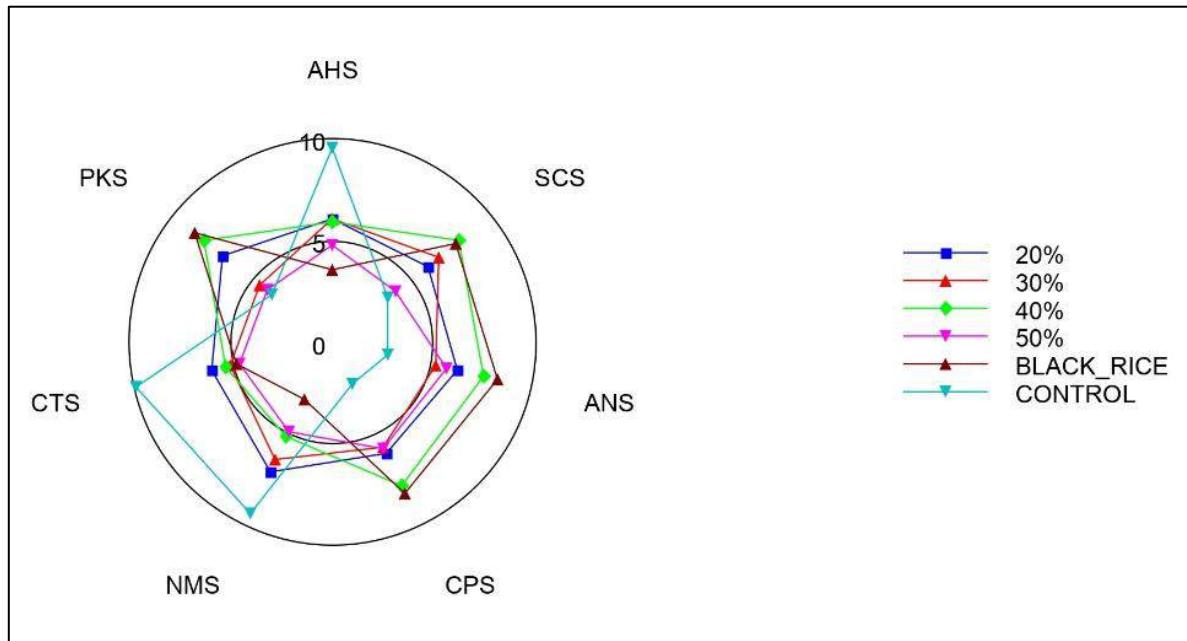


Figure 4.7: PCA of E-Tongue data for cooked samples

The AHS, CTS, NMS, ANS, and SCS represent sensors for sourness, saltiness, umami, sweetness, and bitterness, respectively, while PKS and CPS are general-purpose sensors for complex tastes, respectively, represent sourness, saltiness, umami, sweetness, and bitterness (Xu Y et al., 2021).



Graph 4.5: Radar chart of E-Tongue for different cooked samples

The taste radar map presented in *Graph 4.5* provides valuable insights into the taste profiles of the samples. The map represents the relative intensity of taste attributes on a scale of 0 to 10, allowing for a detailed analysis of the taste differences among the samples. The radar chart

clearly indicates that the samples had better responsive values in terms of AHS, CTS and NMS showing its heightened responsive to sourness, saltiness and umami.

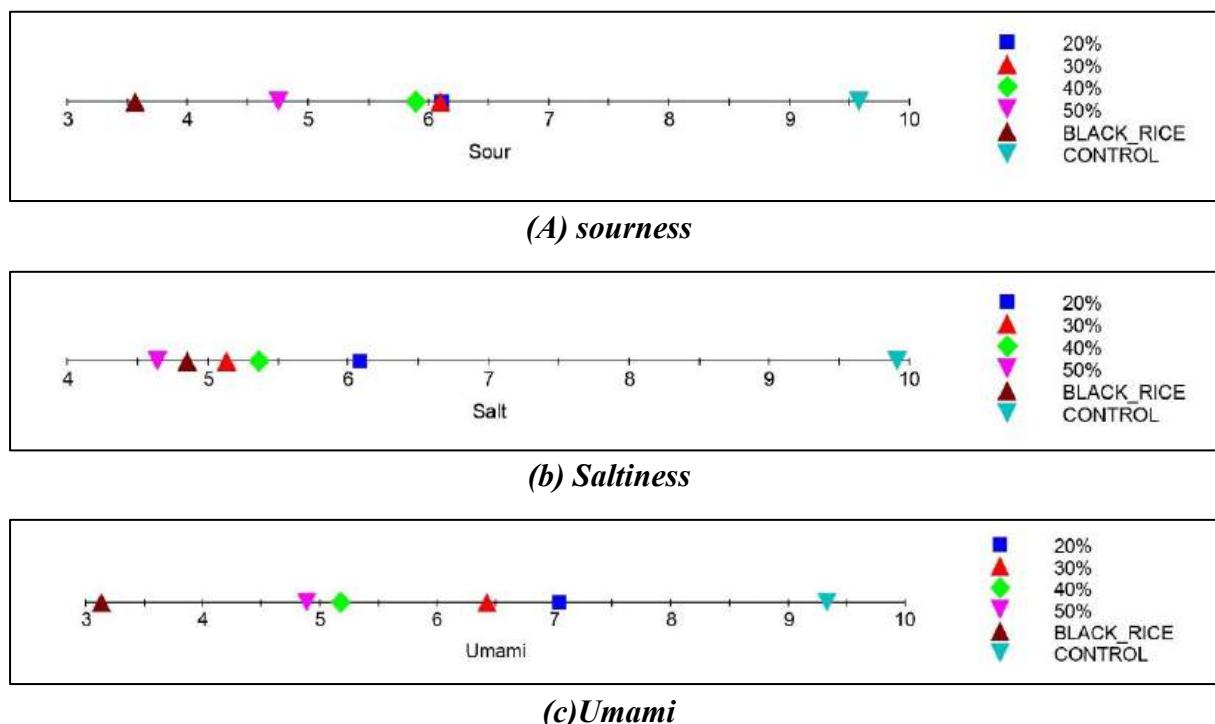


Figure 4.8: Comparison of E-Tongue sensor responses to the cooked samples

On further comparison of E-tongue sensor responses to the cooked samples in terms of sourness, saltiness and umami (Figure 4.8), one prominent observation is that the Umami, saltiness and sourness of the samples have decreased from control to Black Rice. Control exhibited profound response to sourness, saltiness and umami followed by 20% BRF. Black Rice showed the least amount in all these 3 key senses with a subtle variation on the saltiness perception where 50% BRF is the least.

This observation suggests that wheat flour plays a key role in enhancing the saltiness, sourness, and umami Flavors in the products, likely due to its composition and interaction with taste receptors. As black rice flour is introduced and its proportion increases, the intensity of these taste elements decreases, indicating that wheat flour contributes more significantly to these specific sensory attributes. This shift in taste perception could influence product formulation and consumer preferences, depending on the desired balance of Flavors.

CHAPTER – 5

CONCLUSION

CONCLUSION

In conclusion, this study successfully developed rice analogues enriched with Black Rice flour (BRF), leveraging extrusion technology to replicate the natural attributes of traditional rice. By employing formulations of Black Rice flour (BRF) and wheat flour (WF) in varying ratios (20%, 30%, 40%, and 50% BRF), the research underscores the intricate interplay of ingredients, formulation processes, and analytical parameters needed to create nutritionally enhanced rice analogues. The incorporation of BRF was found to significantly influence the structural, functional, and sensory properties of the analogues, demonstrating its potential as a versatile and innovative ingredient.

Key findings revealed the superior nutritional profile of BRF, with enhanced dietary fiber, ash, and antioxidant content. However, challenges such as elevated cooking losses and textural modifications were also observed in the rice analogues, requiring a thoughtful balance between functional improvements and structural stability. The increasing concentration of Black Rice flour in the formulations introduced notable changes, including longer cooking times, greater water absorption, and higher cooking yields. While higher BRF concentrations diluted gluten proteins, resulting in increased cooking losses, these formulations still showcased shorter cooking times compared to Black Rice itself—offering a convenient and nutritionally enriched alternative.

Sensory evaluations further reinforced the appeal of formulations with higher BRF concentrations, particularly those with 40% and 50%, which successfully combined convenience, enhanced nutrition, and a taste reminiscent of traditional rice. Although thermal processing partially degraded bioactive compounds compared to raw materials, the study highlights the importance of optimizing processing techniques to preserve these functional benefits. External factors such as pH, temperature, and environmental conditions during processing and storage were found to influence anthocyanin stability, emphasizing the need for precision in production methods.

In addition, advanced analytical tools like the Electronic Nose (E-Nose) and Electronic Tongue (E-Tongue) provided valuable insights into the aroma and taste profiles of the analogues, further validating their consumer appeal.

These results collectively demonstrate that the 40% BRF rice analogue, in particular, offers a nutritious and convenient alternative to traditional Black Rice. With improved cooking times, retained health benefits, and strong potential for commercial production, these rice analogues address the global demand for healthier staple foods.

CHAPTER – 6

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CHAPTER – 7

APPENDICES

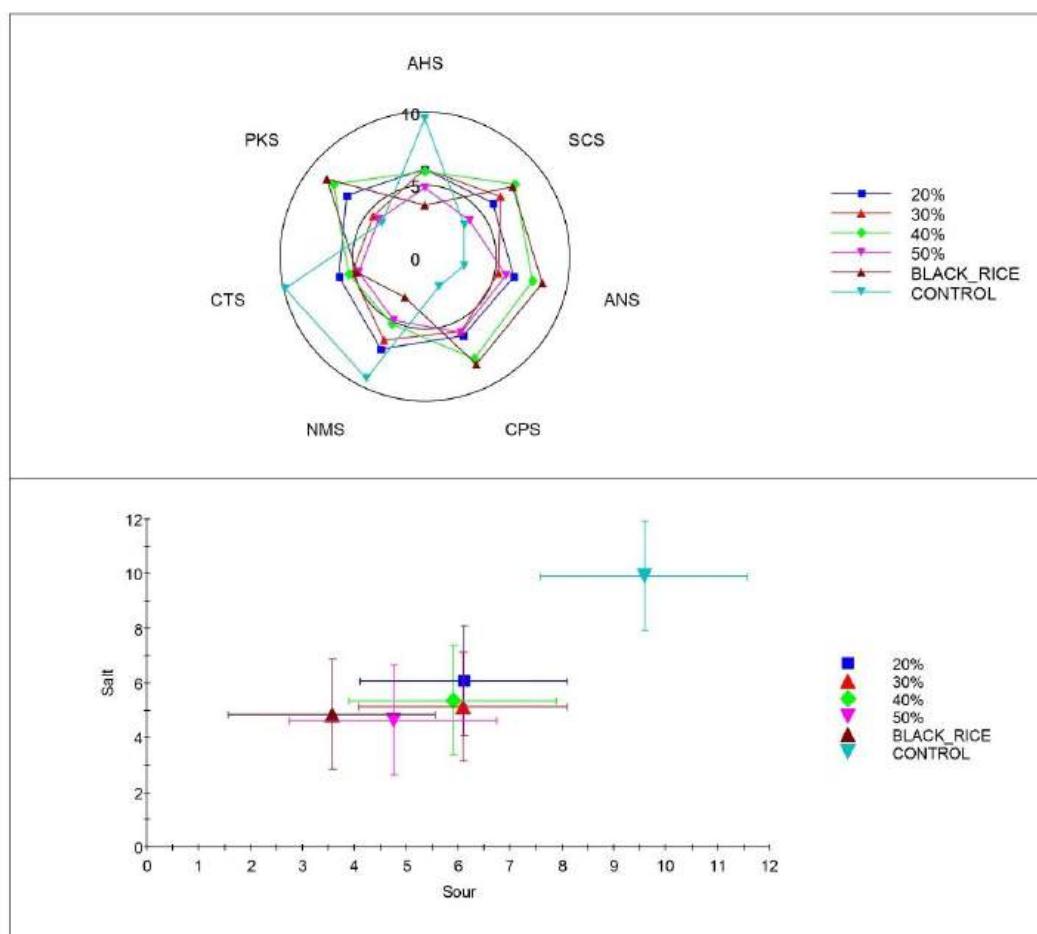
APPENDICES

1. Statistical report of E-Tongue for different cooked samples

Statistics report							
<i>Model supervisor</i> Instrument: Astree Ref AST-059 AM Ref 1814043018925				<i>Model creation date:</i> 3/26/2025 3:57:45 PM <i>Library:</i> COOKED RICE 26-3-2025.lbx			
Preprocessing method AverageValue Time1 = 100 Time2 = 120 NoPretrait				Data processing method Taste screening			
	AHS	PKS	CTS	NMS	CPS	ANS	SCS
20%	6.10	6.90	6.10	7.00	6.10	6.30	6.00
30%	6.10	4.50	5.10	6.40	5.70	5.20	6.70
40%	5.90	8.00	5.40	5.20	7.90	7.70	8.00
50%	4.80	4.10	4.60	4.90	5.80	5.80	4.00
BLACK_RI	3.60	8.70	4.90	3.10	8.30	8.30	7.80
CONTROL	9.60	3.80	9.90	9.30	2.30	2.80	3.50
<i>Note:</i>							
						<i>Visa:</i>	
<i>User:</i> XXXXXXXX	<i>Date:</i> 3/26/2025 3:57:45 PM				<i>Page</i> 1		
<i>Company name</i> ☎ xx xx xx xx xx							

Statistics report

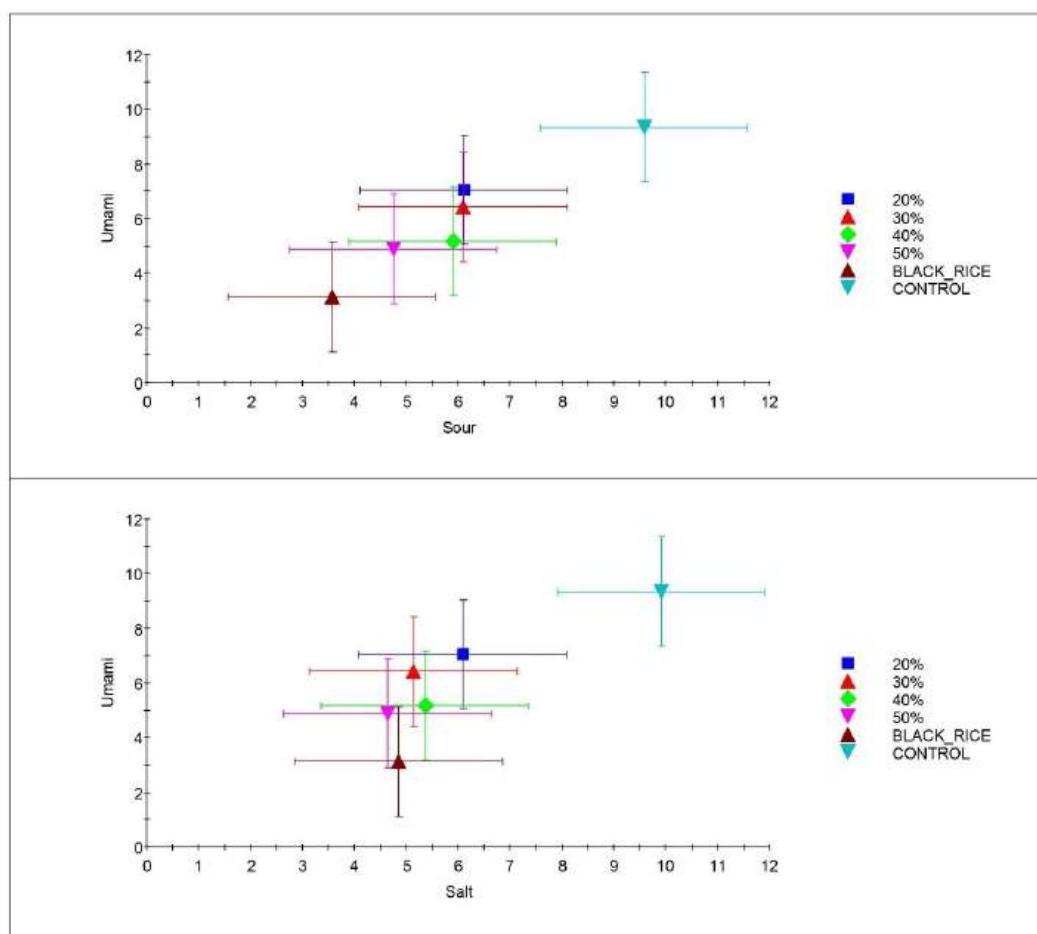
<i>Model supervisor</i>	<i>Model creation date:</i> 3/26/2025 3:57:45 PM
<i>Instrument:</i> Astree Ref AST-059 AM Ref 1814043018925	<i>Library:</i> COOKED RICE 26-3-2025.lbx
Preprocessing method AverageValue Time1 = 100 Time2 = 120 NoPretrait	Data processing method Taste screening



<p><i>Note:</i></p> <div style="border: 1px solid black; height: 100px; width: 100%;"></div>	<p><i>Visa:</i></p> <div style="border: 1px solid black; height: 100px; width: 100%;"></div>
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Statistics report

<i>Model supervisor</i>	<i>Model creation date:</i> 3/26/2025 3:57:45 PM
<i>Instrument:</i> Astree Ref AST-059 AM Ref 1814043018925	<i>Library:</i> COOKED RICE 26-3-2025.lbx
Preprocessing method AverageValue Time1 = 100 Time2 = 120 NoPretrait	Data processing method Taste screening



<p><i>Note:</i></p> <div style="border: 1px solid black; height: 100px; width: 100%;"></div>	<p><i>Visa:</i></p> <div style="border: 1px solid black; height: 100px; width: 100%;"></div>
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2. List of all the identified compounds in the sample

(a) Black Rice

Retention Time (min)	Name	Sensory Descriptors
17.54	Propanal	Acetaldehyde; Cocoa; Earthy; Ethereal; Nutty; Plastic; Pungent; Solvent
	Dimethyl Sulfide	Cabbage; Corn; Fruity; Gaseous; Gasoline; Green; Moldy; Onion; Sulfurous; Sweet; Tomato; Vegetable soup
18.84	2-Propanol	Acetone; Alcoholic; Ethanol; Ethereal; Musty; Pleasant; Rubbing alcohol; Woody
	2-Methylpropanal	Aldehydic; Baked potato; Burnt; Floral; Fresh; Fruity; Green; Malty; Pungent; Sharp; Spicy; Toasted
44.88	Methyl 2-Methylbutanoate	Apple; Chewing gum; Fatty; Fruity; Green; Lily; Powdery; Solvent; Spirit
	Hexanal	Acorn; Aldehydic; Fatty; Fishy; Fresh; Fruity; Grassy; Green; Herbaceous; Leafy; Sharp; Strong; Sweaty
	Butyl Acetate	Banana; Bitter; Ethereal; Fruity; Green; Pear; Pineapple; Pleasant; Solvent; Strong; Sweaty; Sweet
	Propyl Propanoate	Apple; Chemical; Fruity; Fruity (sweet); Pineapple; Pungent; Sharp; Sweet; Winey

(b) Control

Retention Time (min)	Name	Sensory Descriptors
16.25	Acetaldehyde	Aldehydic, Ethereal, Fresh, Fruity, Pleasant, Pungent
	Propanal	Acetaldehyde, Cocoa, Earthy, Ethereal, Nutty, Plastic, Pungent, Solvent
17.57	Dimethyl Sulfide	Cabbage, Corn, Fruity, Gaseous, Gasoline, Green, Moldy, Onion, Sulfurous, Sweet, Tomato, Vegetable
18.87	2-Propanol	Acetone, Alcoholic, Ethanol, Ethereal, Musty, Pleasant, Rubbing alcohol, Woody
23.95	Acetic Acid	Acetic, Acidic, Odorless, Pungent, Sharp, Sour, Vinegar
29.81	N-Butanol	Alcoholic, Amyl alcohol, Banana, Cheese, Fermented, Fruity, Fusel, Harsh, Medicinal, Oil
33.05	2,3-Pentanedione	Almond, Apple, Burnt, Butter, Butterscotch, Caramelized, Cheese, Creamy, Fruity, Malty
41.27	Ethyl Isobutyrate	Alcoholic, Ethereal (sweet), Fruity, Fusel, Rubber, Strawberry, Sweet
44.89	Hexanal	Acorn, Aldehydic, Fatty, Fishy, Fresh, Fruity, Grassy, Green, Herbaceous, Leafy
46.41	Furfural	Almond, Baked, Bread, Sweet, Woody
55.21	Heptanal	Aldehydic, Citrus, Fatty, Fish (dry), Fresh, Fruity, Green, Heavy, Oily, Ozone, Pesticide
	(Z)-4-Heptenal	Biscuit, Creamy, Dairy, Fatty, Fishy, Green, Milky, Oily, Potato (boiled), Sweet
	2-Heptanone	Banana, Cheese, Coconut, Fruity, Gaseous, Gravy, Ham, Musty, Nutty, Pear drop, Soapy, Spicy
60.09	Dimethyl Trisulfide	Alliaceous, Cabbage, Fishy, Meaty, Onion (cooked), Rotten food, Sulfurous
	Benzaldehyde	Almond, Bitter, Burnt sugar, Cherry, Fruity, Sharp, Sweet, Woody

62.77	Ethyl Hexanoate	Anise, Apple, Banana, Fruity, Pineapple, Strawberry, Sweet, Waxy
65.33	1,8-Cineole	Camphor, Herbaceous, Licorice, Medicinal, Mentholic, Minty, Pine, Sweet
	Benzyl Alcohol	Aromatic, Balsamic, Floral, Fruity, Phenolic, Rose, Sweet
70.65	N-Nonanal	Aldehydic, Citrus, Fatty, Floral, Fresh, Fruity, Green, Lavender, Melon, Orange, Soapy
	Ethyl 3-(Methylthio) Propanoate	Fruity, Metallic, Pineapple, Sulfurous, Tomato
	Nonan-2-One	Baked, Cheese, Earthy, Fatty, Fresh, Fruity, Green, Ketonic, Sweet
72.51	Benzyl Acetate	Burnt, Floral, Fresh, Fruity, Jasmine, Sweet, Zucchini (boiled)
	Benzoic Acid	Balsamic, Odorless, Pleasant, Winey
74.27	Terpinen-4-Ol	Fruity, Herbaceous, Licorice, Nutmeg, Pine, Spicy, Sweet, Woody
77.39	Decanal	Aldehydic, Burnt, Citrus, Fatty, Floral, Green, Sweet, Waxy
	Methyl Salicylate	Berry, Minty, Peppermint, Sweet, Warm, Winey

(c) 20% BRF

Retention Time (min)	Name	Sensory Descriptors
16.27	Acetaldehyde	Aldehydic; Ethereal; Fresh; Fruity; Pleasant; Pungent
17.59	Dimethyl Sulfide	Cabbage; Corn; Fruity; Gaseous; Gasoline; Green; Moldy; Onion; Sulfurous; Sweet; Tomato; Vegetable soup
18.89	2-Propanol	Acetone; Alcoholic; Ethanol; Ethereal; Musty; Pleasant; Rubbing alcohol; Woody
	2-Methylpropanal	Aldehydic; Baked potato; Burnt; Floral; Fresh; Fruity; Green; Malty; Pungent; Sharp; Spicy; Toasted
21.75	Butanal	Chocolate; Cocoa; Green; Malty; Musty; Pungent
23.99	Butane-2,3-Dione	Butter; Caramelized; Chlorine; Creamy; Fruity; Pineapple; Pungent; Spirit; Strong; Sweet
28.79	N-Butanol	Alcoholic; Amyl alcohol; Banana; Cheese; Fermented; Fruity; Fusel; Harsh; Medicinal; Oil; Sweet
29.87	Pentan-2-One	Acetone; Banana; Ethereal; Fruity; Sweet; Woody
33.11	Ethyl Propanoate	Acetone; Fruity; Solvent
	Pentanal	Acrid; Almond; Berry; Fermented; Fruity; Green; Herbaceous; Malty; Nutty; Pungent; Rubber
	Ethyl Acrylate	Acrid; Fruity; Harsh; Plastic; Pungent; Sour
	2,3-Pentanedione	Almond; Apple; Burnt; Butter; Butterscotch; Caramelized; Cheese; Creamy; Diacetyl; Fresh; Fruity; Sweet
41.31	Ethyl Isobutyrate	Alcoholic; Ethereal (sweet); Fruity; Fusel; Rubber; Strawberry; Sweet
	Propanoic Acid	Acidic; Pungent; Rancid; Vinegar
	Hexanal	Acorn; Aldehydic; Fatty; Fishy; Fresh; Fruity; Grassy; Green; Herbaceous; Sharp

44.91	Butyl Acetate	Banana; Bitter; Ethereal; Fruity; Green; Pineapple; Pleasant; Solvent; Sweet
55.25	Heptanal	Citrus; Fatty; Fish; Fruity; Green; Heavy; Ozone; Pungent; Smoky; Solvent
61.69	Dimethyl Trisulfide	Alliaceous; Cabbage; Fishy; Onion; Rotten food; Sulfurous
65.35	Benzaldehyde	Almond; Bitter; Cherry; Fruity; Oil; Sharp; Sweet
	Octanal	Citrus; Fatty; Floral; Fruity; Green; Lemon; Meat; Orange; Rancid; Waxy
70.63	N-Nonanal	Aldehydic; Chlorine; Citrus; Fatty; Floral; Fresh; Fruity; Gaseous; Green; Orange peel; Soapy; Sweet; Waxy
	Ethyl 3-(Methylthio) Propanoate	Fruity; Metallic; Pineapple; Sulfurous; Tomato
	Nonan-2-One	Baked; Cheese; Earthy; Fatty; Fresh; Fruity; Green; Musty; Soapy; Sweet; Varnish
	2-Phenylethanol	Floral; Honey; Lilac; Rose; Spicy
	Maltol	Baked; Caramelized

(d) 30% BRF

Retention Time (min)	Name	Sensory Descriptors
16.23	Acetaldehyde	Aldehydic; Ethereal; Fresh; Fruity; Pleasant; Pungent
17.53	Dimethyl Sulfide	Cabbage; Corn; Fruity; Gaseous; Gasoline; Green; Moldy; Onion; Sulfurous; Sweet; Tomato; Vegetable soup
18.85	2-Propanol	Acetone; Alcoholic; Ethanol; Ethereal; Musty; Pleasant; Rubbing alcohol; Woody
	2-Methylpropanal	Aldehydic; Baked potato; Burnt; Floral; Fresh; Fruity; Green; Malty; Spicy; Toasted
26.65	3-Methylbutanal	Aldehydic; Almond; Apple; Chocolate; Fatty; Fruity; Green; Malty; Toasted
44.85	Hexanal	Acorn; Aldehydic; Fatty; Fishy; Fresh; Fruity; Grassy; Green; Herbaceous; Leafy; Strong
	Butyl Acetate	Banana; Bitter; Fruity; Green; Pleasant; Solvent; Sweet
55.17	Heptanal	Citrus; Fatty; Fresh; Fruity; Green; Pungent; Rancid; Smoky; Solvent
	2,6-Dimethylpyrazine	Baked potato; Chocolate; Cocoa; Coffee; Nutty; Sweet; Roast
61.69	Dimethyl Trisulfide	Alliaceous; Cabbage; Cauliflower; Fishy; Meaty; Onion; Rotten food; Sulfurous
	Myrcene	Balsamic; Ethereal; Fruity; Geranium; Lemon; Metallic; Musty; Resinous; Spicy; Sweet; Woody
	Butyl Butanoate	Banana; Cherry; Fruity; Pineapple; Sweet; Tropical
	Ethyl Hexanoate	Anise; Apple; Berry; Fruity (sweet); Pineapple; Strawberry; Waxy; Wine gum
62.69	Hexanoic Acid	Cheese; Fatty; Pungent; Rancid; Sweaty
65.27	L-Limonene	Citrus; Minty; Pine; Terpenic; Woody
	1, 8-Cineole	Camphor; Medicinal; Minty; Pine; Sweet
70.55	N-Nonanal	Aldehydic; Citrus; Fresh; Fruity; Orange peel; Sweet; Waxy
	Ethyl 3-(Methylthio) Propanoate	Fruity; Metallic; Pineapple; Sulfurous
	Nonan-2-One	Earthy; Fatty; Fruity; Musty; Sweet
72.39	N-Nonanal	Floral; Fresh; Fruity; Lavender; Sweet

(e) 40% BRF

Retention Time (min)	Name	Sensory Descriptors
16.26	Acetaldehyde	Aldehydic; Ethereal; Fresh; Fruity; Pleasant; Pungent
17.58	Dimethyl Sulfide	Cabbage; Corn; Fruity; Gaseous; Green; Onion; Sulfurous; Sweet; Vegetable soup
18.88	2-Propanol	Acetone; Alcoholic; Musty; Pleasant; Rubbing alcohol; Woody
	2-Methylpropanal	Aldehydic; Baked potato; Burnt; Floral; Green; Malty; Sharp; Toasted
44.90	Hexanal	Acorn; Aldehydic; Fatty; Fishy; Grassy; Green; Sharp; Tallowy
	Butyl Acetate	Banana; Bitter; Ethereal; Fruity; Green; Pineapple; Solvent; Sweet
46.42	Furfural	Almond; Baked; Benzaldehyde; Bread; Sweet; Woody
55.22	Heptanal	Citrus; Fatty; Green; Heavy; Smoky
61.72	Hexyl Acetate	Acidulous; Apple; Banana; Citrus; Fruity; Sweet
62.70	Hexanoic Acid	Cheese; Fatty; Goat; Pungent; Sweaty
65.26	Octanal	Citrus; Fatty; Floral; Green; Lemon; Orange peel; Waxy
70.56	N-Nonanal	Aldehydic; Citrus; Floral; Fresh; Lavender; Sweet; Waxy

(f) 50% BRF

Retention Time (min)	Name	Sensory Descriptors
16.24	Acetaldehyde	Aldehydic; Ethereal; Fresh; Fruity; Pleasant; Pungent
17.56	Dimethyl Sulfide	Cabbage; Corn; Fruity; Gaseous; Gasoline; Green; Onion; Sulfurous; Sweet; Tomato; Vegetable soup
18.82	2-Propanol	Acetone; Alcoholic; Ethanol; Musty; Pleasant; Rubbing alcohol; Woody
	2-Methylpropanal	Aldehydic; Baked potato; Burnt; Fruity; Green; Sharp; Toasted
26.68	3-Methylbutanal	Aldehydic; Almond; Apple; Fruity; Green; Malty
33.08	Ethyl Propanoate	Acetone; Fruity; Solvent
44.88	Hexanal	Acorn; Aldehydic; Fatty; Fresh; Fruity; Grassy; Green; Sharp; Sweaty
	Butyl Acetate	Banana; Bitter; Ethereal; Fruity; Green; Pineapple; Solvent; Sweet
55.14	Heptanal	Citrus; Fatty; Fresh; Green; Smoky
	Pentanoic Acid	Acidic; Beefy; Cheese; Pungent; Sweaty
61.56	Benzaldehyde	Almond; Bitter; Burnt sugar; Fruity; Sharp; Sweet; Woody
	Z-3-Hexen-1-Ol, Acetate	Apple; Banana; Fruity (sweet); Grassy; Green; Sweet
	Butyl Butanoate	Banana; Cherry; Fruity; Juicy; Pineapple; Sweet; Tropical
	Ethyl Hexanoate	Anise; Apple; Banana; Berry; Green; Pineapple; Strawberry; Sweet
62.62	Hexanoic Acid	Cheese; Fatty; Goat; Pungent; Sweaty
65.18	1, 8-Cineole	Camphor; Medicinal; Minty; Pine; Sweet
	Acetyl Pyrazine	Biscuit; Chocolate; Nutty; Popcorn; Roast
70.44	N-Nonanal	Aldehydic; Citrus; Fresh; Gravy; Green; Lavender; Melon; Orange peel; Waxy
	Ethyl 3-(Methylthio) Propanoate	Fruity; Metallic; Pineapple; Sulfurous
	Nonan-2-One	Baked; Cheese; Earthy; Fresh; Fruity; Sweet