# NUTRITIONAL AND ANTIOXIDANT POTENTIAL OF MEDICINAL RICE VARIETY *RAKTHASHALI*

By

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(2017-16-002)



# DEPARTMENT OF COMMUNITY SCIENCE COLLEGE OF HORTICULTURE VELLANIKKARA, THRISSUR - 680 656 KERALA, INDIA

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# DECLARATION

I, hereby declare that the thesis entitled "**Nutritional and antioxidant potential of medicinal rice variety** *Rakthashali*" is a bonafide record of research work done by me during the course of research and the thesis has not previously formed during the basis for the award to me of any degree, diploma, associate ship, fellowship or other similar title, of any other University or Society.

Vellanikkara Date: Aiswarya Girijan

# CERTIFICATE

Certified that the thesis entitled "**Nutritional and antioxidant potential of medicinal rice variety** *Rakthashali*" is a bonafide record of research work done independently by **Ms. Aiswarya Girijan** under my guidance and supervision and that it has not been previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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Introduction

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### **1. INTRODUCTION**

Rice – the grain of life, vitality and vigour is the principal means for fulfilment of life for millions of people worldwide. Rice is an ancient grain which has been used as food by man over 10,000 years and has fed a great number of people over time than any other crop (Sharma, 2014). Rice plays a fundamental role in world's food security and socio-economic development. It provides employment for millions of rice farmers, processors and traders worldwide (Fresco, 2005). India has the largest acreage (44 million ha) under rice with a production of 100 million tonnes (FAO, 2017). Globally, India is ranked first in terms of rice area and second in rice production (Nayak *et al.*, 2014). According to FAO (2018) the global paddy production in the year 2017-2018 was 503.9 million tonnes and in Kerala, the rice production was 1, 71,398 Ha during the year of 2016-2017.

Worldwide, rice provides 27 per cent of the dietary energy and 20 per cent of the dietary protein intake. In Asia alone, more than two billion people obtain 60 to 70 per cent of their energy from rice and its derived products (FAO, 2004). Grain quality has always been an important consideration in rice variety selection and development. It is the primary determinant for marketability and consumer acceptability.

Rice has relished a unique status in Ayurveda as food and medicine. There are some of the medicinal rice varieties grown by farmers for their medicinal purposes are *Njavara, Chennellu, Kunjinellu, Erumakkari, Karuthachembavu* and *Kavunginpoothala* (Kumary, 2004). Several studies validated the medicinal properties of these rice varieties and traditionally they are either used as medicine or as ingredient in medicinal preparations.

Kerala is well known for the rich genetic diversity of the traditional rice varieties which includes many medicinal rice varieties also. There are several other red rice varieties which are indigenous to Kerala like *Karimbalan*, Karuthan, Kalladiyaran, Kavunginpoothali, Kumkumashali, Kunjicheera, Kunjunji, Kunjootti and Kuttiveliyan.

Coloured rice varieties are reported as potent sources of antioxidants and encouragements as viable sources of antioxidants. There are several pigmented rice varieties which include black, red and dark purple, dark blue, red brown, black purple, or dark red-purple grains. It contains various phytochemicals like flavones, tannin, phenolics, sterols, tocols,  $\gamma$  -oryzanols, amino acids, and essential oils (Nakornriab *et al.*, 2008). Pigmented rice has contribute to various therapeutic properties including antioxidant, anticarcinogenic, antiatherosclerosis and antiallergic activities.

In the recent years, because of high nutritional and therapeutic potential of pigmented rice varieties, researchers and consumer preferences have received increased attention towards pigmented types.

Red rice is available as unhulled or partially hulled and it has a characteristic flavour with high nutritional qualities. There are different kinds of red rice includes red cargo rice, *Rakthashali* rice, Bhutanese red rice, camargece red rice and *matta* rice. *Shali* rices are the ones which mature in winter and are tasty and have so many medicinal properties (Sampong *et al.*, 2011). The founding fathers of ancient Ayurveda- Susruta, Charaka and Vagbhata refer to the medicinal value of *shali*, *vrihi* and *shastika* rice varieties in relation to top most. Charaka, the author of *Charaka Samhita* classify *Rakthashali* or *lohit shali* as the most efficacious to subdue the three deranged doshas – *the vata, the pitta and the kapha* (Nene, 2005).

*Rakthashali* (*Oryza sativa*. *L*) is an indigenous pigmented medicinal rice variety which is reported to be good for health, skin, eyesight, diuretic and has antibacterial, antidiarrheal, antidysenteric, antifungal, antiinflammatory, antioxidant, antithyroid, antiviral, antitumor and antihyper cholesterolemic activities. It is traditionally considered as good for fevers and ulcers.

In *Rakthashali* rice, the presence of beneficial components like flavonoids and phenols possess characteristic antioxidant and anti-inflammatory properties. Anthocyanins and proanthocyanidins are the common pigments in red rice. The antioxidant and the radical scavenging properties of coloured rice cultivars helps to promote human health by reducing the concentration of free radicals present in the body. Phenolic compounds in red rice varieties have the ability to donate hydrogen and act as reducing agents and singlet oxygen quenchers. Hence, red rice have protective effects on cell constituents against oxidative damage. Such antioxidant characteristics of phenolics in red rice has proven to prevent cancer, cardiovascular and nerve diseases. Bioactive compounds like phenolics and flavonoids, anthocyanins, proanthocyanidins, vitamin E and gamma oryzanol are found in the outer and inner layer of coloured rice. Due to its antioxidant action, it is drawing immense interest in research world as an inhibitor of cell proliferation in cancer.

In addition to nutritive and medicinal value, red rice varieties possess many other special features. The red and black rice varieties are comparatively more resistant to storage insect pests than brown rice varieties.

Considering the above, the present study is to evaluate the 'Nutritional and antioxidant potential of medicinal rice variety *Rakthashali*' has been undertaken with the following objectives:

1. To evaluate the nutritional, cooking, physicochemical and antioxidant properties of medicinal rice variety *Rakthashali* 

2. To develop functional food products based on it.

# Review of Literature

### 2. REVIEW OF LITERATURE

Literature pertaining to the study entitled 'Nutritional and antioxidant potential of medicinal rice variety *Rakthashali*' is reviewed under the following headings.

- 2.1 Pigmented and medicinal rice varieties
- 2.2 Physical qualities of rice varieties
- 2.3 Nutritional qualities of rice varieties
- 2.4 Health benefits of rice varieties
- 2.4 Antioxidant properties of rice varieties
- 2.5 Rice based products

## 2.1 Pigmented and medicinal rice varieties

Over 2.7 billion people in the world depend on rice as the staple and hence it is considered as the most important food crops in the world. In India, area under rice is 44.6 m ha with total output of 80 million tonnes with an average productivity of 1855 kg/ha. It is grown in West Bengal, Uttar Pradesh, Madhya Pradesh, Bihar, Orissa, Andhra Pradesh, Assam, Tamil Nadu, Kerala, Punjab, Maharashtra and Karnataka and contribute to total 92 per cent of area and production (Mukesh *et al.*, 2013).

Pigmented rice varieties are consumed from ancient time in Asia, especially in India, China, Japan, Korea and many other countries in Southeast Asia (Tananuwong *et al.*, 2010). Rice varieties are classified as black rice, red rice and brown rice with dark red, dark purple, dark blue, red brown, black purple or dark red purple grains depend upon the colour of the kernel (Sampong *et al.*, 2011). Compared to white rice, coloured rice are more nutritious and it has a characteristic flavour as well as medicinal properties.

The *Garuda Purana* mentioned the importance of the medicinal uses of red *shali* as a destroyer of the three doshas. *Mahashali* is referred to as highly restorative (Kumar, 1988). Red rice is highly valued as they had the power to redress the imbalance in the *tridosha* which are the basic operating principles that govern the psychobiological aspect of the body (Ahuja *et al.*, 2007).

In *Kashyapiyakrishisukti*, one of the first Indian treatises on agriculture, the compiler Kashyapa (800 AD) describes various *shali* rices namely *shali*, *kalama*, and *sambaka* type (Ayachit, 2002). According to '*Sushrutha Samhithan*' rice can be classified into two broad categories called *Shali* rice and *Vreehi* rice. *Shali* rice are winter grown, characterized by red husk and white kernals, whereas *Vreehi* rice are autumn grown with different husk colours and red kernals. Ayurveda considers *shali* rice as "*Laghu*" meaning light or easily digestible, cooling, diuretic capable of alleviating thirst.

Kerala owned a rich treasure of diverse land races or varieties, but the well-known genetic diversity of rice in Kerala has been lost due to the advent of modern rice varieties (Shylaraj *et al.*, 2018).

*Rakthashali* is considered equivalent to *Njavara* in its medicinal value (Menon, 1976). The well-known ayurvedic health practitioners of history, like Susruta (400 BC), Charaka (700 BC) and Vagbhata (700 AD) considered *Rakthashali* rice as the best one amoung *shali* varieties (Nene, 2005).

Traditional rice varieties of Kerala, *Rakthashali, Chennellu, Kunjinellu, Erumakkari, Varakan, Poovali, Thanavala* are believed to be *shali* rice varieties of which *Chennellu* is considered be the best. *Vreehi* rice are considered "*Guru*" (heavy) compared to *shali* rice. Varieties like *Njavara, Karimkuruva, Perunellu, Uimkathi, Valanellu, Chitteni, Modan, Aarunellu etc* are *Vreehi* varieties of Kerala (Nair, 2004).

Aanakomban, Aayiramkanna, Aayiramkanni, Aayirammeni, Edavaka are some of the indigenous red rice varieties in Kerala. Kanakam, Kanali, Kaduk Kalvani, Chendhondi, Chembakam, Paalthondi are some of the different brown rice varieties in Kerala. There are several other red rice varieties which are indigenous Kerala like to Karimbalan, Karuthan, Kalladiyaran, Kavunginpoothali, Kumkumashali, Kunjicheera, Kunjunji, Kunjootti, Kuttiveliyan, Kunnukulamban, Kurumbali, Kuruva, Kuruvatti, Kothambari kayama, Kodhandan, Chadayan velumbala, Chennal thondi, Chettuveliyan, Thaichingan, Thondi, Thonnooram thondi, Paal kayama, Paal thondi, Punnadan thondi, Mannu veliyan, Peruvazha, Marathondi, Valiya chennellu, Veliyan, Velumbala, Vellathooval, Vedhandam (Lathadevi et al., 2007).

Njavara, Chennellu, Chembavu, Kalamappari, Neduvali, Velvali, Narikari Kunjinellu, Erumakkari, Varakan, Poovali, Tanavala, Karuthachembavu and Kavunginpoothala are some of the different traditional as well as medicinal rice varieties in Kerala (Kumary, 2004). Some other medicinal rice varieties still available in Kerala are Jaathi, Suggi, Jeeraka, Kamaal Kolaran, Naron, Nalla Chennellu, Vadakkan and Vattan (Mohanty et al., 2013).

According to Dikshit *et al.* (2004), a survey conducted by National Bureau of Plant Genetic Resources (NBPGR) from 1991 to 1998 revealed that about 35 per cent and 21 per cent red rice varieties were found in Orissa and Manipur respectively.

Sasruta (Circa 400 BC), followed by Kashypia-Krishisukti (800-900 AD) and Acharya Bharamisra (15-16<sup>th</sup> century) were some of the earliest to mention rice varieties having medicinal properties (Kumary, 2016).

Traditionally cultivated rice varieties in Kerala are *Kamadara*, *Mukinnaveluthu*, *Dhandan*, *Vettuveliyari*, *Churulakkari*, *Sampathalan*, *Vazhakkannan* (Nair, 2004). *Chennellu* and *Kunjinellu* are indigenous varieties in North Kerala, among them *Chennellu* is the best one, it has bright red coloured grains grown as an upland variety in the areas of Kannur district. *Kunjinellu* is a variant of red coloured *Erumakkari, Karuthachembavu* and *Kavunginpoothala* are the indigenous traditional rice varieties in South Kerala and *Karuthachambavu* has black grains, blackish red kernels and black endosperm (Kumary, 2016).

Kerala, with its age old traditions in the ayurvedic system of medicine, is also famous for the diversity of rice varieties possessing medicinal properties. Rice varieties of medicinal value are still grown by farmers due to their therapeutic potential (Kumary, 2004).

Rice varieties of therapeutic value has been known since ages from ancient scriptures. Exclusive rice varieties of health value were existed in rice growing regions of the world (Siddiq, 2004). *Njavara* rice (*Oryza sativa* L.) is a medicinal red rice variety endemic to Kerala in India. Morphologically, *Njavara* is similar to ordinary rice with husk colour varying from golden yellow to brownish black (Menon, 2004). Two types of *Njavara* have been identified, the white glumed and black glumed, both of which are used in Ayurvedic treatments (Mohanlal, 2011). *Njavara* is known as "Gold with Fragrance" because it is high valued rice with characteristic medicinal properties and unique taste.

Chuvannamodan, Cheriyaryan, Modan, Karuthamodan, Kattamodan, Kuttimodan, Parambuvattan, Karanellu etc are several other indigenous rice varieties and resistant to insects and pests (Kalode *et al.*, 1977). Mundakan, Karamundakan, Karimundakan, Vellamundakan, Athikkirazhi mundakan and Oarumundakan are also different types of traditional rice varieties (Kumary, 2004).

According to Chaudhary (2003), black rice was more consumed in China (62%) followed by Sri Lanka (8.6%), Indonesia (7.2%), India (5.1%), Philippines (4.3%) and Bangladesh (4.1%). Black rice is a good source of natural antioxidant and it possess strong radical scavenging and anti-oxidation effects (Zhang *et al.*, 2006). Black rice has unusual colour, sweet characteristic flavour and it is more common in Asian countries like Korea, Japan, Thailand and China. Flavour is an

important quality in rice in terms of consumer preference (Yang *et al.*, 2008). Black rice contains black bran covered with endosperm and it takes higher cooking time, become sticky and turns to deep purple colour on cooking (Kang *et al.*, 2011).

Ahuja *et al.* (2008) reported that in Ayurveda, rice is grouped according to the growing seasons and the rice transplanted between July to December referred to as *shali* and was considered sweet in taste, cooling in potency, light in digestion and capable of imparting strength.

Later authorities like *Charakasamhitha* classify *Rakthashali* rice is best for medicinal purposes and is considered the most efficacious, and subdues the three deranged doshas – *the vata, the pitta and the kapha*. It also act as diuretic, spermatophytic, refrigerant, eye-invigorating, cosmetic, tonic, good for fever, ulcers and antitoxic and more nutritious and medicinal than other varieties (Kumar, 1988; Krishnamurthy, 1991).

Many of the landraces of Kerala have got medicinal properties which are used in Ayurvedic system of medicine. *Chennellu* and *Kunjinellu* are aboriginal cultivars of North Kerala with medicinal principles whereas *Erumakkari* and *Karuthachembavu* are indigenous to Southern Kerala. *Chennellu*, grown as an upland variety in certain parts of Kannur district is used for treating vomiting and diarrhoea. *Kunjinellu*, known for its small red grains grown in Kannur district is used in treatment of jaundice. *Karuthachembavu* with red kernels and black endosperm is used in the treatment of nausea, vomiting and stomach pains. *Erumakkari* and *Kavunginpoothala* indigenous to Palakkad district is also used for the treatment of diabetics and cough (Kumary, 2004).

Commonly red rice varieties helps in curing blood pressure, fever and also helps in treating several health complications (Nagnur *et al.*, 2006).

Certain types of rice varieties are useful in the treatment of various ailments. *Shali* rice, *Sashtika* rice, *Laja* rice, *Njavara* rice etc are such type of

varieties in which *Shali* and *Sashtika* rice are used for the treatment of piles, anaemia, chest pain and fractures. *Laja* rice is good for fever, diarrhoea and vomiting whereas *njavara* rice had several medicinal properties other than these varieties. It is good for the treatment of cervical spondylitis, low backache, paralysis, rheumatoid arthritis and neuromuscular disorders (Ahuja *et al.*, 2008).

Simi and Abraham (2008) reported that, *Njavara* is regarded as a special rice variety with beneficial properties for circulatory, respiratory, digestive and nervous system according to the Indian indigenous system of medicine and Ayurveda.

Regular consumption of *Njavara* rice gruel ensures longevity and increase the milk production in lactating mothers. A sweet dish made of this rice in jiggery, ghee and cow's milk is also good for lactating mothers. *Njavara* rice is recommended for acute complaints of piles and is considered as a safe food for snake-bitten patients and people with stomach ulcer. It is also useful in urinary complaints of children where the *Njavara* roots is employed in the form of decoction. Traditional healers suggests the consumption of *Njavara* rice flakes, pounded with Aswagandha and sugar increases vitality, vigour, body weight and acts as an aphrodisiac. It is said that *Njavara* rice which act as a semen enhancer and increase fertility in males. *Njavara* gruel (*Njavara kanji*) is a rejuvenating drink especially for children, aged and underweight (Sreejayan *et al.*, 2003).

Smooth paste of *Njavara* cooked rice and *Njavarakizhi* helps in reducing the adverse condition of *vatha*. It is also used for clearing body channels, preventing skin diseases and premature aging, improves blood circulation and corrects mental irregularities (Nair *et al.*, 2013).

Rahman *et al.* (2006) is of the opinion that *Njavara* can be used for diabetes, fever, cough, respiratory problems. It can also be used for de-worming. It is also useful in nervous diseases, body aches, numbress and wasted muscles due to polio myletes and motor neuron diseases (Swaminathan, 2004).

In India, medicinal rice varieties like *Aalcha, Karhani* and *Dhanwar* from Madhya Pradesh, *Mehar* and *Sairphol* from Orissa, *Katheri* from Himachal Pradesh, *Lal dhan* and *Matali* from Uttar Pradesh, *Karibhatta, Atikaya, Mullarya* from Karnataka, are well known used for treating different ailments (Ahuja *et al.*, 2008). Mohanty and Rout (2001) stated that several traditional rice varieties with medicinal properties were identified in the survey made during 1970s and 1980s in Chattisgarh.

Savitha and Singh (2011) found that pre-menopausal women eating the most whole grain fibre (at least 13g/day) had a 41 per cent reduced risk of breast cancer compared to those with the lowest whole grain fibre intake (4g or less /day). It was found that diet rich in fibre from whole grains such as brown rice offered significant protection against breast cancer for pre-menopausal women.

There are several health benefits of black rice includes prevention of cancer, diabetes, heart diseases, alzheimer's disease, gallstones and it also possess potent antioxidants nutraceutical properties. (Yoshida *et al.*, 2011).

*Kafalya* is a rice variety Himachal Pradesh and Uttar Pradesh, is used for treating leucorrhea and abortion complications. Karnataka rice varieties like *Kari kagga* and *Atikaya* are used for coolness while *Neelam samba* from Tamil Nadu is good for lactating mothers (Arumugasamy *et al.*, 2001).

*Kavun*i is a traditional rice variety from Tamil Nadu, known for its antidiabetic properties (Valarmathi *et al.*, 2015).

According to Hedge *et al.* (2013) many traditional red rice varieties were grown for food and in medications. They have the power to redress the physiological imbalances and also red rice varieties are used in increasing milk secretion in lactating mothers. *Matali* and *Lal dhan* of Himachal Pradesh are used for curing blood pressure and fever. *Aalcha* for the treatment of pimples, *Maharaji*, which gives strength and stamina to ladies immediately after delivery, *Baisoo*r for epilepsy and *Laicha* for pregnant women to deliver healthy children are the traditional rice landraces used by the indigenous system of medicine in Chattisgarh. The *Baisoor* variety is used for the treatment of headache and boils. It is also used for skin diseases, ringworm infection, itching and conjunctivitis and as a face pack with neem leaves. Ayurvedic treatise like Indian *Materia medica* has mentioned many rice landraces with medicinal properties like *Navara* and *Gathuran* were used in the treatment of arthritis, whereas varieties *Kalama, Pundarika, Panduka, Sugandhalaka, Kardamak, Maetunaka* and *Mahashali* have different medicinal properties (Mohanty *et al.*, 2013).

## 2.2 Physical qualities of rice varieties

Milling of rice has an intensive effect on its nutrient composition. Milling and polishing of rice resulting in the losses of nutrients which vary considerably depending on the number of layers removed during processing (Murthy and Govindaswamy, 1967). According to Bandyopadhyay and Roy (1992) the factors like moisture content, pretreatments like parboiling, processing equipments and methods etc will affect the milling quality. According to Nirmala (1997) thousand grain weight is an important indicator of milling quality with respect to its kernel size and density. Babu *et al.* (2012) indicated that grain quality characteristics like milling percentage, grain appearance, cooking quality and nutritional components are very important in rice.

Francies *et al.* (2013) observed the total milling per cent of 76 per cent in *Samyuktha* variety. A study conducted by Ponnappan *et al.* (2017) among four different pigmented rice varieties, namely glutinous white rice, black rice and two red rice varieties (TPS-1 and TKM-9), revealed that red rice variety (TPS-1) attained the higher milling per cent of 66.21 per cent.

Nandini (1995) observed a higher head rice yield in traditional varieties. The highest rice yield was observed for variety *Chuvannari, Thavalakannan* followed by *Vyttila-3*. Lakshmi (2011) and Sathyan (2012) observed a head rice recovery of 62.16 per cent and 33.66 per cent in parboiled as well as raw rice of *Jyothi*. Chandhini (2015) reported the head rice recovery of 41.08 per cent for *Jyothi* variety. According to Nadh (2018), *Jyothi* attained the head rice recovery of 52.46 per cent.

Head rice recovery of *Samyuktha* rice variety was found to be 75 per cent. *Harsha* and *Kanchana* obtained the head rice per cent of 75 and 69 respectively (Francies *et al.*, 2013). Ponnappan *et al.* (2017) observed the head rice recovery of 60.18 in red rice variety TPS-1.

Ganesan *et al.* (1998) reported that thousand grain weight of rice varied from 15.76 to 29.24 g. Thomas *et al.* (2013) reported the thousand grain weight of rice varieties ranged from 16.97 to 19.43 g. Lakshmi (2011) noticed *Jyothi* rice has a grain weight of 26.56 g. Sathyan (2012) observed the grain weight of *Jyothi* as 25.67 g. Francies *et al.* (2013) reported the grain weight of 27.9 in *Samyuktha* rice variety. Ponnappan *et al.* (2017) observed higher thousand grain weight in white glutinous rice varieties. Raghuvanshi *et al.* (2017) reported the thousand grain weight of 18.3 g in red rice variety.

Lakshmi (2011) noticed the thousand grain volume of 3 mm<sup>3</sup> in *Jyothi* variety. Volume weight of 25.8 mm<sup>3</sup> was also observed in *Jyothi* rice variety.

The grain size and shape may vary among different cultivars. According to Slaton *et al.* (2001) rice varieties are classified as short, medium or long grains by rough kernel dimension ratio. Rice buyers, millers and consumers, judge the quality by the uniformity of its size and shape as well as the appearance and overall size-shape relationship (Armstrong *et al.*, 2005). Consumer's preference varies based on the type of rice and their origin Azabagaoglum and Gaytancioglu (2009) and Musa *et al.* (2011).

Gupta and Agarwal (2000) opinioned that the morphological characteristics like grain length, L/B ratio, grain size etc. appeared to be quite stable and could be used as primary quality traits for classifying paddy varieties. The kernel length and breadth of rice varied from 5.0–7.5 mm and 1.9–3.0 mm respectively.

According to Nanda *et al.* (1976) the length and breadth of high yielding rice varieties from India varied from 5.2–6.8 mm and 1.9–2.5 mm respectively. Japonica varieties are generally shorter and bolder grains. Reddy (2000) reported that the mean grain length of *Njavara* rice variety ranged from 7.6 mm to 9.3 mm.

Highest length and breadth ratio was recorded for the local white rice (3.75) whereas, the lowest ratio was recorded for brown rice (2.09) (Thomas *et al.*, 2013).

Siebenmorgan *et al.* (1998) observed that when the long rice grains were exposed to varying air temperatures, the extent of physical damage that persist during each and every exposure situation may vary.

Lakshmi (2011) reported that parboiled *Jyothi* rice variety had a grain length of 6.70 mm, grain width of 2.50 mm and length and breadth ratio of 2.66 and is of long medium. Sathyan (2012) observed grain length of 6.65 mm, grain width of 2.20 mm and length and breadth ratio of 3.02 in *Jyothi* variety which specifies the grain as long slender.

There is a particular variation in the size of rice grain which is ranging from long bold to short bold (CRRI, 1999). Maisuthisakul and Changchub (2014) studied the physical qualities of three black rice varieties (*Hawm nil*, *Hawm kanya* and *Kum*) two red rice varieties (*Sangyod* and *Red Jasmine*) and four white rice varieties (*Hawm ubon*, *Lao tek*, *Jasmine* rice 105 and *Sin lek*) in Thailand. Black rice variety *kum* attained the L/B ratio of 2.71 and for *Hawm kanya* it was 3.00. Varieties like *Hawm ubon*, *Laotek*, *Jasmine105*, *Sinlek*, and red *Jasmine* comes under long grain (7.0 mm) and *Hawm kanya, Kum* and *Sangyod* varieties were medium rice grains (6.27–6.47 mm long).

Saikia *et al.* (2012) evaluated the physical dimensions of two pigmented and two non-pigmented aromatic black rice varieties and observed that pigmented aromatic rice recorded the higher kernel weight, longer and slender in shape.

The range in breadth of unhulled grain was from 1.9 to 3.7 mm and the thickness ranged from 1.5 to 2.2 mm (Reshmi, 2012). Thomas *et al.* (2013) investigated the physical qualities of six different rice varieties (white, brown, bario, black, glutinous and basmati rice) marketed in Malaysia. The L/B ratio of these investigated varieties ranged between 2.09 to 3.75. The mean grain width of *Njavara* rice variety ranged from 2.65 mm to 3.33 mm.

Francies *et al.* (2013) observed the kernel length and width of *Samyuktha* variety and it was found to be 5.7 mm and 2.7 mm respectively. *Harsha* and *Kanchana* are long bold red rice varieties which has the L/B ratio of 2.11and 2.73.

A study conducted by Sugeetha (2010) among eight pre-release rice cultures of KAU for various quality attributes found that, the mean length of grain was found to be highest for MO8-20-KR (6.88 mm) and lowest for MO-87-5 (5.60 mm) rice varieties. The width was found to be the highest for the variety OM-3 (2.73) and lowest for MO-95-1 (2.29) variety.

Grain length and grain width of Pakistan rice varieties ranged from 5.38 – 6.38 mm and 1.90 - 2.24 mm. Raghuvanshi *et al.* (2017) justified that the length of red rice grain was less than that of white rice. The physical properties of red rice suggested that it has more density than the white rice.

Thomas *et al.* (2013) reported the bulk density varied between 0.81- 0.86 g/ml in the investigated rice varieties like white, brown, bario, black, glutinous and basmati rice varieties.

According to Samyor *et al.* (2016) bulk density and true density were found to be highest in red rice (TPS-1, TKM-9) followed by black rice. Raghuvanshi *et al.* (2017) noticed red rice had higher bulk density (0.82 gm/l) than white rice.

Mohapatra and Bal (2006) reported when bulk density increases water uptake ratio also increases.

The water absorption index of 17.60 and 22.07 was noticed in parboiled as well as raw rice of *Jyothi* variety (Lakshmi, 2011 and Sathyan, 2012). The authors also found water solubility index of 0.20 and 0.12 in parboiled and raw rice of *Jyothi* variety. According to Nadh (2018) the water absorption index of 25.56, 25.31, 25.61, 23.71, 22.91 and 22.46 was observed for *Jyothi*, *Aathira*, *Aiswarya*, *Ezhome-4*, *Kanchana and Samyuktha*. *Jyothi*, *Aathira and Aiswarya* varieties respectively. Chandhini (2015) recorded the water absorption index of 24.65 and 25.11 for *Jyothi* and *Uma* rice varieties. *Ezhome -1* and *Ezhome -2* obtained the water absorption index of 22.17 and 23.90 respectively.

Lakshmi (2011) and Sathyan (2012) found the water solubility index of 0.20 and 0.12 in parboiled and raw rice of *Jyothi* variety. According to Chandhini (2015) the water solubility index of *Jyothi* and *Uma* obtained to be 0.49 and 0.42. *Vyshak* and *Vyttila*-8 recorded the water solubility index of 0.61 and 0.78. Revathy (2015) studied the water solubility index in red rice varieties were ranged from 0.45 (*Aathira*) to 0.57 (*Ezhome-4*).

Aroma of cooked rice is an important attribute of rice as it determines the market price of the rice and shows the local and national identity (Fitzgerald *et al.*, 2009).

Texture of cooked rice is a key indicator of rice quality as it affects the consumer acceptance (Lyon *et al.*, 2000). It is also affected by milling and duration of cooking.

Lightly milled rice samples resulted in lower moisture uptake of rice during cooking, thus harder cooked rice. Longer cooking duration results in greater moisture uptake of rice, producing softer cooked rice (Saleh and Meullener, 2007).

According to Bandayopathyay and Roy (1992) the gelatinisation temperature influences the cooking behaviour. Sugeetha (2010) reported that highest gelatinization temperature of 66.88 was observed in MO8-20-KR variety when compared to the other seven varieties.

Sugeetha (2010) reported the highest cooking time of 48.16 minutes in MO-87-5 and lowest of 32.33 minutes in MO-2. Lakshmi (2011) observed cooking time of 44.67 minutes in parboiled *Jyothi*. According to Sathyan (2012) *Jyothi* obtained cooking time of 29.33 minutes. Black rice along with the brown rice showed the longest cooking time.

According to Malik and Chaudhary (2001) amylose determines the cohesiveness, tenderness, volume expansion and appearance of cooked rice.

Lii *et al.* (1996) reported that amylose content is considered as the single most important characteristic for predicting cooking and processing behaviours of rice. Rice with high amylose content (25-30 %) tends to cook firm and dry, whereas rice with an intermediate amylose content (20-25%) tends to be softer and stickier and rice with a low amylose content (<20%) is generally quite soft and sticky (Yadav *et al.*, 2007). The amylose content determines the flakiness or stickiness of cooked rice (Hussian *et al.*, 2009).

Kishine *et al.* (2008) reported that *Nerica* rice varieties of Africa had high amylose content. According to Thomas *et al.* (2013) the lowest amylose content of 3.36 per cent was found in brown rice followed by black rice (5.11%) and it was the highest (27.71%) in white rice.

Kang *et al.* (2011) investigated the amylose content in black rice (*Dragon eyeball 100, Heukjinjubyeo, Heukgwangbyeo, Heuknambyeo,* and *Josaengheukchal*) and white rice (*Hwayoungbyeo*) varieties in Korea. The author observed that black rice varieties have lower amylose content than white rice.

Thomas *et al.* (2013) studied the amylose content of six rice cultivars (white, bario, glutinous, black, brown and basmati rice). Amylose content was the highest in white rice (27.71%) and lowest in brown rice variety (3.36%).

Water uptake ratio is an important parameter which affects the cooking quality (Horigane *et al.*, 2000; Mohapatra and Bal, 2006).

Nandini (1995) reported that among 60 rice varieties of Kerala CSRC collection, *Matta Triveni* and *Aranmula* local were found to be having water uptake ratio of 5.25. The highest water uptake of 1.52 was reported in OM-4 and the lower of 1.40 in M-108-262-1 variety among eight KAU varieties (Sugeetha, 2010).

Water uptake ratio is an important parameter which affects the cooking quality. If the bulk density is higher, then correspondingly water uptake ratio will also be high (Horigane *et al.*, 2000; Mohapatra and Bal, 2006).

According to Cagampang *et al.* (1973), gel consistency measures the tendency of the cooked rice to harden on cooling. Varieties with a softer gel consistency are preferred and the cooked rice and this feature is particularly evident in high amylose rice. Hard cooked rice tends to be less sticky.

Elongation ratio is the ratio between the length of cooked to that of raw rice grain. There were indication that rice with soft gel consistency had greater elongation ratio than medium and hard gel consistency (Singh *et al.*, 2005). Lakshmi (2011) mentioned that *Jyothi* rice had grain elongation ratio of 1.43 per cent and Sathyan (2012) observed grain elongation ratio of 1.80 per cent in *Jyothi* rice variety.

### 2.3 Nutritional qualities of rice varieties

The nutritional quality of rice grain is affected by the chemical composition like starch, moisture, carbohydrate, protein, fat and mineral content.

Moisture content of rice is an important factor which significantly affects the rice grain quality. Moisture readsorption causes starch to expand and produce compressive stresses that can lead to fissured grain which usually break during milling (Kunze, 2001).

As the rice supplies starch, protein and majority of micro minerals to humans, it is considered as key food for human nutrition, especially in Asia (Meena *et al.*, 2013). In addition, it also contain carbohydrate and B complex vitamins.

Nandini (1995) reported that traditional rice varieties of Kerala contains higher values of calories. The traditional variety *Thekkancheera* was found to have higher calorific value of 358 Kcal. Chandhini (2015) reported the energy value of red rice varieties *Ezhome-1*, *Ezhome-2* and *Pratyashya* was 353.57, 363.55 and 318.70 Kcal respectively. The energy value of other varieties like *Jyothi* and was *Uma* 335.81 Kcal and 319.71 Kcal respectively.

Deepa *et al.* (2008) observed the carbohydrate content in *Njavara* variety was 73.5 g/ 100g. Chandhini (2015) analysed the total carbohydrate content of *Pratyashya, Vytilla-8, Vaishak* and *Ezhome-1* was 71.01, 78.24, 78.07 and 78.37 g/ 100g respectively. According to Shijagurumayum *et al.* (2018) the carbohydrate content in pigmented red rice varieties ranged from 64 g/ 100g to 80 g/ 100g.

Starch content of eight KAU varieties was evaluated and found the highest starch content of 76.25 per cent in MO8-20-KR variety (Sugeetha, 2010). A starch content of 75.13 per cent and 79.61 per cent was observed in parboiled and raw rice of *Jyothi* variety (Lakshmi, 2011 and Sathyan, 2012). Starch content reported by Nadh (2018) in red rice varieties was found to be in the range of 65.83 g/ 100g to 70.50 g/ 100g. A huge variation in starch content was observed in red rice from 44.06 per cent in *Amona* to 65.31 per cent in *Rongakurmi* (Shijagurumayum *et al.*, 2018).

The quantity and quality and type of protein are important factor in nutrition. Rice is reported to be a moderate source of protein. However, rice is considered to be a major source of dietary protein in Indian diets (Kennedy and Burlingame, 2003).

Protein content is considered as index for nutritional quality of rice and protein content of 10 per cent and above is considered as cultivar with high protein content (Ressurection *et al.*, 1993). Mean brown rice protein of *"Japonica"* rices was higher than that of *Indica* rices, with a means of 11.8 per cent and 9.8 per cent respectively and coefficients of variation of 16 per cent and 21 per cent (Gomez, 1999). According to IRRI (2002) protein content of brown rice ranged from 4.3 to 18.2 per cent.

According to Chandhini (2015) protein content of 8.75g, 8.95g, 7.5g, 7.0g, 8.1g was observed in various varieties like *Ezhome-1, Ezhome-2, Prathyasha, Vyttila-8* and *Vaishak* respectively. Nadh (2018) observed the protein content of 5.47, 5.00, 4.94, 5.50, 5.34 and 4.70 g/ 100g in *Jyothi, Aathira, Aiswarya, Ezhome-4, Kanchana and Samyuktha* varieties.

Govindaswami *et al.* (1969) reported 6 per cent to 12.6 per cent crude protein in three hundred improved rice varieties in India. Verma and Srivastav (2017) reported the crude protein content of nine aromatic rice of Assam ranged from 9.17 to 11.77 per cent. High protein content was found in indigenous cultivars of north eastern hill states with a range of 6.14 to 12.07 per cent (Devi *et al.*, 2008).

Protein content of brown rice varied from 7.1 per cent to 15.4 per cent in weight depending on cultivars (Tanaka, 1997). Singh *et al.* (2000) reported the protein content of brown rice varieties ranged from 6.6 to 7.3 per cent.

Pathak *et al.* (2017) obtained the protein content of 11.42 g/ 100g in pigmented rice varieties. According to Dasgupta and Handique (2018) among red rice, crude protein varied from 8.20 per cent to 13 .96 per cent which has been recorded for Assam varieties like *Kabrabadam* (KB) and *Biroi* (BI) respectively. For white rice (NPR) landraces, the range of variation was 8.70 per

cent to 11.18 per cent which has been recorded in the sample *Bismuthi* (BM) and *Laki* (LK) respectively.

Rice fat consists essentially of unsaturated fatty acids. It has great influence on rice appearance and eating quality. The fat content decreases when the rice is milled because lipids are more concentrated in the peripheral region of the grains (Saleh *et al.*, 2013). Singh *et al.* (2000) evaluated the fat content of six brown rice varieties and it ranged from 2.1 to 3.2 per cent. According to Sugimoto *et al.* (1998) the fat content of brown rice ranged from 2.1 to 3.2 g/ 100g. Fat or lipid content of rice is approximately in the level of 1 to 5 per cent on dry weight basis (Anon, 2001; Zhou *et al.*, 2003; Kitta *et al.*, 2005). Nadh (2018) noticed that the fat content ranged from 0.31 g/ 100g (*Samyuktha*) to 0.48 g/ 100g (*Aathira*).

Baruah *et al.* (2006) observed the presence of lipid in the range of 2.42 per cent to 4.64 per cent in indigenous deep water red rice varieties. A wide variation in the lipid content was observed in a study involving 14 local landraces of Manipur and Nagaland. Variation was as high as in the range of 1.2 per cent to 4.2 per cent with a mean of 2.49 per cent.

Pathak *et al.* (2017) reported the fat content of pigmented rice varieties ranged from 4.37 to 5.27 g/ 100g. It was found that, among the red rice varieties, lipid content was observed as 1.72 per cent in *Biroi* and 3.75 per cent in *BilBao* indicating wide variation in between the cultivars in Assam (Dasgupta and Handique, 2018). Among the white rice cultivars lowest lipid content were reported in *Bismuthi* and *Laki* with 2.17 per cent and 2.31 per cent respectively. A high lipid content of 3.46 per cent was recorded in *Suhagmani* followed by *Boro* with 3.09 per cent.

A fat content in the range of 0.54 to 0.82 was reported in Indian cultivars according to Yadav and Jindal (2007). Singh *et al.* (1998) stated the fat content of brown rice varieties ranged from 21. To 3.2 per cent. Ravi *et al.* (2012) evaluated indigenous rice cultivar - *Salem samba* based on quality aspects and

found that the protein content was 0.7 per cent. The fat content in the bran and rice was 22.6 per cent and 1.18 per cent in *Salem samba* rice variety. The fibre content in the bran was 8.3 per cent compared to 4.3 per cent in raw rice.

According to Gopalan *et al.* (2012) the thiamine content of brown rice ranged from 0.35 to 0.44 mg/ 100g. Rai (2009) reported that rice as a principal source of B vitamins like thiamine and riboflavin. Chandhini (2015) reported that the thiamine content of KAU red rice varieties ranged from 0.02 to 0.07 mg/ 100g. Nadh (2018) reported that the thiamine content in *Jyothi* and *Samyuktha* variety was 0.060 mg. She also reported the thiamine content of *Aathira* (0.063 mg), *Aiswarya* (0.080 mg) and *Kanchana* (0.070 mg).

Deepa *et al.* (2008) reported the iron content in *Njavara* was 1.93 mg/ 100g. Babu *et al.* (2009) studied the impacts of rice milling in its nutritional properties and found that the iron content was 1.9 mg and 0.5 mg in brown rice and polished rice respectively. According to Chandhini (2015) iron content in red rice varieties were 0.41mg, 0.51mg, 0.61mg, 0.44mg and 0.47mg in *Ezhome-1, Ezhome-2, Prathyasha, Vyttila-8* and *Vaishak* respectively.

The hybrid derivative *Vyttila-3* (11.25 mg/100g) is known for its highest calcium content (11.25 mg/100g) and for hybrid derivative *Bhadra* had a calcium content of 9.80mg/100g (Nandini, 1995). Nadh (2018) noticed the calcium content of 5.46, 5.63, 4.90, 5.40, 5.76 and 5.43 in *Jyothi, Aathira, Aiswarya, Ezhome-4, Kanchana* and *Samyuktha* rice varieties.

Chandhini (2015) revealed that the calcium content in red rice varieties *Ezhome-1, Ezhome-2, Pratyashya, Vyttila-8, Vaishak, Jyothi* and *Uma* were 4.92, 5.27, 6, 5.7, 4.94, 6.6 and 5.26 mg/ 100g respectively.

Nandini (1995) reported that hybrid rice varieties recorded the calcium content ranged from 9.85 to 11.25 mg/ 100g. Kang *et al.* (2011) observed the mineral content of black rice and white rice varieties in Korea. It showed that black rice *Heukjinjubyeo* and *Heukgwangbyeo* contained the highest calcium content of 32 and 36 mg/ 100g. Chandhini (2015) studied the calcium content of

6.6, 5.26 and 6.00 mg/ 100g in *Jyothi, Uma* and *Pratyashya* varieties. According to Nadh (2018) the calcium content in *Aiswarya, Kanchana* and *Samyuktha* were 4.90, 5.76 and 5.43 mg/ 100g repectively.

Deepa *et al.* (2008) reported a higher phosphorus content of 324 mg/ 100g in *Jyothi* variety. According to Chandhini (2015) the phosphorus content of *Jyothi, Uma, Ezhome-2* and *Pratyashya* obtained as 133.2, 101.35, 90.29 and 135.41 mg/100g respectively. Nadh (2018) observed the phosphorus content of 130.10, 127.60, 129.83 and 131.36 mg/100g in *Jyothi, Aathira, Kanchana* and *Samyuktha* varieties.

Rice is a moderate source of fibre especially in white rices ranged from 0.2 to 1.0 g/ 100g. Deepa *et al.* (2008) noticed the fibre content of *Njavara* rice variety varied from 4.96 to 8.08 g/ 100g. Babu *et al.* (2009) discussed a higher fibre content in brown and black rice varieties. Where as in milled rice, Gopalan *et al.* (2012) observed the fibre content of 0.2 g/ 100g. Chandhini (2015) reported the fibre content of 0.7, 0.64, 0.35 and 0.20 g in *Ezhome-1, Ezhome-2, Pratyashya* and *Uma* varieties. Fibre content reported by Nadh (2018) ranged between 0.18 g (*Jyothi*) to 0.31 g (*Samyuktha*).

## 2.4 Health benefits of rice varieties

The pigmented compound such as Cyanidin-3-O- $\beta$ -D-glucopyranoside is present in large content in pigmented rice varieties, which is related with multiple functional attributes such as, protection against cytotoxicity, antineurodegenerative activity, inhibition of glycogen phosphorylase, and having antioxidant and scavenging activity more than white rices and hybrid rice varieties (Oki *et al.*, 2005).

Mohan *et al.* (2014) reported that brown rice is rich in phytochemicals such as polyphenols, oryzanol, phytosterols, tocotrienols, tocopherols and carotenoids as well as vitamins and minerals that have a protective effect against heart disease and cancer. Red and black rice varieties are considered as functional foods, because of the presence of the pigment anthocyanins which have medicinal properties like antioxidant, anti-inflammatory and anticarcinogenic effects (Bhat and Riar, 2015).

Phenolics have the potential to give hydrogen and thereby act as reducing agents. Phenolics also function as singlet oxygen quenchers and free radical hydrogen donors and because of these characteristics, phenolics protect cell compounds against oxidative damage. Such antioxidant properties of phenolics have been indicated in hygienic studies to prevent cancer, cardiovascular and nerve diseases (Kehrer, 1993). Rao *et al.* (2010) reported that total phenolic content of *Njavara* is significantly high when compared to *Jyothi, Yamini* and *Vasumathi*.

Anthocyanins have been reported to be the phenolic compounds present in pigmented rice (Iqbal *et al.*, 2005; Zhang *et al.*, 2006; Yawadio *et al.*, 2007). Cyanidin-3-glucoside and peonidin-3-glucoside, malvidin, pelargonidin-3, 5diglucoside, cyanidin-3-glucoside and cyanidin-3, 5-diglucoside, cyanidin-3glucoside and pelargonidin-3-glucoside are the most common anthocyanins found in the pigmented rice varieties (Yawadio *et al.*, 2007). Moongngarm and Saetung (2010) and Kim *et al.* (2012) reported the phenol content in germinated black rice and red rice were more than non-germinated rice.

The substances removed from pigmented rice (anthocyanins; cyanidin-3glucoside, pelargonidin-3-glucoside) is known for its effects in diabetic prevention because of its aldose reductase inhibitory activity (Yawadio *et al.*, 2007). It also has been reported that a marked decline in the cholesterol, LDL cholesterol and concentration of triacylglycerol in plasma of rats was obtained with a diet containing black rice extracts (Zawistowski *et al.*, 2009). Red and black rice varieties are the richest source of anthocyanins which it has medicinal properties such as antioxidant, anti-inflammatory and anticarcinogenic effects (Bhat and Riar, 2015). It also reduce the risk of colon cancer and induces DNA repair and synthesis of damaged cells and inhibits the proliferation of cancer cells (Vethavarshini *et al.*, 2013). The pigmented germplasm in coloured rice bran possess anthocyanin content (mg cyanidine 3-O glucoside /100g) ranging from 3.58 to 7.86, total phenolic content (mg GAE/100 g) from 67.89 to 89.43, flavonoid content (mg QE/100g) from 57.75 to 78.74 and antioxidant activity from 19.56 to 29.29 per cent (Sampong, 2011).

Total flavonoid content of the methanolic extract was markably higher in *Njavara* compared to *Vasumathi, Yamini and Jyothi* as noticed in quercetin equivalents. Total flavonoid content of *Vasumathi, Yamini, Jyothi* and *Njavara* were 1.68, 2.57, 5.33 and 8.5 mg QEE/g bran respectively. Total flavonoid content of brown rice was more than the total flavonoid content of polished rice as mentioned by Rao *et al.* (2010).

According to Rohrer and Siebenmorgen (2004) rice bran is a part of whole grain nearly 10 per cent which constitutes the bran layers (pericarp, seed coat, nucellus and aleurone) and the germ. Rice bran contains enormous source of phytochemicals that may be working both synergistically and in parallel with each other to promote health and fight disease (Boateng *et al.*, 2009).

Rice bran typically contains 16-32 per cent oil of which 90 per cent of the total fatty acid is made up by palmitic, oleic and linoleic acids (Saunders, 1990).

Keith and Hargrove (1994) reported that rice bran has many applications in diet due to nutritional profile and functional characteristics. It contains high in dietary fibre and low in saturated fat.

Bioactive food substances in rice bran may include  $\gamma$ -oryzanol, tocopherols, tocotrienols, polyphenols (ferulic acid and  $\alpha$ -lipoic acid), phytosterols ( $\beta$ -sitosterol, campesterol, and stigmasterol) and carotenoids ( $\alpha$ carotene,  $\beta$ -carotene, lycopene, lutein, and zeazanthin). Rice bran also contains essential amino acids (tryptophan, histidine, methionine, cysteine, and arginine) and micronutrients (eg, magnesium, calcium, phosphorous, manganese and Bvitamins. Rice bran oil is one of the finest quality vegetable oils present in terms of its cooking qualities, shelf life and fatty acid composition (Sayre and Saunders, 1990). Crude rice bran oil contains approximately 1.5per cent  $\gamma$ -oryzanol, which is a component unique to rice bran.  $\gamma$ -Oryzanol consists of a group of ferulate esters of triterpene alcohols and phytosterols (Xu and Godber, 1999).

Due to its higher saturation, rice bran oil is comparatively stable to oxidation when related to other vegetable oils. Lower levels of linoleic acid along with phenolic compounds and vitamin E derivatives are effective in decreasing low density lipoprotein and total serum cholesterol (Mazza, 2007; Choi *et al.*, 2009).

See tharamaiah and Chandrasekhara (1989) found the ability of rice bran oil to reduce cholesterol absorption and decrease early atherosclerosis, inhibit platelet aggregation and increase excretion of fecal bile acids, is due to the presence of  $\gamma$ -oryzanol.

Coloured rice bran found to reduce the risk of atherosclerotic plaque more than white rice. It also reduce the risk of cancers of the upper gut and colon cancer cell reduction by 30 to 50 per cent (Zhang, 2006).

The phytic acid in rice bran has the ability to inhibit abnormal cell proliferation and prevent metastasis (Vucenik and Shamsuddin, 2003). Both rice bran and the bran oil has bioactive compounds that helps in decreasing the risk of chronic degenerative diseases (Sing *et al.*, 2015).

The high antioxidant capacity of red and light brown rice bran is due to its lipophilic antioxidants, namely gamma-oryzanol, tocopherol and tocotrienols (Jang and Xu, 2009).

Rice is a source of various bioactive amino acids also. Menon and Potty (1999) reported that black and golden yellow glumed *Njavara* cultivars grown under wet land conditions contains free amino acids in the range of 0.316mg/g and 0.089mg/g respectively. DL-2 –amino-n-butyric acid and DLiso–leucine are the amino acids found in black glumed *Njavara* while, golden yellow

glumed *Njavara* contained L-Histidine monochloride, L-ornithine monochloride and DL-isoleucine.

Molecular studies in *Njavara* revealed the presence of Bowmem-Brik Inhibitor (BBI) protein, which is effective especially against breast cancer. This protein exhibits antiinflammatory and antiallergic properties in animals and also imparts resistance to fungal pathogens and pests in crops.

# 2.5 Antioxidant properties of rice varieties

Antioxidants may be defined as compounds that are present in low concentrations which significantly inhibits the rate of oxidation of the target within the biological systems (Lai *et al.*, 2010). The free radicals are produced as a result of consumption of oxygen by aerobic cells. These free radicals are involved in different disorders like ageing, cancer, cardiovascular disease, diabetes, rheumatoid arthritis, epilepsy and degradation of essential fatty acids (Singh *et al.*, 2009).

Preventive antioxidants will inhibit the initial production of free radicals. Superoxide dismutase (SOD), glutathione reductase, glutathione peroxidase, catalase are the vital enzymes in the free radical scavenger enzyme system (Surapaneni and Sadagopan, 2007).

Choi *et al.* (2007) and Adeolu *et al.* (2009) opined that search for effective and natural antioxidant has become crucial due to the adverse side effects of synthetic anti-oxidants leading to carcinogenicity.

DPPH is a free radical compound which has been extensively used to determine the free radical scavenging activity. It is widely used to assess antioxidant efficacy within a very short time (Marxen *et al.*, 2007; Li *et al.*, 2009). According to Reshmi and Nandini (2018) *Njavara* yellow variety had shown highest DPPH scavenging activity compared to black varieties, with an  $IC_{50}$  value of 31.52 µg/ml for *Njavara* yellow, 33.73 µg/ml for *Njavara* black. According to Rao *et al.* (2010) *Njavara* had the highest DPPH scavenging activity with an  $IC_{50}$  value of 30.85 µg/ml.  $IC_{50}$  values of the other three

varieties were 48.88, 70.58 and 87.72 µg/ml for *Jyothi*, *Yamini* and *Vasumathi* respectively.

*Njavara* black variety has higher scavenging activity with 2, 2-diphenyl-1-picryl hydrazyl radical with an IC<sub>50</sub> value of 84.66 µg/ml (Shalini *et al.*, 2012). *Njavara* was found to have the highest DPPH scavenging activity in a comparative study of four tested rice varieties like *Njavara* (IC<sub>50</sub> value of 30.85 µg/ml), *Vasumathi* (IC<sub>50</sub> value of 87.72 µg/ml), *Yamini* (IC<sub>50</sub> value of 70.58 µg/ml) and *Jyothi* (IC<sub>50</sub> value of 48.88 µg/ml) (Rao *et al.*, 2010).

The hydroxyl radical is considered as reactive oxygen species (ROS) with a short half-life that is responsible for the biological damage intrinsic to free radical pathology (Hochestein and Atallah, 1998).

According to Reshmi and Nandini (2018), the highest hydroxyl radical scavenging activity was observed in *Njavara* yellow with an IC<sub>50</sub> value of 46.00  $\mu$ g/ml followed by *Njavara* balck (IC<sub>50</sub> value of 55.68  $\mu$ g/ml) and *Hraswa* had IC<sub>50</sub> value of 60.78  $\mu$ g/ml Among the three rice varieties *Njavara* black showed the highest value for superoxide activity. The IC<sub>50</sub> value of *Njavara* black was 48.78  $\mu$ g/ml, whereas *Njavara* yellow has an IC<sub>50</sub> value of 55.43  $\mu$ g/ml and *Hraswa* control variety had an IC<sub>50</sub> value of 60  $\mu$ g/ml.

Reshmi and Nandini (2018) shown that the reducing power of *Njavara* black, yellow and *Hraswa* varieties increased as the concentration increased from 100  $\mu$ g to 500  $\mu$ g/100ml.

Rao *et al.* (2010) estimated 0.5 mg/ml of the methanolic rice bran extracts which indicated the absorbance values of 0.59, 1.04, 1.93 and 2.98 similar to *Vasumathi, Yamini, Jyothi* and *Njavara* respectively.

According to Ismail *et al.* (2004) total antioxidant activity is based on the antioxidant potential of pure compounds to quench and inhibit the formation of a coloured radical cation produced by the action of the reagent solution.

Rao *et al.* (2010) reported that total antioxidant activity of *Njavara* rice bran extracts increased with rise in concentration and a potential change was observed at 0.02 to 0.1 mg/ml concentration of the extract. At concentration of 0.1 mg/ml of the methanolic rice bran extracts, the absorbance values of *Vasumathi, Yamini, Jyothi* and *Njavara* were found to be 1.65, 2.21, 2.52 and 2.93 respectively.

Rao *et al.* (2010) estimated superoxide scavenging activity in *Njavara*, *Jyothi*, *Yamini* and *Vasumathi* and found that the  $IC_{50}$  values were 41, 48, 102 and 140 µg/ml respectively.

According to Rao *et al.* (2010) *Njavara* extract was found to have the highest nitric oxide scavenging activity compared to the other three rice bran methanolic extracts in a moderate dose dependent inhibition of nitric oxide with an IC<sub>50</sub> value of 52.25  $\mu$ g/ml. IC<sub>50</sub> values of *Jyothi, Yamini* and *Vasumathi* were 71.41, 107.18 and 102.48  $\mu$ g/ml respectively. Chakuton *et al.* (2012) showed a potential correlation with pigmented varieties and antioxidant activity. Antioxidant activity of coloured rice bran varieties were found to be superior than that of non coloured rice bran.

Hong *et al.* (2009) found that "takju" a Korean traditional rice wine exhibited a powerful radical scavenging activity against a variety of oxidative systems. The author also pointed out that takju should be regarded as an antioxidant since its functional compound reduces oxidative stress.

#### **2.6. Rice based products**

Food preferences and practices of food are the result of cultural heritage and economic and social factors (Aneena, 2009). Product and by products of rice are used by humans in numerous ways.

Processing of raw rice into new products has been undertaken in response to new consumer needs with the development of instant rice (Roy *et al.*, 2008).

Rice powder and starch, cakes and puddings, baked bread and crackers, breakfast cereals, rice snacks and noodles, baby or weaning foods, rice milk, fermented foods and beverages and bran products are some of the rice based value added products (Chumniwkri and Peuchkamut, 2016).

Rice noodles are historically prepared popular dish, with very smooth texture, soft mouth feel and white colour, broadly consumed in most of the South-East Asian countries (Thomas *et al.*, 2014).

Jomduang and Mohamed (1994) reported that the expanded rice is a convenience food commonly eaten in India either as such or with jaggery, roasted bengal gram and shredded vegetables and spices. The product is majorly produced in home or cottage sector by skilled artisans.

Ready to eat breakfast cereals, baby foods and snacks are the important preparations in which the rice flour is used as a major ingredient (Hsich *et al.*, 1990). Ready to eat breakfast cereals in various forms is a popular food (Vaclavik and Christian, 2014). The major reason for using rice flour as an ingredient because it is gluten free and it has a bland taste, white colour, high digestibility and hypo allergic property (Rosell and Marco, 2008).

Medium grain low amylose rice is used for producing baby foods and breakfast cereals. Rice starch is used as thickening agent in food preparation including infant formula. The granular size of rice starch is comparatively small. Intermediate amylose (20-25 per cent) varieties are utilized for producing fermented cakes and in making canned soups. High amylose rice (>25 per cent) is used for produced extruded rice noodles (Puspita and Panulu, 2015).

Rice vinegar results from the completion of the rice starch fermentation and is a traditional Japanese and Chinese product (Iwuoha and Eke, 1996).

Rice wines are fermented alcoholic drink made from cereals. The maximum percentage of alcohol in original pure rice wine is below 20 per cent, usually 15-16 per cent.

Hogan (1977) observed that rice alone or in combination with other cereal grain products is precooked, dried, flaked or formed into dough then expanded or puffed and toasted.

Durgadevi *et al.* (2018) developed five value added products from *Rakthashali* rice which includes rice flakes, extruded snack, rice noodles, rice vermicelli and rice pasta with greater acceptability to the products.

Ready to eat pre-cooked pasta or rice based dish having sauce which does not require cooking and just treated or toasted or grilled was suggested by Brennan *et al.* (2013).

Flaked rice is a major product in India. It is known by a number of names, including aval (Tamil), avalakki (Kannada), atukulu (Telugu) and poha (Hindi). The process involved in the production of flaked rice are cold or hot soaking, roasting, flaking, sieving and packing. In flaked rice production, generally freshly harvested paddy is preferred as it gives more whiteness (Sulochana *et al.*, 2007).

Flaked or brown rice and parboiled milled rice may be transformed to puffed rice by either roasting in hot sand or heating in hot air (Juliano and Hicks 1996).

Sharif *et al.* (2009) prepared biscuit and cookies with wheat flour and rice bran. Sensory characteristics like appearance, colour, flavour, texture and overall acceptability was acceptable at 10 per cent incorporation of rice bran.

Rani (2015) prepared rice bran and soy flour incorporated cookies and organoleptic evaluation was conducted and revealed that the cookies with 10 to 20 per cent incorporation of rice bran was most acceptable.

'Hang' rice is the rice product from the folk perception of historical northeast region of Thailand (Phattayakorn *et al*, 2016). It was a germinated product prepared from black waxy rice, red non-waxy rice (red Jasmine) and white non-waxy rice and it possess high amount of  $\gamma$ -aminobutyric acid (GABA)

and antioxidants such as phenolic compounds,  $\gamma$ -oryzanol and vitamin E (Moongngarm and Saetung, 2010; Kim *et al.*, 2012).

Extrusion provides the viability of changing functional characteristics of food materials and furnishes a broad range of novel and precooked and technical foods. Usually extruded products are processed with cereal flours (Kothakota *et al.*, 2013).

Soup is the one of the historical food which can be classified as a starter, warm food during cold and sick. In the modern world commercially prepared instant soup such as canned, dehydrated, and frozen soups have replaced homemade soup as preparing a soup is a time consuming process (Abeysinghe and Illeperuma, 2006). Instant soup can become a substitute food for breakfast because it could fulfil the adequacy of energy and nutrient required by the body, feasible for preparing with easiness in preparation (Sunyoto *et al.*, 2012).

Lakshmy (2011) standardised instant soup mix by incorporating temph flour at 50 per cent level and the organoleptic scores obtained was above 7 which means that the temph based soup mix was highly acceptable.

Niththiya *et al.* (2014) developed vegetable added instant dried soup mixes had an acceptable sensory, nutritional and microbial quality and it can be stored under ambient condition without affecting the quality characters.

Singh *et al.* (2015) formulated instant soup mix with fresh mushroom and organoleptic qualities obtained a higher mean scores for all the sensory qualities.

# Materials and Methods

# **3. MATERIALS AND METHODS**

The study entitled 'Nutritional and antioxidant potential of medicinal rice variety *Rakthashali*' was carried out with the objective to evaluate the nutritional, cooking, physicochemical and antioxidant properties of medicinal rice variety *Rakthashali* and to develop functional food products based on it.

The materials used and the methods followed in the present study are given under the following headings.

3. 1. Collection of rice variety

3. 2. Quality evaluation of rice variety

3. 3. Shelf life studies of rice, roasted rice flour and rice bran

3. 4. Assessment of antioxidant and antiproliferatory properties of rice and rice bran

3. 5. Standardisation of instant soup mix with rice flour

3. 6. Organoleptic evaluation of instant soup mix

3.6.1. Nutritional qualities of the selected instant soup mix

3. 7. Standardisation of rice bran cookies

3. 7.1. Organoleptic evaluation of rice bran cookies

3.7.2. Nutritional qualities of the selected cookies

3.8. Cost of production

3. 9. Statistical analysis

# **3. 1. Collection of rice variety**

Grain of indigenous medicinal rice variety *Rakthashali* was procured from farmer in Pantharangadi of Malapuram district. The paddy grains were dehulled in rubber roll sheller. One half of the dehulled grains of the *Rakthashali* was powdered and sieved to obtain fine rice flour, while the other half was subjected



Rakthashali rice variety



Whole grains of Rakthashali rice



Rice bran from *Rakthashali* 

Plate 1. Rice variety selected for the study

to polishing by using a lab level mini rice miller for 30 seconds to collect the bran. The polishing were collected and sieved to get fine rice bran. All the quality evaluation studies were conducted in the rice flour and rice bran separately and is mentioned in plate1.

# 3. 2. Quality evaluation of Rakthashali rice grains and rice bran

Whole rice grains and the rice bran obtained by milling were cleaned and sieved to get fine flour. Various quality parameters like physical qualities and cooking qualities are studied in the whole rice grains. Biochemical and nutritional qualities of *Rakthashali* were assessed in both whole grain and rice bran.

# 3. 2. 1. Physical qualities of rice

Milling qualities like total milled rice per cent and head rice per cent were assessed. Quality parameters like grain size, thousand grain weight and thousand grain volume were measured following standard procedures. Shape and appearance of the grain were assessed by visual observations.

# 3. 2. 1. 1. Total milled rice per cent

Milling per cent includes the weight of head rice and broken rice and is calculated as follows.

Milled rice (%) = 
$$\frac{\text{Weight of milled rice}}{\text{weight of paddy}} \times 100$$

# 3. 2. 1. 2. Head rice recovery

Whole grains (head rice) were separated from the milled rice using a winnower. The resulting head rice was weighed to get head rice recovery (Adair, 1952).

Head rice recovery (%) =  $\frac{\text{Weight of head rice}}{\text{weight of paddy}} \times 100$ 

# 3. 2. 1. 3 Thousand grain weight

Thousand grain weight was measured by standard procedure of Redding *et al.* (1991). This was the method of counting and weighing of 1000 randomly selected polished rice kernels. It is expressed as g/ 1000 grain.

#### 3. 2. 1. 4 Thousand grain volume

Thousand grain volume was determined by Ali *et al.* (1993). Thousand grains of milled rice kernels were dropped into a 500 ml graduated cylinder filled with 100 ml of water and tapped the cylinder to remove the air bubbles and measured the total volume of rice and water. For getting the volume weight, the initial volume of water is subtracted to get the volume of rice and is expressed as mm<sup>3</sup>.

# 3. 2. 1. 5 Grain shape and grain size

Grain appearance depend upon the size and shape of the kernel. Length and width of grain were measured using grain vernier and using the following scale, grain size and shape was determined. For size, the grain was classified as;

Extra-long	g -	>7.50 mm
Long	-	6.61 - 7.50 mm
Medium	-	5.51 - 6.60 mm
Short	-	<5.50 mm

For shape, based on length-to-width ratio, grain was classified as detailed below.

Slender	-	>3.0 mm	
Medium	-	2.1 - 3.0 n	nm
Bold	-	1.1 - 2.0 n	nm
Round	-	<1.0 mm	(IRRI, 2002)

# 3.2.2 Physical qualities of rice flour

Rice sample was washed and strained to remove excess water, powdered and sieved to get a fine rice flour. The following physical qualities of rice flour were studied.

#### 3.2.2.1 Bulk density

The bulk density of rice flour was measured by the method forwarded by Okaka and Potter (1977). The sample of fifty gram was taken and drop it into a 100 ml graduated cylinder. Tapped the cylinder 50 times and determined the bulk density by calculating the weight per unit volume of the sample.

# 3.2.2.2 Water Absorption Index (WAI) and Water Solubility Index (WSI)

WAI and WSI of flour were determined by the method of Anderson *et al.* (1969). The fine flour of sample (2.5g) was mixed with 30 ml distilled water and it was cooked for 90°C for 15 minutes by placed in a water bath. The cooked contents were subjected to cooling and it were transferred in centrifuge tubes and centrifuged it for 10 minutes. WAI and WSI were calculated by the formulas.

$$WAI = \frac{Weight of sediment}{weight of dry solids} \times 100$$
$$WAI = \frac{Weight of dissolved solids in supernatant}{weight of dry solids} \times 100$$

# 3.2.2.3 Retrogradation property

Retrogradation property was evaluated by the method described by Singh *et al.* (2005). Flour paste (9%) was heated to 90°C for 20 minutes and then cooled. These cooked rice flour paste was stored for 3, 6, 9, 12 days at 4°C. Syneresis was measured as percentage amount of water released after centrifugation in 15 minutes.

# 3.2.3 Cooking qualities of rice

The cooking characteristics of rice are mostly determined by the properties of the starch. Hence, cooking qualities of whole rice grains were evaluated.

# **3.2.3.1 Preparation of cooked rice**

Rice were cooked by straining method suggested by Saleh and Meullener (2007). Rice was measured and washed with cold water, strained and repeated washing. Fairly large quantity of water taken in a big pan was boiled and the rice grains were added. Added the rice into the boiling water. After cooking, rice water was drained. Cooking qualities namely, the sensory qualities, gelatinization temperature index, cooking time, water uptake, volume expansion, amylose content, gel consistency and grain elongation were analysed.

#### **3.2.3.2 Selection of judges**

A series of organoleptic trials were done by using simple triangle test at laboratory level. According to Jellenick (1985) organoleptic evaluation was conducted using a panel of fifteen judges were selected in the age group between 18 to 35 years.

## 3.2.3.3 Preparation of score card

For the organoleptic evaluation of prepared products, score card was prepared containing six sensory quality parameters namely appearance, colour, flavour, texture and taste and overall acceptability. Each of the sensory quality attributes were assessed by using nine point hedonic scale. Overall acceptability was computed separately using the average of above mentioned five quality attributes. The score card prepared was mentioned in Appendix I.

#### **3.2.3.4 Gelatinisation temperature index**

Gelatinisation temperature index was observed by alkali digestion and it was suggested by Little *et al.* (1958). It is measured by observing the degree of spreading individual rice kernels in a weak alkali solution (1.7% KOH). Six whole rice kernels without cracks were selected and placed in a petridish. Ten ml of 1.7 per cent potassium hydroxide (KOH) solution was added. The sample were aligned to provide enough space between kernels to allow for spreading. The

petridishes were covered and incubated for 23 hours at 30°C in an oven. Starchy endosperm was rated visually to index the degree of spreading in alkali.

Complete disintegration of kernels shows low gelatinisation temperature index whereas partial disintegration of kernels shows intermediate gelatinisation temperature. Rice with a high gelatinisation temperature remains largely unaffected in the alkali solution. The spreading and clearing of kernels as noted in seven point scale described by Jennings *et al.* (1979) and was expressed as an average of six grains.

Sl/no.	Spreading	Score	Clearing
1	Kernel not affected	1	Kernel chalky
2	Kernal swollen	2	Kernel chalky, collar powdery
3	3 Kernel swollen, collar incomplete		Kernel chalky, collar cloudy and narrow
4	Kernel swollen, collar complete	4	Centre cottony, collar cloudy and wide
5	5 Kernel split or segmented collar complete and wide		Centre cottony, collar cloudy
6	Kernel dispersed, merging with collar	6	Center cloudy, collar cleared
7	All kernels dispersed and intermingled	7	Center and collar cleared

Table 1. Alkali spreading and clearing of kernels

# Classification

# **Gelatinisation temperature**

High - >74 °C

High, intermediate - 71 - 74 °C

Intermediate	-	70 - 74 °C
Low	-	<70 °C

#### 3.2.3.5 Cooking time

Cooking time was estimated by the method suggessted by Juliano and Bechtel (1985). About 10g of whole rice samples were dropped into a 250 ml beaker containing 100 ml distilled water and was boiled for 98 °C. Cooking duration was measured and after 10 minutes and every minutes thereafter, one or two grains of rice were removed and pressed between two clean glass plates. Measure the cooking time when at least 90 per cent of the grains no longer had opaque core. The rice was then allowed to simmer for about two minutes to ensure that the grains had been gelatinised and the optimum cooking time was measured with the additional two minutes of simmering.

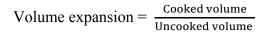
#### 3.2.3.6 Water uptake

Water uptake was estimated by the method suggested by Zhou *et al.* (2007). A known weight of whole rice grains (10g) was cooked with excess cooking water (100 ml) in a beaker. The volume of excess residual cooking water was measured by using a pipette after the cooking process. The water uptake capacity of cooked grains was calculated from the difference between the total cooking water and residual cooking water after the cooking process and as expressed in milli litre per gram of grain.

Water uptake = Total cooking water – Residual cooking water

# 3.2.3.7 Volume expansion

Volume expansion was estimated by the method described by Pillaiyar and Mohandas (1981). It was calculated from the ratio between the cooked volume of rice to that of uncooked rice. The volume of the 10 milled kernels was noted initially and after cooking in a graduated test tube. The volume expansion is calculated from the ratio between cooked volume to the uncooked volume.



#### 3.2.3.8 Amylose content

Amylose content was determined by using the standard procedure of Sadasivam and Manikkam (1992). To 100 mg of powdered rice sample, one ml of 1 N NaOH were added and kept overnight and and the volume was made up to 100 ml. From this, 2.5 ml of extract was taken by the addition of 20 ml of distilled water and phenolphthalein indicator (3 drops). After this, 0.1 N HCl was added drop by drop until the pink colour disappears. One ml of iodine reagent was added to this and made up the volume to 50 ml in standard flask. The intensity of the colour observed was measured in a spectrophotometer at 590 nm.

#### 3.2.3.9 Gel consistency

Gel consistency was measured by the method suggested by Cagampang *et al.* (1973). Rice sample for measuring gel consistency were stored in the same room for 2 days so as to equalize the moisture content of the grain. Whole rice grains were ground to give a fine flour (100 mesh). The powdered sample of 100 mg was weighed into test tubes containing Ethyl alcohol (0.2 ml of 95 per cent) and 2.0 ml of 0.2 M KOH using a pipette. The contents were mixed well. To prevent steam loss and to reflux the samples, test tube were covered with glass marbles and vigorously boiled the contents in a water bath for eight minutes until the contents reached the 2/3 height of the test tube. The test tube was removed from the water bath and allowed to stand in room temperature for five minutes and after that the test tubes was cooled in an ice-water bath for 20 minutes. Laid the test tube horizontally on a laboratory table lined with a graph paper and the total length of the gel was measured from the bottom to the gel front. The gel length is expressed as millimetre.

Gel consistency of rice varieties were classified as

Soft - > 61-100 Medium - 41-60

Medium ha	rd -	36-40
Hard	-	< 26-35

#### **3.2.3.10** Grain elongation

Grain elongation was measured by evaluating the degree of the elongation of cooked rice and was suggested by Azeez and Shafi (1966). Whole rice kernels (25) were taken in a beaker and soaked for 30 minutes in 20 ml distilled water. Then it was placed in a water bath at 98°C for 10 minutes. The cooked rice was transferred to a petridish lined with filter paper. Cooked rice was transferred to petridish and ten cooked grains were selected and measured by placing it linearly on a graph paper. The elongation ratio was measured by the ratio of the average length of cooked grains to the average length of raw grains.

# 3.2.4. Chemical and nutritional qualities of rice and rice bran

Biochemical and nutritional qualities of *Rakthashali* rice and rice bran like moisture, starch, carbohydrate, protein, total fat, energy, calcium, zinc, iron and phosphorus were assessed and also the *in vitro* digestibility of starch and *in vitro* availability of calcium, zinc, iron and phosphorus were also evaluated using standard procedures. Analysis was carried out in triplicate samples for the following parameters and are discussed below.

#### **3.2.4.1.** Moisture

Moisture content was determined in rice and rice bran and was estimated by the method of A.O.A.C (2012). Samples (5 g) were taken in a petridish and dried in a hot air oven at 60°C-70°, cooled and then weighed. This was repeated until a constant weight was observed. The moisture content was expressed in percentage and it was calculated from the loss in weight during drying.

# 3.2.4.2. Starch

The starch content was estimated colorimetrically using anthrone reagent (A.O.A.C, 2012). The rice and rice bran were powdered and the powders (0.5g) were extracted with 80 per cent ethanol. The residue was dried in a water bath containing 5 ml of water and 6.5 ml of 52 per cent perchloric acid and again extracted at 0°C for 20 minutes. The supernatant was collected and made up to 100 ml using standard flask and 0.2 ml of the supernatant was pipetted out. In this, 1 ml of water and 4 ml of anthrone reagent was added and heated for eight minutes. The intensity of colour was measured in a spectrophotometer at 630 nm.

#### **3.2.4.3.** Carbohydrate

The carbohydrate content was measured colourimetrically using anthrone reagent (Sadasivam and Manikkam, 1992). Powdered samples (rice and rice bran) of 0.1 g was hydrolysed with 5 ml of 2.5 N HCl, cooled and the residue was neutralized with solid sodium carbonate. Made up the content to 100 ml standard flask and centrifuged. Pipetted 0.1 ml of supernatant by the addition of 1 ml distilled water and 4 ml anthrone reagent. Heated the contents for eight minutes on cooling and the intensity of colour from green to dark green was read at 630 nm. The amount of total carbohydrate present in the sample was estimated from the standard graph and is expressed in grams.

# 3.2.4.4. Protein

Protein content was measured by the method of A.O.A.C (2012). Rice and bran (0.2g) was digested in a digestion flask with 6 ml Con.  $H_2SO_4$ , 0.4 g of CuSO<sub>4</sub> and 3.5 g K<sub>2</sub>SO<sub>4</sub> until it forms green colour. After digestion, it was diluted with water and 25 ml of NaOH and then titrated with 0.2 N HCl to get the nitrogen content. The protein content was calculated by the nitrogen content obtained and was multiplied with a factor of 6.25. It is expressed in percentage.

# 3.2.4.5. Total fat

Fat content of rice and bran were determined by the method of A.O.A.C (2012). Five gram of samples were powdered and extracted with petroleum ether for six hours by gentle heating in a soxhelt apparatus. Extraction flask was then cooled and ether was removed by heating and weight was taken. The fat content was expressed in percentage.

# 3.2.4.6. Energy

The energy content was worked out from the amount of total carbohydrate, protein and fat present in the sample.

Total carbohydrate, protein and fat were estimated by the method as described in 3. 2. 4. 3, 3. 2. 4. 4 and 3. 2. 4. 5. Finally multiply the amount of total carbohydrate, protein, and fat by 4, 4 and 9 respectively. Then the results are added together to get the energy. Energy content was expressed as kilo calorie (Kcal).

# 3.2.4.7. Calcium

Calcium content was estimated with the diacid extract prepared from the sample by using atomic spectrophotometer (Perkin and Elmer, 1982). The diacid was prepared by mixing 70 per cent perchloric acid in the ratio 9:4. Two gram of sample was digested in this diacid and the extract was made up to 100 ml. This solution was read directly in atomic absorption spectrophotometer. Calcium content was expressed in mg 100 g of the sample.

#### 3.2.4.8. Zinc

The zinc content of the sample was estimated by atomic absorption spectrophotometric method (Perkin and Elmer, 1982). The diacid solution was directly read in atomic absorption spectrophotometer. The zinc content is expressed in mg per 100 g of sample.

# 3.2.4.9. Iron

Iron content of the sample was estimated by atomic absorption spectrophotometric method using the diacid extract prepared from the sample (Perkin and Elmer, 1982). The reading of diacid solution was taken in atomic absorption spectrophotometer and is expressed in mg per 100 g sample.

# 3.2.4.10. Phosphorus

The phosphorus content was estimated by the procedure suggested by Jackson (1973) which gives yellow colour with nitric acid vandate molybdate reagent. To 5 ml of predigested aliquot, 5 ml of nitric acid vandate molybdate reagent was added and made up to 50 ml with distilled water. After 10 minutes, the OD was read at 420 nm. The content of phosphorous present in the sample was estimated from the standard graph prepared using serial dilution of standard phosphorous solution and expressed in mg per 100 g.

#### 3.2.4.11. In vitro digestibility of starch

Starch digestibility was estimated as suggested by Satterlee *et al.* (1979). One gram of the sample was powdered and gelatinised in 100 ml water and boiled for one hour and filtered. One ml of gelatinised solution was taken and 1 ml of the enzyme solution (saliva diluted with equal quantity of water) was added. The mixture was incubated at 37°C for 1-2 hours. The reaction was stopped by adding 1 ml of NaOH. Later, glucose was estimated by the method suggested by Somoygi (1952) and IVSD was computed.

# 3.2.4.12. In vitro availability of minerals

#### **HCl extractability**

Rice and rice bran was extracted with 0.03N hydrochloric acid by shaking the contents at 37°C for 3 hours. The clear extract obtained after filtration with Whatman No.42 filter paper was oven dried at 100°C and wet acid digested. The amount of the HCl extractable calcium, zinc, iron and phosphorus in the digested sample were determined by the methods as described for the estimation of minerals.

Mineral extractability =  $\underline{\text{Mineral extractability in } 0.03\text{N} \text{HCl}} \times 100$ 

Total mineral

# 3.3. Shelf life studies of rice, roasted rice flour and rice bran

# **3.3.1. Enumeration of microbial population**

The rice, rice flour and rice bran samples was evaluated for the presence of bacteria, yeast and fungi initially and at monthly intervals for three months. The method suggested by Agarwal and Hasija (1986) were microbe's serial dilution and plate count method. Ten grams of sample was added to 90 ml of sterile water and shaken for 20 minutes. From that 1 ml of solution was transferred to a test tube containing 9 ml of sterile water to get 10<sup>-2</sup> dilution and similarly 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup> and 10<sup>-6</sup> dilutions were also prepared. Enumeration of total micro flora was carried out using nutrient agar media for bacteria, Potato dextrose agar media for fungi and Sabouraud's dextrose agar media for yeast. The dilution for bacteria was 10<sup>-6</sup> and for yeast 10<sup>-3</sup> and for fungi 10<sup>-3</sup>.

# **3.3.2. Insect infestation**

Presence of storage insects was assessed by examining the flour and starch under the microscope. Samples were sieved first with 60 BL sieve and observed under microscope. The insect infestation in stored samples was assessed initially and after a period of three months by visual examination.

# 3.3.3. Peroxide value

Peroxide values of rice, rice flour and rice bran was estimated to find the rate of rancidity during storage. It was estimated by the procedure given by Sadasivam and Manickam (1992). One gram of extracted sample was taken in a boiling tube and to that one gram of potassium iodide and 20 ml solvent mixture

(glacial acetic acid and chloroform) were added. The tube was placed in boiling water for 30 seconds and the contents were transferred to a conical flask containing 20 ml of 5 per cent potassium iodide solution. The tubes were washed twice with 25 ml water and collected in a conical flask. This was titrated against N/500 sodium thiosulphate solution until yellow colour disappears. Later 0.5ml of starch solution was added and titrated till the appearance of blue colour. A blank solution was also prepared and peroxide value was calculated and expressed in milli equivalent per kg of the samples.

# **3.4.** Assessment of antioxidant and antiproliferative properties of rice and rice bran

Antioxidant properties like free radical scavenging activities, antiproliferatory studies in rice flour and rice bran were analysed.

# **3.4.1.** Antioxidant properties

The free radical scavenging properties of rice flour and rice bran were assessed. The antioxidant activities such as DPPH (1,1- diphenyl 1-2-picrylhydrazyl) radical scavenging activities, reducing power (RP) assay, nitric oxide (NO) scavenging, superoxide and hydroxyl scavenging activity and total antioxidant activity were assessed in rice flour and rice bran extracts. The total phenol content (TPC) and total flavonoid content (TFC) were also be analysed.

# **3.4.1.1. Preparation of extract**

The rice and rice bran was dried and powdered using grinder and sieved. The sieved powders (5 g) were extracted thrice with 100 ml methanol for 24 hrs in an electrical shaker at 40°C. The extracts were filtered through Whatman No.1 filter paper and evaporated under vaccum using a rotary evaporator (Heidolph, Germany). The residual crude methanolic extracts were weighed and dissolved in dimethyl sulphoxide (DMSO), and filtered through a 0.45  $\mu$ m of nylon membrane filter and stored at -20°C until further analysis.

# 3.4.1.2. DPPH radical scavenging assay

The methanolic extract of the rice flour and rice bran was tested for its scavenging activity against the stable free radical DPPH (2, 2-diphenyl-1picrylhydrazyl) by the method of (Aquino *et al.*, 2001). DPPH, in its radical form, has an absorption band at 517nm, which disappears on its reduction. Different concentrations of the methanolic extract of *Rakthashali* flour and bran were separately added to 0.375ml of freshly prepared DPPH solution in methanol. The volume was made up to 2ml with methanol. The reaction mixture was incubated in the dark condition for 20mins and the absorbance was measured at 517nm. The percentage inhibition was calculated and the concentration needed for IC<sub>50</sub> was estimated.

#### 3.4.1.3. Superoxide radical scavenging assay

Superoxide scavenging activity of the extract was determined by nitro blue tetrazolium (NBT) reduction method (Mc Cord and Fridovich, 1969). It depends on the light induced superoxide generation by riboflavin and the corresponding reduction of NBT. Various concentrations of the rice flour and rice bran extract was added to the reaction mixture consisting of 0.1 M EDTA containing 0.3 mM NaCN, 0.12 Mm riboflavin, 1.5 mM NBT and 0.067 M phosphate buffer making up the volume to a total of 3ml. The tubes were uniformly illuminated with an incandescent lamp for 15 min and the optical density was measured at 560 nm before and after the illumination. The percentage inhibition of superoxide generation was evaluated by comparing the absorbance values of the control and the experimental tubes.

# 3.4.1.4. Estimation of total flavonoid content

Flavonoids in the methanolic extract of *Rakthashali* rice flour and rice bran was estimated according to the method of Chang *et al.* (2002). About 0.5 ml of rice extracts was mixed with 1.5 ml of methanol, 0.1 ml of 10 per cent aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water.

The reaction mixture was incubated at room temperature for 30-35 minutes and the absorbance of the reaction mixture was measured at 415nm. The calibration curve was prepared by using Quercetin at concentrations of 50 to  $250\mu$ g/ml in methanol. Flavonoid content were expressed as  $\mu$ g/mg Quercetin equivalent (QE) of dry extracts.

#### **3.4.1.5.** Estimation of total phenolic content

The total phenolic content in the methanolic extracts of *Rakthashali* was determined by using Folin-Ciocalteu reagent following a slightly modified method of Ainsworth (2007). Gallic acid (20-100  $\mu$ g/ml) was used as a reference standard for plotting calibration curve. A volume of 0.5 ml of the rice extracts (100  $\mu$ g/ml) was mixed with 2 ml of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 4 ml of sodium carbonate solution (7.5%, w/v). The reaction mixture was incubated at room temperature for 30 minutes with intermittent shaking for colour development. The absorbance of the resulting blue colour was measured at 765 nm using double beam UV-VIS spectrophotometer. The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of the phenolic compounds was expressed as  $\mu$ g/mg gallic acid equivalent (GAE) of dry extracts.

# **3.4.1.6.** Hydroxyl radical scavenging activity

The hydroxyl radical generated in the Fe<sup>3+</sup>-ascorbate – EDTA-H<sub>2</sub>O<sub>2</sub> system (Fenton reaction) degrade 2-deoxyribose the product of which condense with Thiobarbituric acid Reacting Substances (TBAR's) forming pink coloured complex (Elizebeth *et al.*, 1990). The efficacy of test compounds to interfere with the reaction is assessed. The reaction mixture contained, in a final volume of 1.0 ml, 2-deoxy-2-ribose (2.8 mM);KH<sub>2</sub>PO<sub>4</sub>-KOH buffer (20mM, pH 7.4); FeCl<sub>3</sub> (0.1 mM); EDTA (0.1 mM); H<sub>2</sub>O<sub>2</sub> (0.1 mM); ascorbic acid (0.25-4.0 µg/ml) of rice extracts. After incubation for 1 hour at 37C, 0.4 ml of the reaction mixture was transferred to test tubes and added with 1.5 ml (0.8%) TBA, 1.5 ml (20% pH 3.5) acetic acid, 200 µl (8.1%) SDS and made up the volume 4.0 ml with distilled

water. The incubation was carried out at 100 C for 1 h. After cooling, the absorbance was measured at 532 nm against an appropriate blank solution. Vitamin C was used as a positive control in this assay.

# 3.4.1.7. Ferric reducing antioxidant power assay (FRAP)

The antioxidant capacity of rice flour and rice bran were estimated according to the procedure by Pulido (2000) with some modifications. The method measures the ferric reducing ability (FRAP). When a ferric tripyridyl-s-triazine (Fe<sub>3+</sub>  $T_pT_z$ ) complex is reduced to ferrous (Fe 2) form at low pH (which has an intense blue colour) can be monitored by measuring the change in absorption at 595 nm. FRAP reagent (25 ml acetate buffer (300 mmol/l), pH 3.6; 2.5 ml TpTz (2,4,6-tripyridyl-s-triazine) (10 mmol/l) in 40 mmol HCl and 2.5 ml FeCl<sub>3</sub>.6H<sub>2</sub>O solution), prepared freshly and 900 µl was mixed with different concentrations of rice flour and rice bran extracts and made the volume up to 1.0 ml using distilled water. Then the tubes were incubated at 37 C for 15 min and read against distilled water at 595 nm. The effective concentration was evaluated by comparing the absorbance values of control and experimental tubes.

#### **3.4.1.8.** Nitric oxide scavenging activity

Different concentrations of sample solution were prepared in 100 ml volumetric flask. 0.1489 g of sodium nitroprusside (final concentration 5 mm) was added to this and kept for incubation. At different time points, 5.6 ml was taken, 0.2 mL of reagent A was added and kept for incubation at 30°C for 10 min. After incubation, 0.2 ml of Griess reagent was added and kept for incubation at 30°C for 20 min and the absorbance was measured at 542 nm against blank.

# 3.4.1.9. Total antioxidant activity

To determine total antioxidant capacity (TAC) of rice flour and rice bran was determined by phosphomolybdate assay. Ascorbic acid was used as a standard. A stock solution of ascorbic acid (10mg/ml) was prepared in distilled water, from which dilutions were made. In a test tube, 300  $\mu$ l rice extracts were mixed with 3 ml phosphomolybdate reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The test tube was covered with aluminum foil and incubated at 95 °C for 90 min. The mixture was then allowed to reach room temperature when its absorbance was recorded at 765 nm. Blank was run using the same procedure but containing an equal volume of methanol in place of the samples. The antioxidant capacity was reported as  $\mu$ g of ascorbic acid equivalents (AAE) per ml.

### 3.4.2. Antiproliferatory activities

Antiproliferative assay in human adenocarcinoma cell lines using MTT [3-(4, 5-dimethylthiazolyl-2)-2, 5- diphenyltetrazolium bromide] assay was done separately in rice and rice bran.

### 3.4.2.1. Cell line and maintenance

Human adenocarcinoma cell line (MCF-7) was purchased from National Centre for Cell Sciences, Pune. The cells were maintained in Dulbecco's Modified Eagle Media (DMEM), with 10% FBS and 100 U/L penicillin and streptomycin under standard concentration.

#### **3.4.2.2.** Cytotoxicity analysis

Approximately,  $1 \times 10^{6}$  cells/ml of MCF-7 were seeded in flat bottomed 48 well plates containing 0.25 mL DMEM. The cells at 80 – 90 per cent confluency were incubated with different concentrations of rice flour and rice bran extracts  $(25 - 125 \ \mu\text{g/ml})$  for 48 h. After treatment, 20  $\mu$ l of 5 mg/mL MTT (3-(4, 5 – dimethyl-2-thiazoyl)-2, 5 – diphenyl – 2 H - tetrazolium bromide) was added to all wells and further incubated for 4 hr. The formazan crystals formed were dissolved with 500  $\mu$ l of dimethyl sulphoxide as solubilizing reagent. Absorbance of the coloured product was measured at 570 nm. Cell viability was then calculated by comparing with the absorbance of control well.

# 3.5. Standardisation of instant soup mix with rice flour

An instant soup mix was standardized by incorporating rice flour and mushroom powder in varying proportions. All other ingredients were used as per the standard procedure (Lakshmy, 2011).

# **3.5.1.** Preparation of vegetable mix

Carrot and beans were shredded and blanched in boiling water containing potassium metabisulphite (0.2 %) for 3 minutes. This was dried in a dryer. The dried vegetables were mixed together and this mix was used in soup mix.

#### 3.5.2. Preparation of spice mix

Onion, garlic and ginger were peeled, sliced and blanched in boiling water containing potassium metabisulphate (0.2 %) for 3 minutes. This was then ground to a paste and dried in a dryer. The dehydrated spices were ground to a fine powder and were used in soup mix.

#### 3.5.3. Standardisation of soup mix

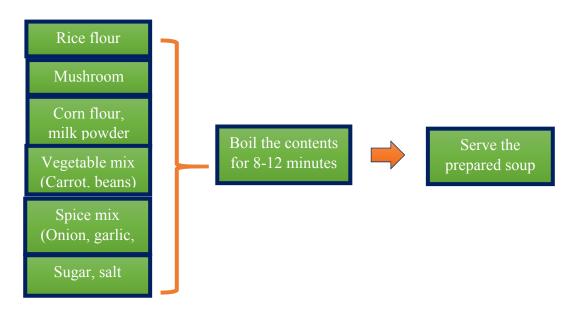
The treatment adopted for the standardisation are detailed below. Instant soup mix was standarised by mixing the rice flour and mushroom powder in different levels. Corn flour, milk powder, sugar, salt, spices and vegetable mixes are added to the flours and blend thoroughly. Soup mix were prepared using 100 per cent rice flour and mushroom powder was taken as control ( $T_0$ ). The quantity of ingredients used for the preparation of instant soup mix are given in Table 2. Soups were prepared using the developed instant soup mixes and the organoleptic evaluation were conducted.

Ingredients (g)	Treatments						
	T <sub>0</sub> MP	T <sub>0</sub> RF	<b>T</b> <sub>1</sub>	$T_2$	<b>T</b> <sub>3</sub>	T <sub>4</sub>	<b>T</b> <sub>5</sub>
Rice flour	-	16	14.4	12.8	11.2	9.6	8
Mushroom powder	16	-	1.6	3.2	4.8	6.4	8
Corn flour	10	10	10	10	10	10	10
Milk powder	50	50	50	50	50	50	50
Vegetable mix	10	10	10	10	10	10	10
Spice mix	2	2	2	2	2	2	2
Sugar	5	5	5	5	5	5	5
Pepper powder	2	2	2	2	2	2	2
Salt	5	5	5	5	5	5	5

 Table 2. Quantity of ingredients used for the standardisation of instant soup

 mix using rice flour

Figure 1. Flow chart for the preparation of soup mix



The quantity of water and time required to make soup mix of acceptable consistency was standardised by repeated trials. For this, 10 g of soup mixes ( $T_0$  to  $T_5$ ) was mixed with varying quantities of water. The soup mix was mixed with water to form slurry without forming lumps and boiled with frequent stirring and cooked till it attained the consistency of soup. The quantity of water and time taken for the preparation of soup mix from the seven treatments under study are represented in Table 3.

	Time (minutes)				
Water (ml/10g)	T <sub>0</sub> RF	T <sub>0</sub> MP			
160	4	6			
150	6	6			
200	8	12			
250	10	5			
300	7	5			

 Table 3. Quantity of water required and time taken for the preparation of soup mix

# 3.5.4. Organoleptic evaluation of instant soup mix

Organoleptic evaluation of soup mix was carried out using score card by a selected panel of fifteen judges as mentioned in 3.2.3.2. The score card prepared was given in Appendix II.

# 3.5.5. Nutritional qualities of instant soup mix

Nutritional qualities like moisture, starch, protein, total fat, calcium, zinc, iron and phosphorus of most acceptable soup mix were assessed using standard procedures mentioned in 3.2.4.1 to 3.2.4.10.

# 3. 7. Standardisation of rice bran cookies

Rice bran cookies were standardised by incorporating rice bran and wheat flour in varying proportions. All other ingredients are used as per standard procedure (Saeed *et al.*, 2009).

# Table 4. Quantity of ingredients used for the preparation of rice bran cookies

Ingredient	Quantity (g)
Whole wheat flour	100
Sugar	25
Butter	50
Baking powder	1
Salt	0.25
Water	As per requirement

The treatments adopted for the standardisation are detailed below.

Treatments	Wheat flour (%)	Rice bran (%)
T <sub>0</sub>	100	_
<b>T</b> <sub>1</sub>	95	5
<b>T</b> <sub>2</sub>	90	10
<b>T</b> <sub>3</sub>	85	15
T <sub>4</sub>	80	20
<b>T</b> <sub>5</sub>	75	25
T <sub>6</sub>	70	30

Table 5. Treatments used for the standardisation of rice bran cookies

To find out the suitable level for the preparation of cookies, rice bran was incorporated with wheat flour in to different proportions. The ratio of composite flour as Wheat Flour (WF): rice bran (RB) was for T<sub>1</sub> (95% WF+ 5% RB), T<sub>2</sub> (90% WF + 10% RB), T<sub>3</sub> (85% WF + 15% RB), T<sub>4</sub> (80% WF + 20% RB), T<sub>5</sub> (75% WF + 25% RB), T<sub>6</sub> (70% WF + 30% RB) respectively.

# 3.7.1. Preparation of rice bran incorporated cookies

Butter and powdered sugar were creamed and the dry ingredients like composite flour (wheat flour + rice bran), baking powder, and salt were mixed thoroughly. After that the dry mixture and the homogenous paste of sugar and butter was mixed thoroughly. This contents were thoroughly kneaded manually by adding required amount of water and kept for proofing for 2 hrs. The prepared dough was moulded in a uniform shapes and cut with the help of a cutter. These cookies were baked at 150 °C for 15-20 minutes in oven (Saeed *et al.*, 2009).

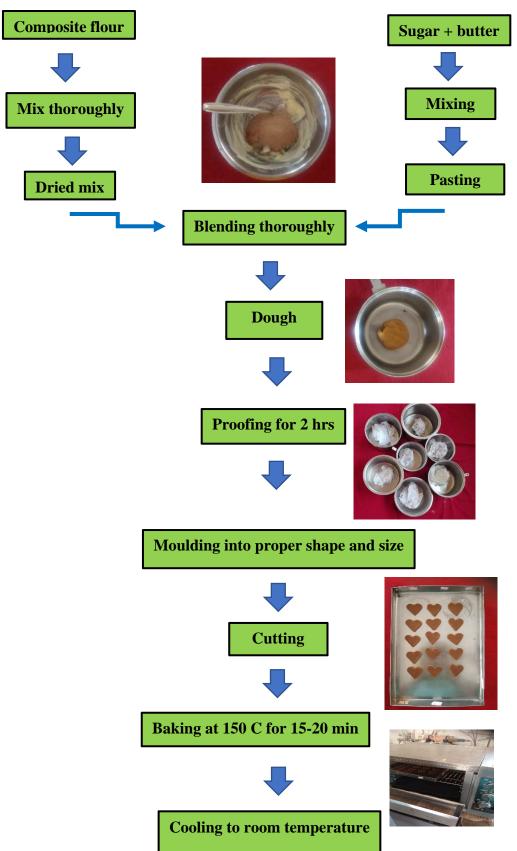


Figure 2. Flow chart for the preparation of rice bran cookies

# 3.7.2. Organoleptic evaluation of rice bran cookies

Cookies were prepared using rice bran and wheat flour and the organoleptic evaluation were conducted according to the procedure explained in 3.2.3.2. The score card used for the organoleptic evaluation of rice bran cookies is given in Appendix III.

# 3.7.3. Nutritional qualities of rice bran cookies

The nutritional qualities like moisture, starch, protein, fat, calcium, zinc, iron, phosphorous of the most acceptable cookies were analysed as per the procedures mentioned from 3.2.4.1 to 3.2.4.10.

# 3.8. Cost of production of the developed products

The cost of production of the developed instant soup mix and cookies were estimated based on the expenses incurred for the preparation of the products. The cost of production was computed based on the market price of the ingredients used for the preparation of products. The cost was calculated for 100 g of the products and the details are presented in Appendix IV.

### 3. 9. Statistical analysis

The data were recorded and analysed as completely randomised design (CRD). Based on organoleptic evaluation, the best treatment was selected using Kendall's Coefficient of Concordance (W). The quality evaluation of rice, rice flour and rice bran and the antioxidant and antiproliferative properties of rice flour and rice bran were compared based on standard deviation (SD).



## **4. RESULTS**

The results of the study entitled 'Nutritional and antioxidant potential of medicinal rice variety *Rakthashali*' are presented under the following heads.

# 4.1. Quality evaluation of rice variety

- 4.1.1. Physical qualities of rice
- 4.1.2. Physical qualities of rice flour
- 4.1.3. Cooking qualities of rice
- 4.1.4. Chemical and nutritional qualities of rice and rice bran
- 4.1.5. Shelf life studies of rice, roasted rice flour and rice bran

# 4.1.6. Assessment of antioxidant and antiproliferative properties of rice flour and rice bran

- 4.1.6.1. Antioxidant activities
- 4.1.6.2. Antiproliferatory assay

## 4.1.7. Acceptability of rice based products

- 4.1.7.1. Standardisation of instant soup mix with rice flour
- 4.1.7.2. Organoleptic qualities of instant soup mix
- 4.1.7.1.1. Nutritional qualities of instant soup mix
- 4.1.7.2. Standardisation of rice bran cookies
- 4.1.7.2.1. Organoleptic qualities of rice bran cookies
- 4.1.7.2.2. Nutritional qualities of rice bran cookies
- 4.1.8. Cost of production of the developed products
- 4.1.9. Statistical analysis

## 4.1. Quality evaluation of rice variety

# 4.1.1. Physical qualities of rice

Physical qualities like total milled rice per cent, head rice per cent, thousand grain weight, volume weight, grain shape and size of rice variety were evaluated and are represented in Table 6. The results pertaining to the physical qualities are as follows.

The total milled rice per cent of *Rakthashali* rice variety was found to be  $70.08 \pm 0.01$  per cent. The head rice recovery of  $56 \pm 0.02$  percent was observed for *Rakthashali* rice variety. Thousand grain weight was observed to be  $11.65 \pm 0.02$  g per 1000 grains. Thousand grain volume of rice variety was assessed and obtained in the range of  $9 \pm 0.1$  mm<sup>3</sup>. The grain shape was determined by measuring the grain length and width of the rice. The grain length of the variety was  $7 \pm 0.4$  mm and grain width was  $3 \pm 0.1$  mm with an L/B ratio of  $2.3 \pm 0.11$ . This indicates that *Rakthashali* rice belong to medium grain classification.

Variety	Total milled rice (%)	Head rice (%)	Thousand grain weight (g)	Thousan d grain volume (mm <sup>3</sup> )	Grain length (mm)	Grain width (mm)	L/B ratio	Grain classifica tion	
Raktha	70.08	56 ±	$11.65 \pm$	$0 \pm 0.1$	$7 \pm 0.4$	$3 \pm 0.1$	2.3 ±	medium	
shali	$\pm 0.01$	0.02	0.02	9 ± 0.1	/ ± 0.4	$5 \pm 0.1$	0.11	mearum	

Table 6. Physical qualities of rice

# 4.1.2. Physical qualities of rice flour

Physical quality is an indicator which influence the consumption and trade. It is important to evaluate the physical composition of rice. Rice flour prepared from whole rice were evaluated for various physical characteristics like bulk density, water absorption index, water solubility index and retrogradation property. The results are presented in Table 7. Bulk density of rice flour of *Rakthashali* rice was found to be  $0.86 \pm 0.16$  g/ml. Water absorption index was  $24.45 \pm 1.45$ , while the water solubility index was  $0.46 \pm 0.03$ .

Table 7. Physical qualities of rice flour

Bulk density (g/ml)	Water absorption index	Water solubility index				
0.86 ± 0.16	24.45 ± 1.45	$0.46 \pm 0.03$				

## 4.1.2.1. Retrogradation property

Retrogradation property of rice flour prepared from *Rakthashali* rice was studied by evaluating the syneresis per cent and is represented in Table 8.

Rice flour of *Rakthashali* rice obtained the highest syneresis on 3<sup>rd</sup> day of observation, which increased gradually on 6<sup>th</sup>, 9<sup>th</sup>, and 12<sup>th</sup> day of observation. The syneresis percentage showed a gradual increase from 3<sup>rd</sup> to 12<sup>th</sup> day of study.

Table 8. Retrogradation property of rice

Days	3 <sup>rd</sup>	6 <sup>th</sup>	9 <sup>th</sup>	12 <sup>th</sup>
Syneresis (%)	$14.25 \pm 1.01$	20.52 ± 1.32	$26.40 \pm 1.50$	$32.05 \pm 0.96$

# 4.1.3. Cooking qualities of rice

Table rice was prepared and the organoleptic qualities were assessed by using standard procedure. Cooking qualities like gelatinization temperature index, cooking time, water uptake, volume expansion, amylose content, gel consistency and grain elongation were also evaluated.

## 4.1.3.1 Organoleptic qualities of table rice

Organoleptic qualities of table rice was evaluated and the mean scores of different quality attributes of cooked rice are given in Table 9. In cooked rice, the mean score for appearance was 7.37 and colour attained a mean score of 7.53 and value obtained for flavour was 7.64. For texture, a mean score of 6.02 was obtained. For taste, the mean score obtained was 7.58. The overall acceptability of 7.02 was obtained for cooked rice. All the organoleptic qualities, except texture, obtained a mean score of above 7. The cooked *Rakthashali* rice is presented in plate 2 also.

Table 9. Mean score for organoleptic qualities of table rice

Variety	Appea	Colour	Flavour	Texture	Taste	Overall
	rance					acceptability
Rakthashali	7.37	7.53	7.64	6.02	7.58	7.02

Various cooking qualities like gelatinization temperature index, cooking time, water uptake, volume expansion, amylose content, gel consistency and grain elongation were evaluated. The results are as follows.

*Rakthashali* rice was subjected to the alkali digestion test and were visually observed to evaluate the degree of disintegration in alkali. After the stipulated time of observation, it was observed that the grains become chalky and the kernels were not affected due to alkali treatment. It means that the *Rakthashali* rice grains had high gelatinization temperature index (>74 °C) and the rating was found to be low and is represented in plate 3. Cooking time was measured using standard procedure and found to be  $40 \pm 3.0$  minutes which is observed to be higher. Water uptake was measured and obtained a higher value of  $9.3 \pm 1.44$  ml. Volume expansion ratio was determined to be  $4 \pm 0.59$  for this rice variety. Amylose content of  $20.38 \pm 1.69$  per cent was observed in *Rakthashali* rice variety. Gel consistency of rice was determined by measuring gel length. The gel



Plate 2. Table rice prepared with *Rakthashali* rice



Plate 3. Gelatinization temperature index of *Rakthashali* rice

length of  $52 \pm 0.98$  mm was obtained for this rice variety and observed that *Rakthashali* rice variety comes under medium grain classification. Grain elongation ratio was assessed according to standard procedure and obtained the value of  $0.60 \pm 0.1$ . Table 5 indicates the cooking qualities of *Rakthashali* rice variety.

Gelatinization temperature index	High
Cooking time (min)	40 ± 3.0
Water uptake (ml)	9.3 ± 1.44
Volume expansion ratio	4 ± 0.59
Amylose content (%)	20.38 ± 1.69
Gel consistency (mm)	52 ± 0.98
Grain elongation ratio	0.6 ± 0.1

Table 10. Cooking qualities of Rakthashali rice variety

## 4.1.4. Chemical and nutritional qualities of rice and rice bran

The nutritional qualities of rice and rice bran like moisture, starch, carbohydrate, protein, total fat, energy, calcium, zinc, iron, phosphorus, *In vitro* digestibility of starch and *In vitro* availability of calcium, zinc, iron and phosphorus of *Rakthashali* rice and rice bran were evaluated. The results on the chemical and nutritional qualities of rice and rice bran are discussed below.

The moisture content was observed to be  $9.30 \pm 0.44$  per cent for rice and in rice bran was found to be  $12.25 \pm 1.98$  per cent. Starch content of rice and rice bran was evaluated and found to be  $65 \pm 1.01$  g/ 100g in rice and  $23.04 \pm 0.46$  g/ 100g in rice bran. Carbohydrate content of rice was found to be  $71.25 \pm 0.85$  g/ 100g and in rice bran it was  $46.08 \pm 1.33$  g/ 100g. The protein content of  $11.60 \pm$ 1.17 g/ 100g was observed in rice while rice bran had  $11.36 \pm 0.88$  g/ 100g of protein. The fat content of rice bran was found to be  $5.70 \pm 0.955$  g/ 100g and a lower fat content of  $3.68 \pm 1.00$  g/ 100g was observed in rice grain. Energy content of  $339.52 \pm 1.52$  Kcal was observed in rice grain and for rice bran it was of  $281.06 \pm 1.96$  Kcal. Fibre content was also estimated in rice and rice bran. Highest fibre content of  $18.59 \pm 0.30$  g/ 100g was observed rice bran. For rice, it was found to be  $9.08 \pm 0.01$  g/ 100g.

Calcium content was determined and observed to be  $5.69 \pm 1.5$  mg/ 100g and  $12.1 \pm 1.89$  mg/ 100g in rice and rice bran respectively. The zinc content of *Rakthashali* was assessed and found to be  $1.42 \pm 1.16$  mg/ 100g for rice grain and for rice bran it was of  $0.88 \pm 0.16$  mg/ 100g. Iron content was determined and found to be  $1.67 \pm 0.83$  mg/ 100g in rice grain and a higher iron content of 31.01  $\pm 1.98$  mg/ 100g was obtained for rice bran. The phosphorus content was higher for rice ( $352 \pm 3.00$  mg/ 100g) compared to rice bran ( $116 \pm 2.00$  mg/ 100g).

In vitro availability of starch from rice and rice bran are given in Table 6. The *in vitro* digestibility was observed in rice was  $60.32 \pm 0.04$  per cent and for rice bran digestibility was found to be  $18.07 \pm 0.19$  per cent. The *in vitro* availability of calcium, zinc, iron and phosphorus was assessed and found as  $70.81 \pm 1.16$  per cent,  $46.65 \pm 1.09$  per cent,  $67.04 \pm 0.93$  per cent, and  $56.32 \pm 0.91$  per cent respectively. Rice bran obtained lower *in vitro* availability of calcium, zinc, iron and phosphorus 65.41 ± 1.40 per cent,  $41.76 \pm 1.09$  per cent,  $61.11 \pm 1.28$  per cent and  $40.07 \pm 1.03$  per cent compared to rice.

Nutritional qualities (per 100 g)	Mean value of rice	Mean value of rice bran
Moisture (%)	$9.30 \pm 0.44$	$12.25 \pm 1.98$
Starch (g/ 100g)	$65.00 \pm 1.01$	$23.04 \pm 0.46$
Carbohydrate (g/ 100g)	$71.25 \pm 0.85$	46.08 ± 1.33
Protein (g/ 100g)	$11.60 \pm 1.17$	$11.36 \pm 0.88$
Energy (Kcal)	$339.52 \pm 1.52$	281.06 ± 1.96
Fat (g/ 100g)	$3.68 \pm 1.00$	$5.70 \pm 0.955$
Fibre (g/ 100g)	$9.08 \pm 0.01$	$18.59 \pm 0.30$
Calcium (mg/ 100g)	5.69 ± 1.5	$12.1 \pm 1.89$
Zinc (mg/ 100g)	$1.42 \pm 1.16$	$0.88 \pm 0.16$
Iron (mg/ 100g)	$1.67 \pm 0.83$	31.01 ± 1.98
Phosphorus (mg/ 100g)	$352.00\pm3.00$	$116.00 \pm 2.00$
In vitro digestibility of starch (%)	$60.32\pm0.04$	$18.07 \pm 0.19$
In vitro availability of calcium (%)	$70.81 \pm 1.16$	$65.41 \pm 1.40$
In vitro availability of zinc (%)	$46.65 \pm 1.09$	$41.76 \pm 1.09$
In vitro availability of iron (%)	$67.04 \pm 0.93$	61.11 ± 1.28
In vitro availability of phosphorus (%)	$56.32 \pm 0.91$	$40.07 \pm 1.03$

 Table 11. Chemical and nutritional qualities of Rakthashali rice variety

## 4.1.5. Shelf life studies of rice, roasted rice flour and rice bran

## 4.1.5.1. Microbial qualities

The rice, roasted rice flour and rice bran was evaluated for bacteria, fungi and yeast initially and at monthly intervals of storage and the results pertaining to microbial enumeration are given in Table 12.

In rice, initially and during first month of storage, the bacterial count was absent. Bacterial growth was observed by the end of second month which gradually increased to  $0.6 \times 10^5$  cfu g<sup>-1</sup> at the end of storage. Fungal and yeast growth were not detected in rice till the end of storage.

Microbial enumeration was also conducted in roasted rice flour for three months. Bacterial growth was not detected upto two months but it was found to be  $0.3 \times 10^5$  cfu g<sup>-1</sup> at the end of storage period. Fungal and yeast growth were not observed throughout the storage period of three months.

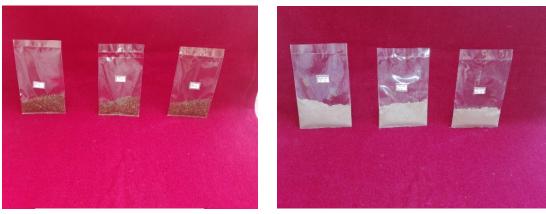
In rice bran also, the bacterial growth was not detected initially of storage period. But the bacterial count of  $0.5 \times 10^5$  cfu g<sup>-1</sup> was observed at first month which increased to  $0.7 \times 10^5$  cfu g<sup>-1</sup> at the end of three months of storage period. Fungal count was observed to be  $0.5 \times 10^3$  cfu g<sup>-1</sup> in rice bran and yeast colonies were not detected in rice bran initially and at the end of storage.

## 4.1.5.2. Insect infestation

Observations on the presence of storage pests and insects were taken in rice, roasted rice flour and rice bran initially and at the end of three moths of storage. No storage pests was observed in rice, roasted rice flour and rice bran. Insect infestation was also not detected in rice, roasted rice flour and rice bran.

## 4.1.5.3. Peroxide value

Peroxide value was not found in rice, rice flour and rice bran initially and at the end of storage period. The stored rice, roasted rice flour and rice bran are mentioned in plate 4.



Rice

**Roasted rice flour** 



**Rice bran** 

Plate 4. Stored rice, roasted rice flour and rice bran

 Table 12. Microbial growth in rice, roasted rice flour and rice bran

Rakthashali	Microbial growth (cfu/g)											
	Initial		1 <sup>st</sup> month		2 <sup>nd</sup> month			3 <sup>rd</sup> month				
	Bacteria	Fungi	yeast	Bacteria	Fungi	yeast	Bacteria	Fungi	yeast	Bacteria	Fungi	yeast
Rice	ND	ND	ND	ND	ND	ND	0.2	ND	ND	0.6	ND	ND
Roasted rice flour	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.3	ND	ND
Rice bran	ND	ND	ND	0.5	ND	ND	0.5	ND	ND	0.7	0.5	ND

ND – Not detected

## 4.1.6. Assessment of antioxidant properties of rice variety

Antioxidant compounds are functional compounds which reduces the oxidative stress. Antioxidant and antiproliferative activities were studied in *Rakthashali* rice and rice bran. The results are as follows.

## 4.1.6.1. Antioxidant activities

Antioxidant activities such as DPPH (1,1-diphenyl 1,2- picrylhydrazyl) radical scavenging activity, reducing power (RP) assay, nitric oxide (NO) scavenging activity, superoxide and hydroxyl scavenging activity, total antioxidant activity were assessed in rice and rice bran extracts. The total phenol (TC) and total flavonoid (FC) were also analysed. The results are discussed below.

## 4.1.6.1.1. DPPH radical scavenging activity

Free radical scavenging activities of the rice flour and rice bran extracts were assessed by the DPPH assay. The stable free radical, DPPH was effectively scavenged by rice flour and rice bran extracts. Rice flour and rice bran extracts are dose dependently showed DPPH radical scavenging activity within the concentrations of (5-40  $\mu$ g/ml) extracts. Methanolic extract of rice bran and rice flour possess DPPH scavenging activity. The free radical scavenging activity was observed by finding the IC<sub>50</sub> (Inhibition coefficient) value. In the present, study both the rice flour and rice bran extracts showed the antioxidant activity effectively by scavenging the free radical with the IC<sub>50</sub> value of 8.50 ± 0.196  $\mu$ g/ml in rice flour and 22.5 ± 1.174  $\mu$ g/ml in rice bran respectively. Figure 1 express the DPPH radical scavenging activity of rice flour and rice bran.

## 4.1.6.1.2. Superoxide radical scavenging activity

Superoxide radical scavenging activity is based on the ability of the extract to inhibit the reduction of nitro blue tetrazolium (NBT) by superoxide, which is generated by the reaction of photo-reduction of riboflavin within the system. Superoxide radicals generated by the photo reduction of riboflavin was effectively scavenged by rice flour than rice bran extract. The extracts were added dose dependent at different concentrations from  $10 - 50 \ \mu g/ml$  and the scavenging efficacy of rice flour was higher with the IC<sub>50</sub> values of  $25.4 \pm 2.47 \ \mu g/ml$ . Rice bran extract does not shown an effective scavenging capacity, but it showed the IC<sub>50</sub> value of  $49.28 \pm 2.8 \ \mu g/ml$  of concentration. Figure 2 represents the superoxide radical scavenging activity of rice flour and rice bran.

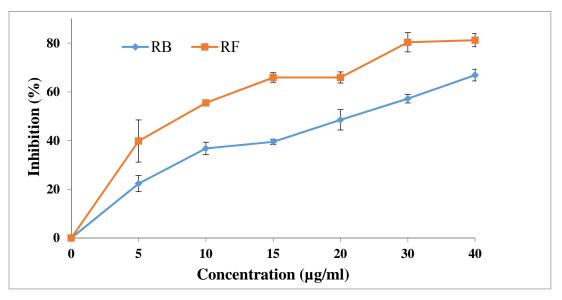
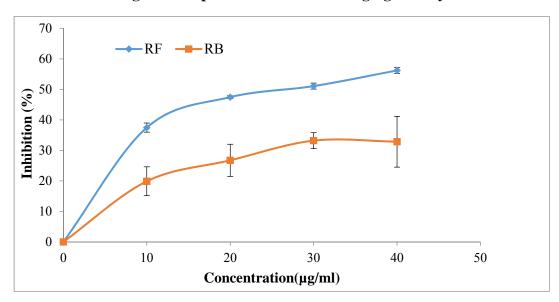


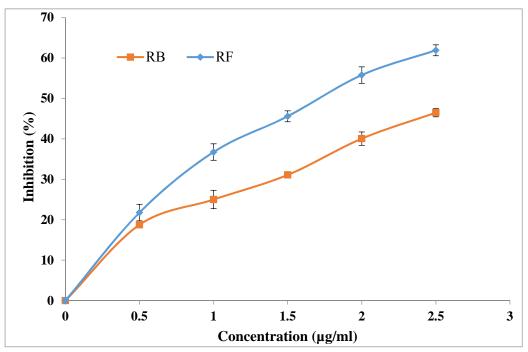
Figure 3. DPPH radical scavenging activity

Figure 4. Superoxide radical scavenging activity



# 4.1.6.1.3. Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the test compounds for hydroxyl radicals generated from Fe<sup>3+</sup>/ ascorbate/ EDTA/ H<sub>2</sub>O<sub>2</sub> systems. The hydroxyl radical attack deoxyribose which eventually results in the formation of thiobarbituric acid reaction substances (TBARS). The efficacy was found to be in dose dependent manner in the concentration of  $0.5 - 3 \mu g/ml$ . The hydroxyl radical scavenging activity was significantly higher in rice flour extract with the IC<sub>50</sub> values of  $1.72 \pm 0.030 \mu g/ml$ . Rice bran extract scavenged the hydroxyl radical with IC<sub>50</sub> values were found to be  $2.47 \pm 0.077 \mu g/ml$  which is slight lower compared to rice flour. Figure 3. represents the hydroxyl radical scavenging activity of rice flour and rice bran.

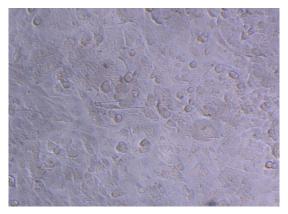




# Antiproliferatory activity of rice flour and rice bran extract towards MCF-7



Plate 5. Control cell line (MCF-7)



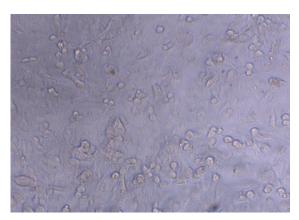


Plate 6. Rice flour extract induced cell death Plate 7. Rice bran extract induced cell death

Antioxidant activity	Rice flour	Rice bran							
IC <sub>50</sub> value (µg/ml)									
DPPH radical scavenging activity	8.50 ± 0.196	$22.5 \pm 1.174$							
Superoxide radical scavenging activity	$25.4 \pm 2.47$	$49.28 \pm 2.8$							
Hydroxyl radical scavenging activity	$1.72 \pm 0.030$	$2.47\pm0.077$							
Nitric oxide radical scavenging activity	ND	ND							

Table 13. IC<sub>50</sub> values for scavenging activities of the rice flour and rice bran extracts

# 4.1.6.1.4. Total antioxidant activity

Total antioxidant activity is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex. The total antioxidant activity of rice flour and rice bran extracts increased with increase of the concentration of extracts. The methanolic extracts of rice flour and rice bran showed a significant change in the absorbance value from 10 to 100  $\mu$ g/ml. Rice flour extract showed the total antioxidant activity with the absorbance value of 0.95 to 9.56. Rice bran extract noticed the total antioxidant activity from 2.13 to 10.30. The total antioxidant activity shown by rice bran extract was found to be significant at 100  $\mu$ g/ml which is equivalent to the 10 mg/g vitamin C. Figure 5 represents the total antioxidant activity of rice flour and rice bran extracts.

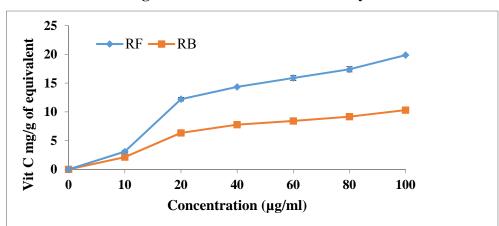
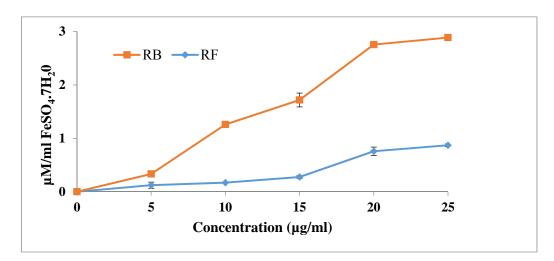
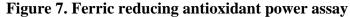


Figure 6. Total antioxidant activity

## 4.1.6.1.5. Ferric reducing antioxidant power assay (FRAP)

Antioxidant activity has been proposed to be related to reducing power. Ferric reducing antioxidant power assay measures the reducing capacity of the test compounds. Therefore, the antioxidant potential of methanol extracts of rice flour and rice bran was estimated for their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The reducing power of the rice flour and rice bran extracts increased with the increase concentration and a significant change was observed at 5- 25  $\mu$ g/ml concentration of the extracts. At 5  $\mu$ g/ml of the methanolic extract of rice bran showed the absorbance value 0.21 which increased to 2.01 and rice flour showed the absorbance value ranged from 0.12 to 0.87 when concentration increases. The extracts of rice flour and rice bran dose dependently shown ferric reducing ability within the range 5- 25  $\mu$ g/ml and the ferric reducing antioxidant activity was higher in rice bran.





### 4.1.6.1.6. Total phenol and total flavonoid content

The total phenolic content of methanolic extract of rice flour and rice bran was found to be to  $3.8 \pm 0.04$  and  $1.9 \pm 0.14$  mg GAE /g extract respectively. Total flavonoid content in the methanolic extract of rice flour and rice bran was estimated and found to be  $90.5 \pm 1.4$  mg QTE /g extract and  $106.9 \pm 4.0$  mg

QTE /g extract respectively. Table 14 shows the total phenol and total flavonoid content.

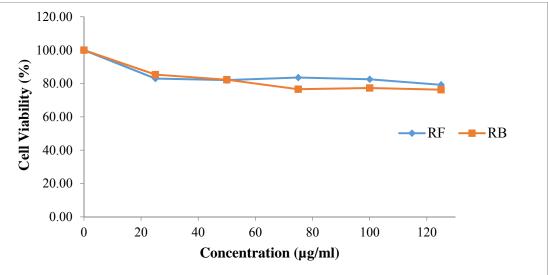
Sample	Total phenol (mg GAE/g extract)extract	Total flavonoids (mg QTE/g extract)extract
Rice flour	3.8 ± 0.04	90.5 ±1.4
Rice bran	$1.9 \pm 0.14$	$106.9 \pm 4.0$

Table 14. Total phenol and total flavonoid content

# 4.1.6.2. Antiproliferative activities

Antiproliferatory assay in adenocarcinoma cancer cell line using MTT [3-(4, 5-dimethylthiazolyl-2)-2, 5- diphenyltetrazolium bromide] assay was done in *Rakthashali* rice and bran extracts. The result are shown in figure 6.

Figure 8. Antiproliferative activity



Antiproliferatory activity was separately studied in rice flour and rice bran. The methanolic extracts were added in different concentrations dose dependent from 20 - 120 ug/ml. In the cytotoxicity analysis towards human adenocarcinoma

cell line (MCF-7), the IC<sub>50</sub> value was not detected in both rice flour and rice bran extracts, but rice bran showed comparable activity with the loss of cell viability in 50 ug/ml was 26.63 per cent. In rice flour extract the loss in cell viability was observed to be 21.18 per cent in the concentration of 25 ug/ml. From this assay, up to a concentration of 120 ug/ml, rice flour and rice bran extracts, no further increase in cytotoxicity towards human adenocarcinoma cell line (MCF-7). Antiproliferatory activity was comparatively higher for rice bran than rice flour extract. In this study, a slight antiproliferatory activity was observed against adenocarcinoma cell line (MCF-7) was observed. The normal cell line and the rice flour and rice bran induced cell death was shown in plate 5, 6 and 7 respectively.

## 4.1.7. Acceptability of rice based products

## 4.1.7.1. Standardisation of instant soup mix with rice flour

Soup mix were standardised by incorporating various proportions of rice flour and mushroom powder. The organoleptic evaluation of soup mix was conducted by the panel of 15 judges.

#### 4.1.7.1.1. Organoleptic qualities of instant soup mix with rice flour

The organoleptic evaluation of various combination of rice flour and mushroom based instant soup mix along with control is represented in Table 15 and the prepared soup mixes are represented in plate 8.

The mean scores for appearance of soup mix prepared with rice flour and mushroom powder ranged from 6.22 (T<sub>1</sub>- 90 % RF+ 10 % MP) to 8.51 (T<sub>4</sub>- 60 % RF + 40 % MP) with a mean rank score of 1.83 and 6.60. Treatments like T<sub>0</sub> (100 % RF), T<sub>0</sub> (100 % MP), T<sub>2</sub> (80 % RF + 20 % MP), T<sub>3</sub> (70 % RF + 30 % MP) and T<sub>5</sub> (50 % RF + 50 % MP) obtained a mean scores of 8.16, 6.31, 6.28, 7.46 and 7.48 with a mean rank score of 5.37, 2.00, 2.53, 4.50 and 4.57 respectively.

The highest mean scores for colour of soup mix was observed in  $T_4$  (8.54) with a mean rank score of 6.63. This was followed by  $T_0$  RF (8.13),  $T_3$  (7.48),  $T_5$ 

(7.46), T<sub>1</sub> (6.64), T<sub>2</sub> (6.60) and T<sub>0</sub> MP (6.37) with a mean rank score of 4.47, 4.57, 4.57, 2.47, 2.27 and 1.47.

The flavour of soup mix prepared with rice flour and mushroom powder, the highest mean score was also obtained in  $T_4$  (8.75) and lowest was in  $T_0$  MP (6.40) with a mean rank scores of 6.87 and 1.53. Treatments like  $T_0$  RF,  $T_1$ ,  $T_2$ ,  $T_3$ and  $T_5$  obtained the mean score of 8.22, 6.51, 6.97, 7.51 and 7.35 with a mean rank scores of 4.53, 1.83, 3.20, 4.50 and 4.13.

The highest mean score for texture of soup mix was obtained in  $T_4$  (8.51) with a mean rank score of 6.67.  $T_0$  RF (100 % RF) obtained a mean score of 8.33 with a mean rank score of 4.67. Other treatments  $T_0$  MP,  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_5$  obtained the mean scores of 6.35, 6.75, 6.95, 7.53 and 7.44 with a mean rank scores of 1.33, 2.30, 2.83, 4.37 and 4.27.

For taste, the highest mean score was noticed in T4 (8.68) with a mean rank score of 6.77. The lowest mean score was obtained in  $T_0$  MP (6.42) with a mean rank score of 2.00. Treatments like  $T_0$  RF,  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_5$  obtained a mean scores of 8.40, 6.44, 6.64, 7.35 and 7.37. The mean rank scores of these treatments are 4.20, 1.80, 1.80, 4.33 and 4.40 respectively.

The overall acceptability for soup mix obtained the highest mean score of 8.75 in  $T_4$  and was followed by 8.06 ( $T_0$  RF), 7.66 ( $T_3$ ), 7.17 ( $T_5$ ), 6.57 ( $T_0$  MP) and 6.24 ( $T_1$  and  $T_2$ ). The mean rank scores of these treatments are 6.90, 4.93, 3.97, 2.17 and 1.33 respectively.

Treatments	Appearance	Colour	Flavour	Texture	Taste	Overall	Total score
						acceptability	
T <sub>0</sub>	8.16	8.13	8.22	8.33	8.40	8.06	49.3
(100 % RF)	(5.37)	(4.47)	(4.53)	(4.67)	(4.20)	(4.07)	
T <sub>0</sub>	6.31	6.37	6.40	6.35	6.42	6.57	38.42
(100 % MP)	(2.00)	(1.47)	(1.53)	(1.33)	(2.00)	(2.17)	
T <sub>1</sub>	6.22	6.64	6.51	6.75	6.44	6.24	38.8
(90 % RF + 10 % MP )	(1.83)	(2.47)	(1.83)	(2.30)	(1.80)	(1.33)	
T <sub>2</sub>	6.28	6.60	6.97	6.95	6.64	6.24	39.68
(80% RF + 20 % MP)	(2.53)	(2.27)	(3.20)	(2.83)	(1.80)	(1.33)	
T <sub>3</sub>	7.46	7.48	7.51	7.53	7.35	7.66	44.99
(70 % RF + 30 % MP )	(4.50)	(4.57)	(4.50)	(4.37)	(4.33)	(4.93)	
T <sub>4</sub>	8.51	8.54	8.75	8.51	8.68	8.75	51.74
(60 % RF + 40 % MP)	(6.60)	(6.63)	(6.87)	(6.67)	(6.77)	(6.90)	
T <sub>5</sub>	7.48	7.46	7.35	7.44	7.37	7.17	44.27
(50 % RF + 50 % MP)	(4.57)	(4.57)	(4.13)	(4.27)	(4.40)	(3.97)	
Kendall's w	**0.814	**0.884	**0.873	**0.867	**0.885	**0.890	

Table 15. Mean scores for organoleptic qualities of instant soup mix with different treatments

Values in parenthesis is mean rank score based on Kendall's W; \*\* Significant at 1% level

RF Rice flour; MP Mushroom powder



 $T_0 - 100\% \text{ RF}, 100\% \text{ MP} - \text{Control}$   $T_1 - 90\% \text{ RF} + 10\% \text{ MP}$   $T_2 - 80\% \text{ RF} + 20\% \text{ MP}$   $T_3 - 70\% \text{ RF} + 30\% \text{ MP}$   $T_4 - 60\% \text{ RF} + 40\% \text{ MP}$   $T_5 - 50\% \text{ RF} + 50\% \text{ MP}$ MP - Mushroom Powder: RF - Rice Flour

Plate 8. Prepared instant soup mixes with rice flour

# 4.1.7.2.2. Chemical and nutritional qualities of instant soup mix with rice flour

Chemical and nutritional qualities like moisture, starch, carbohydrate, protein, fat, calcium, zinc, iron and phosphorus content were analysed in selected instant soup mix and the results are as follows and represented in Table 16.

Nutritional qualities (per 100 g) of	Mean value
soup mix	
Moisture (%)	$4.55 \pm 0.02$
Starch (g/ 100g)	$57.56 \pm 0.06$
Carbohydrate (g/ 100g)	$64.56 \pm 0.67$
Protein (g/ 100g)	$32.21 \pm 0.78$
Fat (g/ 100g)	$2.23\pm0.03$
Calcium (mg/ 100g)	$32.2 \pm 1.94$
Zinc (mg/ 100g)	$0.21 \pm 0.01$
Iron (mg/ 100g)	$0.54 \pm 0.12$
Phosphorus (mg/ 100g)	254.57 ± 1.54

Table 16. Chemical and nutritional qualities of instant soup mix

Moisture content of selected instant soup mix was observed and found to be  $4.55 \pm 0.02$  per cent. The product contained  $57.56 \pm 0.06$  of starch. Carbohydrate content was observed to be  $64.56 \pm 0.67$  g/ 100g in rice flour based soup mix. The protein and fat content of soup mix was found to be  $32.21 \pm 0.78$  g/ 100g and  $2.23 \pm 0.03$  g/ 100g respectively.

Minerals like calcium, zinc, iron and phosphorus were analysed and the calcium content of  $32.2 \pm 1.94$  mg/ 100g was noticed in the selected instant soup mix. Zinc content was analysed for rice flour based soup mix and found out to be

 $0.21 \pm 0.01$  mg/ 100g. Iron content of  $0.54 \pm 0.12$  mg/ 100g was obtained for soup mix. Phosphorus content was found to be  $254.57 \pm 1.54$  mg/ 100g in the product.

## 4.1.7.2. Standardisation of rice bran cookies

Cookies were standardised by incorporating different proportions of wheat flour and rice bran and the organoleptic evaluation was conducted.

## 4.1.7.2.1. Organoleptic qualities of rice bran cookies

Rice bran cookies were prepared with whole wheat flour and rice bran and the mean scores for the different quality attributes are represented in Table 17 and the prepared rice bran cookies are shown in plate 9. The highest mean score for appearance was noticed in cookies prepared with 80 per cent wheat flour and 20 per cent rice bran (T<sub>4</sub>) (8.66) with a mean rank score of 6.73. The lowest mean score for appearance was recorded in T<sub>6</sub> (70 % wheat flour + 30 % rice bran) (6.24) with a mean rank score of 1.10. Cookies prepared with T<sub>0</sub> (100 % wheat flour), T<sub>1</sub> (95 % wheat flour + 5 % rice bran), T<sub>2</sub> (90 % wheat flour + 10 % rice bran), T<sub>3</sub> (85 % wheat flour + 15 % rice bran) and T<sub>5</sub> (75 % wheat flour + 25 % rice bran) obtained a mean scores of 8.00, 8.00, 8.00, 8.33 and 6.77 with a mean rank scores of 5.37, 4.13, 3.17, 5.33 and 2.17 respectively for appearance.

Mean score for colour of cookies ranged from 8.60 (T<sub>4</sub>- 80 % wheat flour + 20 % rice bran) to 6.29 (T<sub>6</sub>- 70 % wheat flour + 30 % rice bran). The highest mean score for colour was observed for cookies prepared with 80 per cent wheat flour and 20 per cent rice bran (T<sub>4</sub>) (8.60) followed by T<sub>3</sub> (8.16), T<sub>0</sub> (7.68), T<sub>1</sub> (7.66), T<sub>2</sub> (7.34), T<sub>5</sub> (6.73) and T<sub>6</sub> (6.29). The mean rank scores obtained for these treatments were found to be 7.00, 5.93, 4.47, 4.07, 3.53, 2.00 and 1.00 respectively.

Among different treatments, cookies prepared with 80 per cent wheat flour and 20 per cent rice bran ( $T_4$ ) obtained the highest mean score of 8.59 for flavour with mean rank score of 7. The second highest mean score for flavour was noticed in cookies prepared with 85 per cent wheat flour and 15 per cent rice bran ( $T_3$ ) (8.17) with a mean rank score of 6. Cookies prepared with  $T_0$  (100 % wheat flour),  $T_1$  (95 % wheat flour + 5 % rice bran),  $T_2$  (90 % wheat flour + 10 % rice bran),  $T_5$  (75 % wheat flour + 25 % rice bran),  $T_6$  (70 % wheat flour + 30 % rice bran) obtained a mean rank score of 4.53, 4.47, 3.00, 2.00 and 1.00 respectively.  $T_6$  (70 % wheat flour and 30 % rice bran) obtained a lower mean score of 6.29 with a mean rank score of 1.

The mean scores for texture of cookies with various treatments were ranged from 8.59 (T<sub>4</sub>) to 6.29 (T<sub>6</sub>) with a mean rank score of 7 and 1. Treatments like T<sub>0</sub> (100 % wheat flour), T<sub>1</sub> (95 % wheat flour + 5 % rice bran), T<sub>2</sub> (90 % wheat flour + 10 % rice bran), T<sub>3</sub> (85 % wheat flour + 15 % rice bran) and T<sub>5</sub> (75 % wheat flour + 25 % rice bran) obtained a mean scores of 7.70, 7.66, 7.34, 8.17 and 6.73 with a mean rank scores of 4.67, 4.13, 3.27, 5.87 and 2.00 respectively.

In the case of taste, the highest mean score was observed in T<sub>4</sub> (80 % wheat flour + 20 % rice bran) (8.59) followed by T<sub>3</sub> (85 % wheat flour + 15 % rice bran) (8.17), T<sub>1</sub> (95 % wheat flour + 5 % rice bran) (8.02), T<sub>0</sub> (100 % wheat flour) (7.70), T<sub>2</sub> (90 % wheat flour + 10 % rice bran) (7.34), T<sub>5</sub> (75 % wheat flour + 25 % rice bran) (6.73) and T<sub>6</sub> (70 % wheat flour + 30 % rice bran) (6.29) respectively. The mean rank scores of 7, 5.73, 5.07, 4.20, 3.00, 2.00 and 1.00 were observed in these treatments.

The overall acceptability for cookies obtained the mean scores ranged from 8.59 (T<sub>4</sub>) to 6.29 (T<sub>6</sub>). The second highest mean score (8.17)was observed in cookies prepared with 85 per cent wheat flour and 15 per cent rice bran which was followed by T1 (95 % wheat flour + 5 % rice bran)(8.06), T<sub>0</sub> (100 % wheat flour) (7.70), T<sub>2</sub> (90 % wheat flour + 10 % rice bran) (7.34), T<sub>5</sub> (75 % wheat flour + 25 % rice bran) (6.73) and T<sub>6</sub> (70 % wheat flour + 30 % rice bran) (6.29). The mean rank scores for these treatments were 7, 5.73, 5.20, 4.07, 3, 2 and 1 respectively.

Treatments	Appearance	Colour	Flavour	Texture	Taste	Overall	Total
						acceptability	score
T <sub>0</sub>	8.00	7.68	7.70	7.70	7.70	7.70	46.48
(100 % WF)	(5.37)	(4.47)	(4.53)	(4.67)	(4.20)	(4.07)	
T <sub>1</sub>	8.00	7.66	7.82	7.66	8.02	8.06	47.22
(95 % WF + 5 % RB )	(4.13)	(4.07)	(4.47)	(4.13)	(5.07)	(5.20)	
$T_2$	8.00	7.34	7.43	7.34	7.34	7.34	44.79
(90 % WF + 10 % RB)	(3.17)	(3.53)	(3.00)	(3.27)	(3.00)	(3.00)	
T <sub>3</sub>	8.33	8.16	8.17	8.17	8.17	8.17	49.17
(85 % WF + 15 % RB )	(5.33)	(5.93)	(6.00)	(5.87)	(5.73)	(5.73)	
T <sub>4</sub>	8.66	8.60	8.59	8.59	8.59	8.59	51.62
(80 % WF + 20 % RB)	(6.73)	(7.00)	(7.00)	(7.00)	(7.00)	(7.00)	
Τ <sub>5</sub>	6.77	6.73	6.73	6.73	6.73	6.73	40.42
(75 % WF + 25 % RB)	(2.17)	(2.00)	(2.00)	(2.00)	(2.00)	(2.00)	
T <sub>6</sub>	6.24	6.29	6.29	6.29	6.29	6.29	37.69
(70 % WF + 30 % RB)	(1.10)	(1.00)	(1.00)	(1.00)	(1.00)	(1.00)	
Kendall's W	**0.882	**0.939	**0.982	**0.937	**0.971	**0.980	

Table 17. Mean scores f	for organoleptic qu	alities of rice bran c	ookies with o	different treatments

Value in parentheses is mean rank score based on Kendall's W

\*\* Significant at 1% level

WF- Wheat Flour

RB- Rice Bran



- $T_0 100\% \text{ WF} \text{control}$   $T_1 95\% \text{ WF} + 5\% \text{ RB}$   $T_2 90\% \text{ WF} + 10\% \text{ RB}$   $T_3 85\% \text{ WF} + 15\% \text{ RB}$   $T_4 80\% \text{ WF} + 20\% \text{ RB}$   $T_5 75\% \text{ WF} + 25\% \text{ RB}$   $T_6 70\% \text{ WF} + 30\% \text{ RB}$
- WF Wheat flour: RB Rice bran

Plate 9. Prepared rice bran incorporated cookies

The nutritional qualities of rice bran cookies was estimated. The nutritional constituents like moisture, starch, carbohydrate, protein, fat, calcium, zinc, iron and phosphorus were analysed and is represented in Table 18.

Nutritional qualities (per 100 g) of	Mean value		
cookies			
Moisture (%)	5.27 ± 0.27		
Starch (g/ 100g)	$44.03 \pm 1.00$		
Carbohydrate (g/ 100g)	$63.87 \pm 0.07$		
Protein (g/ 100g)	8.78 ± 0.20		
Fat (g/ 100g)	$22.74 \pm 0.05$		
Calcium (mg/ 100g)	$18.32 \pm 0.66$		
Zinc (mg/ 100g)	$1.55 \pm 0.01$		
Iron (mg/ 100g)	$0.964 \pm 0.10$		
Phosphorus (mg/ 100g)	$118.87 \pm 0.25$		

Table 18. Chemical and nutritional qualities of rice bran cookies

Moisture content of selected rice bran cookies was observed and found to be  $5.27 \pm 0.27$  per cent. The starch content of 44.03 g/ 100g was attained for rice bran cookies. Carbohydrate content of  $63.87 \pm 0.07$  was observed for rice bran cookies. The protein content of cookies was also evaluated and found to be  $8.78 \pm$ 0.20 g/ 100g. Higher fat content of 22.74 ± 0.05 g/ 100g was observed for rice bran cookies.

Minerals like calcium, zinc, iron and phosphorus were analysed. Calcium content of  $18.32 \pm 0.66$  mg/ 100g was noticed selected rice bran cookies. Zinc content was estimated for rice bran cookies and found out to be  $1.55 \pm 0.01$  mg/ 100g. Higher iron content of  $0.964 \pm 0.10$  mg/ 100g was evaluated for cookies. Phosphorus content was found to be  $118.87 \pm 0.25$  mg/ 100g in cookies.

# 4.1.8. Cost of production of the developed products

The cost of production of instant soup mix and cookies were estimated per 100g of the finished product. The details of the products are represented in Appendix IV. The cost for the preparation of instant soup mix was Rs. 80.14/ 100g. The production cost for rice bran cookies was estimated for Rs. 23.07/ 100g.

# Discussion

## **5. DISCUSSION**

Results of the study entitled "Nutritional and antioxidant potential of medicinal rice variety *Rakthashali*" are discussed in this chapter under the following headings.

5.1. Quality evaluation of rice variety

5.1.1. Physical qualities of rice

5.1.2. Physical qualities of rice flour

5.1.3. Cooking qualities of rice

5.1.4. Chemical and nutritional qualities of rice and rice bran

5.2. Assessment of antioxidant and antiproliferative activities

5.3. Organoleptic and nutritional qualities of instant soup mix

5.4. Organolepticand nutritional qualities of rice bran cookies

### 5.1. Quality evaluation of rice variety

# 5.1.1. Physical qualities of rice

Physical qualities like total milled rice per cent, head rice per cent, thousand grain weight, volume weight, grain shape and size were evaluated in rice grains.

Bandyopadhyay and Roy (1992) observed that the cultivar, moisture content, pretreatments such as parboiling and processing equipments and methods affect the milling quality and milling yield of paddy.

In the present study, the total milled rice per cent of *Rakthashali* rice was observed to be 70.08 per cent. Nadh (2018) discussed the milling per cent of six red rice varieties which ranged between 62.76 (*Aathira*) per cent to 76 per cent (*Ezhome-4*). The study reported that milling per cent of *Samyuktha* variety was 72.21 per cent which is similar to the milling per cent observed in the present

study. According to Chandhini (2015) and Lakshmi (2011) *Jyothi* rice variety obtained a higher milling per cent of 73.8 per cent and 78.3 per cent respectively. Red rice variety *Uma* also obtained a higher milling per cent of 76 per cent (Sathyan, 2012). A lower milling per cent was observed for *Aiswarya* (65.75 %) and *Kanchana* (68.76 %) respectively (Nadh, 2018). The milling per cent of 69 per cent, 68 per cent, and 63.7 per cent was showed in *Ezhome-1, Ezhome-2 and Ezhome-3* varieties respectively which was lower compared to the present study (Chandhini, 2015).

A study by Binodh *et al.* (2006) reported that the milling per cent of rice varieties ranged from 49.20 to 89.50 per cent among the fifty five promising rice varieties in Tamil Nadu.

Head rice recovery of rice varieties depends on the grain type, chalkiness and cultivation practices (Dipti *et al.*, 2003). It may vary according to several factors like temperature, humidity, grain size, shape, hardness, moisture content and storage condition (Rani *et al.*, 2006). In the present study, the head rice per cent of *Rakthashali* rice variety was found to be 56 per cent. Similar observations were made by Rajesh (2016) who obtained the head rice recovery of 56 per cent and 52.34 per cent in *Kanchana* and *Samyuktha* respectively. Nadh (2018) reported the head rice per cent for *Ezhome-4* (59.15 %) followed by *Aathira* (57.90 %), *Samyuktha* (57.11 %), *Kanchana* (54.26 %), *Jyothi* (52.46 %) and *Aiswarya* (49.32 %). Chandhini (2015) noticed the head rice per cent of 49.75 per cent in *Ezhome-2* and 55.49 per cent in *Ezhome-1* respectively. Head rice yield ranged from 67.68 to 78.5 per cent and 21.92-63.58 per cent in aromatic and non-aromatic rice varieties in India (Sneha, 2015).

Acording to Nirmala (1997) thousand grain weight is considered to be a function of kernel size and and its density which determines milling quality. The thousand grain weight of 11.65 g was observed in *Rakthashali* rice grains. According to Reshmi (2012) the thousand grain weight of *Njavara* black and *Njavara* yellow was found to be 16.40 g and 13.00 g respectively. A higher

thousand grain weight of 25.07 g was observed in *Njavara* genotypes (Sanal, 2005). Francies *et al.* (2013) observed the thousand grain weight of *Samyuktha* (27.9 g), *Kanchana* (26.0 g) and *Harsha* (25.9 g) respectively. The thousand grain weight of 20.38 g obtained for *Ezhome-1*, 18.6 (*Ezhome-2*), 20.68 g (*Vaishak*), 19.98 (*Jyothi*) and 16.38 (*Uma*) rice varieties reported by (Chandhini, 2015). Nadh (2018) also discussed the thousand grain weight of some red rice varieties and observed the grain weight of *Kanchana* (26.12) followed by *Jyothi* (26.12), *Samyuktha* (25.44), *Ezhome-4* (25.41), *Aathira* (23.64 g) and *Aiswarya* (22.96 g).

Grain dimension is an important parameter which decide the commercial value (Badawi and EI-Hissewy, 2001). Grain dimension comprises mainly, colour, grain length, width, shape and thickness. In the present study, Rakthashali obtained the mean grain length of 7 mm. Reddy (2000) discussed the mean grain length of 7.6 mm to 9.3 mm in Njavara rice varieties. According to Shylaraj et al. (2018) Jeerakashala obtained the grain length of 7.7 mm which is on par with that of the present study. Deep et al. (2008) observed the grain length of 6.63 mm in Njavara rice variety. Reshmi (2011) reported that the mean grain length of Njavara black was found to be 5.60 and that of Njavara yellow was 5.30 mm. The author also studied the grain length of Harsha variety and it was obtained to be 6.48 mm. Nadh (2018) noticed the grain length of Kanchana, Aathira, Aiswarya and Samyuktha obtained was 6.76 mm, 6.60 mm, 6.40 mm and 6.20 mm respectively. The study also observed the grain length of 9.45 mm and 5.63 mm for Jyothi and Ezhome-4 varieties. Francieset al. (2013) reported the grain length of 5.7 mm (Samyuktha), 6.95 mm (Harsha) and 6.0 mm (Kanchana). The kernel length of paddy varieties ranged from 5.92-12.13 mm and 7.45-9.73 mm in aromatic and non-aromatic groups (Sneha, 2015).

*Rakthashai* rice variety showed the grain width of 3 mm. In this line, Francies *et al.* (2013) observed that *Harsha* attained the grain width of 3.30 mm which is on par with the present study. The mean breadth values of *Njavara* black, yellow and *Harsha* was found to be 2.12 mm, 1.88 mm and 2.42 mm respectively (Reshmi, 2012). According to Deepa *et al.* (2008) the grain width of 2.44 mm was observed in *Njavara* rice variety. Reddy (2000) discussed the grain width of *Njavara* which was ranged between 2.65 mm to 3.33 mm respectively which is in line with the present study. Sanal (2005) reported the mean grain width of *Njavara* was 2.87 mm. Shylaraj *et al.* (2018) reported the grain width of 2.4 mm, 2.3 mm and 3.1 mm in *Jeerakashala, Gandhakashala* and *uma* varieties respectively. The kernel breadth of paddy varieties were ranged from 1.32 to 1.96 mm and from 1.67 to 1.98 for aromatic and non-aromatic groups (Sneha, 2015). The study also reported the grain width of 3 mm in black *Njavara*, golden *Njavara* and *Jyothi* rice varieties.

Nadh (2018) evaluated the grain width of 2.89 mm in *Jyothi* and lowest grain width of 2.08 mm in *Ezhome-4*. The grain width of other varieties were *Kanchana* (2.84 mm), *Aathira* (2.68 mm), *Samyuktha* (2.64 mm) and *Aiswarya* (2.48 mm) respectively. Chandhini (2015) reported that the grain with of some red rice varieties *Vaishak* (PTB-60) (1.86 mm) followed by *Ezhome-1* (1.84 mm), *Vyttila-8* (1.82 mm), *Ezhome-2* (1.21 mm) and *Prathyasha* (1.21 mm). Francies *et al.* (2013) observed the grain width of *Samyuktha* (2.7 mm) and *Kanchana* (2.2 mm).

Veni and Rani (2008) reported that the grain size and shape of rice are important characteristics, which determine the consumer preference as well as the commercial success of a variety. Physical dimensions of grains such as length, breadth or width and thickness as well as the shape of the kernel vary from variety to variety. These are considered to be an important criteria of rice quality especially for developing new varieties for commercial production (Bandhyopadhyay and Roy, 1992; Dela and Kush, 2000).

Grain shape and size are determined by considering the L/B ratio of rice. According to IRRI (2002), L/B ratio from 2.5 to 3.0 has been widely acceptable. In the present study the L/B ratio was measured and observed that *Rakthashali* had the L/B ratio of 2.3 which is on par with *Ezhome-*1 (2.3) which indicate that it belongs to medium grain classification. Nadh (2018) evaluated KAU released red rice varieties, L/B ratio ranged from 2.34 (*Aathira*), 2.71 (*Ezhome-4*), 2.55 (*Aiswarya*), 2.37 (*Kanchana*) and 2.34 (*Samyuktha*) which is similar to the present study.

According to Shylaraj *et al.* (2018) the L/B ratio of black *Njavara*, golden *Njavara* and *Uma* obtained were 3, 3 and 2.39 respectively with medium size and intermediate shape. *Jyothi* obtained the L/B ratio of 3.12 with long size grain and slender shape. According to Chandhini (2015) the L/B ratio of red rice varieties were *Ezhome-2* (3.59), *Vaishak* (2.4) repectively. Francies *et al.* (2013) observed the L/B ratio of 2.11in *Samyuktha* and 2.73 in *Kanchana* respectively. Rajesh (2016) also observed higher L/B ratio in *Aiswarya* (3.47), *Kanchana* (4.62) and *Samyuktha* (3.96) which is higher according to the present study.

## 5.1.2. Physical qualities of rice flour

Gardisser (1998) reported that the medium grain cultivars showed higher bulk density than long grain cultivars. The present study showed a higher bulk density of 0.86 g/ ml due to the median grain classification. Nadh (2018) observed a lower bulk density of 0.72 g/ ml in *Ezhome-4* variety. The other red rice varieties attained the bulk density of 0.65, 0.72, 0.68, 0.71 and 0.66 g/ ml for *Jyothi, Aathira, Aiswarya, Kanchana* and *Samyuktha* rice varieties which was lower compared to the bulk density obtained in the present study. Higher bulk density was observed for *Njavara* black (1.00 g/ ml) and *Njavara* yellow variety (0.99 g/ ml) (Reshmi, 2012).

Yadav and Jindal (2007) reported that the bulk density of Indian rice varieties varied between 0.83 to 0.92 g/ ml. According to Sathyan (2012) and Lakshmi (2011) a bulk density of 0.84 g/ ml was observed in red rice variety *Jyothi*. Bulk density of aromatic rice varieties were ranged from 0.48 g/ ml (*Bakuljoha*) to 0.56 g/ ml (*Ketekijoha*) (Saikia *et al.*, 2012). Higher bulk density was observed in pigmented rice varieties in India ranged between 0.96 to 1.12 g/ ml (Ponnappan *et al.*, 2017).

Water absorption index means the amount of water absorbed by starch and can be used as an index of gelatinization (Morsy*et al.*, 2015). In the present study,

the water absorption index of *Rakthashali* rice flour obtained was 24.45. The rate of moisture migration is dependent of temperature of soaking. Higher the soaking temperature, higher the moisture absorption. According to Nadh (2018) red rice varieties obtained the water absorption index of 25.56, 25.31, 25.61, 23.71, 22.91 and 22.46 was observed for *Jyothi, Aathira, Aiswarya, Ezhome-4, Kanchana and Samyuktha. Jyothi, Aathira and Aiswarya* varieties. A lower water absorption index of 23.71, 22.91 and 22.46 was observed in *Ezhome-4, Kanchana and Samyuktha*. Chandhini (2015) observed the water absorption index of roasted and unroasted rice flour in the range of 22.17 (*Ezhome-1*) to 25.11 (*Uma*) and 22.67 (*Vaishak*) to 25.46 (*Jyothi*) respectively.

Water solubility index determines the amount of polysaccharides release from the granule on the addition of excess of water (Yousf *et al.*, 2017).Water solubility index of *Rakthashali* was observed and found to be 0.46. Water solubility index is an *in vitro* indicator of good starch digestibility as it implies the extent of gelatinisation (Guha *et al.*, 1997). Another study conducted by Nadh (2018) studied the water solubility index in red rice varieties were ranged from 0.45 (*Aathira*) to 0.57 (*Ezhome-4*). Water solubility index of 0.51, 0.49, 0.47 and 0.46 was observed in *Samyuktha, Aiswarya, Jyothi and Kanchana*. Chandhini (2015) reported the water solubility index of 0.34, 0.42, 0.49, 0.55, 0.60, 0.61 and 0.73 in *Ezhome-1, Uma, Jyothi, Pratyasha, Ezhome-2, Vaishak and Vyttila-8* respectively. *Jyothi* and *Uma* observed the water solubility index of 0.42 and 0.68 respectively.

Retrogradation is an important parameter that indicates stiffening or hardness of cooked rice after storage (Philpot *et al.*, 2005). Consumer preference and utility of rice largely depend on the retrogradation properties of rice (Zielinska and Fortuna, 2010). It is usually described as recrystallization during storage after starch pasting. The changes in the rate of retrogradation is due to the presence of amylose and amylopectin molecules and their molecular properties (Villareal *et al.*, 1976). In the present study, retrogradation property of *Rakthashali* was studied and observed syneresis during the 3<sup>rd</sup> day of observation (14.25 %) which gratually increased on 6<sup>th</sup>, 9<sup>th</sup> and 12<sup>th</sup> days. In this line, similar study was reported by Nadh (2018) the highest syneresis percentages of 14.20, 19.60, 26.73 and 33.66 for 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup> and 12<sup>th</sup> days was reported for *Samyuktha* variety. The other varieties showed the increasing trend in syneresis percentages of 10.73, 14.20, 18.26 and 25.60 (*Jyothi*), 12.43, 17.06, 21.23 and 28.03 (*Aathira*), 11.70, 14.93, 19.03 and 26.03 (*Aiswarya*), 12.86, 17.10, 23.53 and 32.16 (*Ezhome-4*) and 13.10, 18.33, 25.26 and 31.60 (*Kanchana*) during 3<sup>rd</sup>, 6<sup>th</sup>, 9<sup>th</sup> and 12<sup>th</sup> day of observation.

According to Chandhini (2015), among rice flour prepared with different rice varieties, *Ezhome*-1 obtained a high syneresis percentage (25%) during the 3<sup>rd</sup> day of observation, which gradually increased to 42 per cent on 12<sup>th</sup> day. During 3<sup>rd</sup> day the syneresis percentage was observed in Uma rice variety was low when compared with other varieties like *Ezhome-1, Pratyasha, Vishak, and Jyothi*. Amylose content tends to degrade after heat treatment and show an increase in the syneresis percentage (Manful *et al.*, 2008).

# 5.1.3. Cooking qualities of rice

Cooking qualities of rice is in relation with the physical and chemical characteristics as well as the starch and the starch components like amylose and amylopectin (Tan *et al.*, 1999).

In the present study the organoleptic qualities of table rice was conducted and indicated that the highest mean score of 7.64 was obtained for flavour followed by taste (7.58), colour (7.53) and appearance (7.37). The lowest mean score of 6.02 was obtained for texture of cooked rice. The whole grain with bran was used to prepare table rice. The coarse nature of bran imparted a hard texture to the cooked grains. Except for texture, all other parameters showed the mean score of above 7 and the overall acceptability was also observed to be 7.02. Similar to this, Chandhini (2015) conducted the sensory evaluation of table rice with different rice varieties. Sensory evaluation of cooked rice indicated the mean score of 7.7 in *Uma* variety for appearance. Nandini *et al.* (1995) reported in varieties like

*Vyttila*-1, *Vyttila*-3 and *Jyothi* showed the mean score of 7.74. Nadh (2018) conduced the sensory evaluation in red rice varieties and obtained the highest mean score of 7.44 in *Jyothi* variety.

Rice texture depend upon amylose content, gelatinisation temperature and cooking time (Simonelli *et al.*, 2017). In the present the lower mean score was obtained for texture that may be because of the high gelatinisation temperature index and cooking time.

The overall acceptability of the cooked *Rakthashali* rice was high. Nadh (2018) reported the mean scores of rice varieties varied from 7.1 (*Pratyashya*) to 7.8 (*Jyothi*). Divakar and Francies (2010) evaluated *Karuna* rice variety was found to be most acceptable for the preparation of table rice.

The gelatinisation temperature seems to be a major determinant in deciding a particular cultivar for its suitability for processing (Govindaswamy, 1985). According to Bandyopathyay and Roy (1992) the gelatinisation temperature influences the cooking behaviour of a particular cultivar.

In the present study, the rice grains were not affected by alkali treatment which showed high gelatinisation temperature index (>74°C). High gelatinisation temperature obtained in the study is because of the presence of bran layer. The crystalline region of the bran do not allow the water to enter inside the layer which impart the breakdown of intermolecular bonds of starch molecules resulting in higher gelatinisation temperature index. The gelatinisation temperature for *Njavara* black and *Njavara* yellow was found to be 85.60 °C and 83.00 °C possess high gelatinisation temperature index which was similar to the present study. According to Nandhini (1995), higher gelatinisation temperature was observed for hybrid varieties (Remya, Thrissur local-1 and Mashuri) (86.54°C) when compared to traditional (82.82°C) and other improved varieties (85.75°C).

During grain development, if a high ambient temperature persists, a high gelatinisation temperature can be observed in grains (Resurreccion *et al.*, 1977). According to Nadh (2018) the disintegration of alkali treatment among red rice

varieties and found that *Ezhome-4*, *Aathira*, *Jyothi*, *Aiswarya* and *Kanchana* showed intermediate gelatinization temperature index as the grains were swollen and collar complete and wide. Chandhini (2015) reported a high gelatinisation temperature index for newly released red rice varieties *Ezhome-1*, *Ezhome-2* and *Prathyasha* respectively. Gelatinisation temperature index was studied in Sri Lankan traditional rice variety *Dik Wee* (red) and *Rathal* (white) showed to be high with the gelatinisation temperature of 74.5-80°C (Abeysekera *et al.*, 2008).

One of the important quality parameter of cooked rice is the cooking time and it varies with the processing and method of cooking. Cooking time of rice varieties depends on coarseness of the grain and its gelatinisation temperature (Dipti et al., 2002). Raghuvanshi et al. (2017) reported the cooking time in Indian rice varieties ranged from 30 to 40 minutes. In the present study, higher cooking time of 40 minutes was observed for *Rakthashali* rice variety. Deepa et al. (2008) observed the cooking time of *Njavara* as 38 minutes. The cooking time of black rice ranged from 40.33 minutes to 43.33 minutes (Chingakham, 2015). Jyothi variety showed the cooking time of 37 minutes (Nandhini, 1995). Nadh (2018) observed the cooking time taken for red rice varieties ranged from 21 minutes (Aiswarya) to 27 minutes (Ezhome-4). Rice varieties having low or intermediate amylose content obtained higher cooking time. In this line Rakthashali which had intermediate amylose content and possess higher cooking time. The cooking time of Jyothi and Uma were 22.20 and 22.42 respectively which is found to be lower and also the cooking time of 22.39 minutes, 23.83 minutes and 24.45 minutes were obtained in Vyttila-8, Vaishak and Ezhome-1 respectively (Chandhini, 2015). Lower milled or whole grain rice was characterised by high cooking time, hardnesss, low adhesiveness, cohesiveness and water uptake ratio (Mohapatra and Bal, 2006).

Water uptake is an important parameter which affects the cooking quality (Horigane *et al.*, 2000 and Mohapatra and Bal, 2006). Hussein and Ahamed (1981) reported that rice having high protein content required more water and long time to cook. In the present study, the protein content was high for

*Rakthashali* rice and water uptake of 9.3 ml/g was observed. Bulk density is significantly correlated with water uptake. Nadh (2018) discussed the water uptake of 8.71 ml/g in *Ezhome-4* followed by *Jyothi* (8.70), *Aathira* (8.56), *Kanchana* (7.58) and *Samyuktha* (7.39) respectively. In ordinary rice variety *Uma*, the water uptake was found to be 6.42 ml/g. The water uptake of 5.74 ml/g, 4.86 ml/g, 3.81 ml/g, 3.72 ml/g and 3.47 ml/g was observed for *Ezhome-2*, *Vyttila-8*, *Vaishak, Pratyashya* and *Ezhome-1* respectively (Chandhini, 2015). The water uptake of black rice varieties were ranged from 1.99 ml/g to 4.25 ml/g (Chingakham, 2015). White rice varieties obtained the water uptake of 2.37 (Jaya) to 3.98 (RYT-2610) (Singh *et al.*, 2005).

In the present study, the medium grain *Rakthashali* obtained the volume expansion ratio of 4. Lesser expansion in brown rice was because of the kernel is covered with thin layer of bran which contains oil, so that the expansion ratio was lower. Chandhini (2015) determined a high volume expansion ratio of 5.60 in Ezhome-2 variety. The other varieties showed the volume expansion ratio of 5.18 (*Ezhome-1*), 4.49 (*Pratyashya*), 4.50 (*Vyttila*), 4.78 (*Vyshak*), 5.62 (*Jyothi*) and 6.07 (*Uma*). Nadh (2018) reported the volume expansion ratio of 5.43 in *Ezhome-4* and 4.35 for *Samyuktha* varieties.

Amylose, an important component of starch is having characteristic function in determining rice quality. Rice consists of soluble and insoluble amylose. Insoluble amylose of rice directly affects kernel firmness and inversely affects stickiness and glossiness of cooked grain (Bhattacharya and Chinnaswamy, 1982). When cooked, rice with low amylose content is sticky and soft, as amylose content increases rice becomes firmer. Amylose content may be classified as low (10-20 %), intermediate (20-24 %) or high (>25 %) (Resurrection *et al.*, 1977). In the present study, amylose content of 20.38 per cent was observed in *Rakthashali* rice variety which was categorised under intermediate amylose content. Yadav *et al.* (2007) stated that the amylose content in Indian rice varieties were ranged from 2.25 per cent to 22.21 per cent. Red rice varieties like *Ezhome-1* and *Ezhome-2* observed the amylose content of 15.31 per cent and 15.61 per cent respectively (Chandhini, 2015). For common rice varieties like *Jyothi* and *Uma*, the amylose content was found to be 22.17 per cent and 23.32 per cent respectively. According to Deepa *et al.* (2008) amylose content of *Njavara* was 22.7 per cent and for *Jyothi* it was of 22.9 per cent.

Reshmi (2012) reported the amylose content in *Njavara* black and *Njavara* yellow and was found to be 21.64 per cent and 24.37 per cent respectively.*Harsha* rice variety showed the amylose content of 24.81 per cent. According to Nadh (2018) recorded the amylose content of 24.90 (*Aathira*) rice variety followed by *Ezhome-4* (24.58 %), *Aiswarya* (24.52 %), *Jyothi* (24.20), *Kanchana* (23.98 %) and *Samyuktha* (23.23 %). Brazilian white rice varieties showed the amylose content of 25.1 per cent (Micro 547), 25.4 per cent (Micro 208) and 25 (IRGA) respectively (Livore *et al.*, 2004).

Gel consistency is an important characteristic which determines the product stability. Gel consistency measures the hardness of cooked rice on cooling (Kanlayakrit and Maweang, 2013). In the present study, gel consistency was measured and gel length was obtained to be 52 mm. Similar to this Chemutai *et al.* (2016) reported in Indian rice varieties *Sharbati* and *HBC*-19 observed the gel length of 54 mm and 58 mm. Medium gel consistency represents the shorter gel length. Cooked rice with medium gel consistency hardens faster than soft one (Sidhu, 1975). In the present study, cooked rice had harder texture which support the statement. Reshmi (2012) also reported the gel length of *Njavara* black and *Njavara* yellow varieties observed to be 54.40 mm and 54.00 mm respectively which is similar to the present study. The author also reported that the variation in the gel consistency is due to the genetic background of rice genotypes. According to Biswas and Juliano (1988) gel consistency of Indian rice cultivars ranged from 26 mm to 58 mm.

Sathyan (2012) and Chandhini (2015) observed a medium gel length of 48.10 mm and 37.2 mm in *Jyothi* variety. Chandhini (2015) reported the gel length of 57.16 mm and 56.91 mm in *Ezhome-1* and *Vaishak* respectively. Nadh (2018) observed that in red rice varieties like *Kanchana, Aiswarya, Aathira, Jyothi*,

*Ezhome-4* and *Samyuktha* recorded the gel length of 61.47 mm, 60.44 mm, and 58.80 mm, 57.30 mm, 55.76 mm and 62.21 mm respectively.

Sarkar *et al.* (2004) observed the gel consistency between 22 to 78 mm for scented and non-scented rice varieties.

Grain elongation was considered as an important quality parameter contributing to finer appearance (Dipti *et al.*, 2003). In the present study, the grain elongation ratio of 0.6 was observed in *Rakthashali* rice variety. The lower the grain elongation ratio may be due to the lesser size of the grain and the presence of bran layer, which may restrict water enter in to the kernel. Pilliayar (1988) conveyed that the increase in milling per cent by 80 per cent normally increased the elongation ratio. In the present study, the milling percentage of *Rakthashali* rice was lower (70.08 %) which is truthful to this statement. Similar to this study Chandhini (2015) observed a grain elongation ratio of 0.72 in *Ezhome-1* and *Vaishak* rice varieties. The author also noticed the grain elongation ratio of 0.9 in *Ezhome-2* and *Pratyashya*. Other varieties showed the grain elongation ratio of 0.81, 0.99 in *Vyttila-8* and *Jyothi* varieties.

According to Nadh (2018) the grain elongation ratio varied from 1.47 (*Samyuktha*) to 1.79 (*Aiswarya*). Lakshmi (2011) reported that the grain elongation ratio varied from 1.30 to 1.43 in *Jyothi*.

# 5.1.4. Chemical and nutritional qualities of rice and rice bran

Rice grain quality is mainly determined by its moisture content (Sotelo *et al.*, 2000). In the present study, moisture content was observed to be 9.30 per cent for rice and for rice bran it was of 12.25 per cent. The higher moisture content of the rice bran is due to the hygroscopic nature and it absorbs the moisture and produce stress fissures. The moisture content of rice grains vary depending upon the field conditions. Similar to this, the moisture content reported by Khatoon and Prakash (2007) for rice varieties ranged from 9.00 to 10.50 per cent. Reshmi (2012) noticed the moisture content of *Njavara* black and *Njavara*  yellow to be 11.60 per cent and 13.00 per cent respectively. Moisture content of 13.1 per cent was observed for *Njavara* rice variety (Deepa *et al.*, 2008).

Nandini (1995) observed the moisture content of 13.69 per cent in *Harsha* and 12.75 per cent in *Njavara*. Nadh (2018) reported that the moisture content varied from 10.5 to 12.5 per cent. Chandhini (2015) obtained the moisture content of 8.5, 10.3, 10.4, 10.6 and 11.6 per cent in *Ezhome-2, Uma, Vyttila-8, Vaishak and Ezhome-1* respectively. The moisture content of 9.70 and 9.60 per cent observed in white and brown rice varieties (Otegbayo *et al.*, 2001). White rice varieties showed the moisture content ranged from 11.64 to 12.72 per cent. Yodmanee*et al.* (2011) reported the moisture content in Thailand pigmented rice variety ranged from 5.96 to 6.79 per cent.

Starch is a polysaccharide found in nature by the condensation of a large number of glucose molecules. Starch forms 90 per cent of rice by weight (Jane *et al.*, 1999). In the present study, the starch content of rice and rice bran was found to be 65 g/ 100g and 23.04 g/ 100g respectively. Reshmi (2012) reported a starch content of 76.14 g/100g in *Njavara* black and for *Njavara* yellow rice, it was of 74.45 g/ 100g. Starch content reported by Nadh (2018) in red rice varieties was found to be in the range of 65.83 g/ 100g (*Jyothi*) to 70.50 g/ 100g (*Aiswarya*). The author also observed the starch content of 68.23, 69.36, 67.26 and 68.33 g/ 100g in *Aathira, Ezhome-4, Kanchana and Samyuktha*.

Chandhini (2015) observed the starch content of 63.18 g/ 100g in *Jyothi*. According to Khatoon and Prakash (2007) the starch content of rice ranged from 79.90 to 83.00 g/ 100g.

In the present study, the carbohydrate content obtained for *Rakthashali* rice and rice bran was 71.25 g/ 100g and 46.08 g/ 100g respectively. Vathavarshini *et al.* (2013) reported the carbohydrate content of brown rice was 49.7 g/ 100g. The carbohydrate content of red rice varieties ranged from 64 g/ 100g to 80 g/ 100g (Dasgupta and Handique, 2018). Deepa*et al.* (2008) reported the higher carbohydrate content in *Njavara* rice (73.5) and in *Jyothi* and *IR* 64

obtained the carbohydrate content of 72.8 g/ 100g and 74.1 g/ 100g respectively. Chandhini (2015) obtained the carbohydrate content of 71.45, 75.6, 78.07, 78.24, 71.01, 81.43 and 78.37 g/ 100g in *Uma, Jyothi, Vaishak, Vyttil-8, Prathyashya, Ezhome-2 and Ezhome-1*. Mudoi and Das (2018) studied in traditional brown rice varieties *Jul Bao, Dal Bao, KotiaBao, Bogaguri* and *Kolaguni* in Assam and noticed the carbohydrate content of 80.62, 88.09, 88.10, 89.01 and 87.52 per cent respectively. Aromatic rice variety (*Bakuljoha*) observed 76.6 per cent of carbohydrate. Raghuvanshi*et al.* (2017) reported the carbohydrate content of 70.19 g/ 100g in Indian red rice.

Protein content is considered as index for nutritional quality of rice and protein content of above 10 per cent is considered as cultivar with high protein content (Ressurection et al., 1979). Protein is the second highest constituent of rice, it makes a fundamental contribution to nutritional quality (Juliano and Betchtel, 1998). Among cereal proteins, rice protein is the richest by virtue of its true availability (Rai, 2009). In the present study, the protein content of 11.60 g/ 100gwas observed in rice whereas in rice bran it was of 11.36 g/ 100g. Similar to this study, Reshmi (2012) noticed the protein content of 11.80 g/ 100g in Njavara yellow and 12.15 g/ 100g in Njavara black variety. The author also observed the protein content of 8.77 g/ 100g which was lower compared to the present study. Govindaswami et al (1996) reported 6 to 12.6 per cent crude protein in three hundred improved rice varieties in India. Pigmented rice varieties showed the protein content from 5.43 to 13.83 per cent (Mudoi and Das, 2018). Aromatic rice variety Bakuljoha obtained the protein content of 7.7 per cent only. Red rice showed the protein content of 10.49 g/ 100g according to Raghuvanshi et al. (2017). Yodmanee et al. (2011) reported that the protein content of pigmented rice varieties ranged from 6.63 g/ 100g to 8.44 g/ 100g.

Nadh (2018) observed the protein content of 5.47, 5.00, 4.94, 5.50, 5.34 and 4.70 g/ 100g in *Jyothi, Aathira, Aiswarya, Ezhome-4, Kanchana and Samyuktha* varieties. Chandhini (2015) reported that higher protein content was observed in *Ezhome-2* (8.95 g/ 100g) and *Jyothi* (7.5 g/ 100g). Deepa *et al.* 

(2008) obtained the protein content of 9.52 g/ 100g and 7.97 g/ 100g in *Njavara* and *Jyothi* respectively.

Fat content in brown rice were significantly different among the varieties. Fat content *Rakthashali* rice was obtained to be 3.68g/ 100g and for bran it was 5.70 g/ 100g. The higher fat content in rice bran is due to the presence of fatty acids such as oleic, linoleic, stearic and behenic acids. According to Raghuvanshi *et al.* (2017) the fat content of 1.81 g/ 100g was noticed in red rice which was low when compared to the present study. Coloured rice varieties of Assam showed that the fat content ranged from 1.12 to 3.70 per cent (Mudoi and Das, 2018). *Njavara* and *Jyothi* attained the fat content of 2.48 and 2.60 g/ 100g respectively (Deepa *et al.*, 2008). Nadh (2018) noticed that the fat ranged from 0.31 g/ 100g (*Samyuktha*) to 0.48 (*Aathira*). Chandhini (2015) reported the fat content of 0.3, 0.35, 0.42, 0.53, 0.64 and 0.71 in *Ezhome-2, Uma, Jyothi, Prathyasha, Ezhome-1 and Vaishak*. Baruah *et al.* (2006) working with red rice cultivars observed lipid in the range of 2.42 per cent to 4.64 per cent. Fat content of 1.17 g/ 100g was reported by Vethavarshini *et al.* (2013). Fat content in pigmented rice variety in Thailand ranged between 1.44 to 2.17 g/ 100g (Yodmanee *et al.*, 2011).

Minerals are essential for the normal metabolic functions and are required components in a balanced diet. In the present study, calcium content of 5.69 mg/ 100g and 12.1 mg/ 100g was obtained for rice and rice bran respectively. Similar to this, Nadh (2018) discussed the calcium content of 5.46, 5.63, 4.90, 5.40, 5.76 and 5.43 in *Jyothi, Aathira, Aiswarya, Ezhome-4, Kanchana* and *Samyuktha*rice varieties. Dutta and Barua (1982) reported that the calcium content of rice varied from 15.77 to 29.70 mg/ 100g. *Njavara* obtained the calcium content of 11.6 g/ 100mg (Deepa *et al.*, 2008). Sreedevi (1989) reported a lower calcium content in traditional rice varieties. Raghuvanshi*et al.* (2017) reported the calcium content of 8.71 mg/ 100g in red rice. Chandhini (2015) revealed that the calcium content in red rice varieties *Ezhome-1, Ezhome-2, Pratyashya, Vyttila, Vaishak, Jyothi* and *Uma* were 4.92, 5.27, 6, 5.7, 4.94, 6.6 and 5.26 mg/ 100g respectively.

Zinc content observed in the present study was found to be 1.42 mg/ 100g for rice grain and for rice bran it was of 0.88 mg/ 100g.According to Reshmi (2012) the zinc content of *Njavara* black and *Njavara* yellow was found to be 0.88 and 0.90 mg/ 100g respectively which is on par with the present study. Rice varieties *Ezhome-2, Vyttila-8, Prathyasha, Ezhome-1* and *Vaishak* observed the zinc content of 1.31, 1.28, 1.17, 1.08 and 1.01 mg/ 100g respectively (Chandhini, 2015). *Jyothi* and *Uma* obtained higher zinc content of 1.11 to 1.07 mg/ 100g (Nadh, 2018). The highest zinc content of 2.97 mg/ 100g were found in Irri-6 (Ahuja *et al.*, 2007). Zinc content of brown rice varieties ranged from 13.91 ppm to 37.3 ppm.

In the present study, higher iron content of 1.67 mg/ 100g and 0.31 mg/ 100g was observed in both rice grain and rice bran. *Njavara* black and *Njavara* yellow rice varieties obtained lower iron content of 0.36 mg/ 100g. But Deepa *et al.* (2008) reported an iron content of 1.93 mg/ 100g in *Njavara* rice variety which is similar to the present study. Lakshmi (2011) and Sathyan (2012) observed the iron content of 1.97 mg/ 100g and 1.94 mg/ 100g in *Jyothi* variety. Chandhini (2015) noticed the iron content of 0.41, 0.51, 0.61, 0.44, 0.47, 0.57 and 0.56 mg/ 100g in *Ezhome-1, Ezhome-2, Pratyashya, Vyttila-8, Vaishak, Jyothi* and *Uma. Jyothi, Aathira, Aiswarya, Ezhome-4, Kanchana*and *Samyuktha* obtained the iron content of 0.61, 0.45, 0.52, 0.50, 0.39 and 0.44 mg/ 100g respectively (Nadh, 2018). Druvasree (2013) observed the iron content among brown rice varieties, the highest iron content was recorded in *IR*-64 (2.66 mg/ 100g) and lowest was recorded in *KRH*-4 (1.6 mg/ 100g).

In the present study, the phosphorus content of 352 mg/ 100g was observed in rice and 116 mg/ 100g in rice bran. According to Reshmi (2012) the phosphorus content was 352.40 mg/ 100g in *Njavara* black and 351.40 mg/ 100g in *Njavara* yellow rice varieties. Deep *et al.* (2008) discussed the phosphorus content of 304, 268 and 248 mg/ 100g in *Njavara, Jyothi* and *IR-64*. Nandhini (1995) reported the phosphorus content of rice varied from 116 to 155.50 mg/ 100g. Lakshmi (2011) and Sathyan (2012) the phosphorus content of 161.83 mg/

100g and 158.60 mg/ 100g was observed in *Jyothi* variety. Phosphorus content noticed in *Harsha* was lower (135.40 mg/ 100g). Chandhini (2015) noticed the phosphorus content of 128.17, 135.41, 122.87, 95.87, 90.29, 133.2 and 101.35 mg/ 100g in *Ezhome-1, Ezhome-2, Pratyasha, Vyttila, Vaishak, Jyothi and Uma*. The phosphorus content of 131.96, 129.83 and 131.36 mg/ 100g was reported in *Aiswarya, Kanchana* and *Samyuktha* variety (Nadh, 2018).

Starch digestibility is the ability of starch to be digested and absorbed in the body. In the present study, the *in vitro* digestibility was found to be 60.32 per cent in rice and in rice bran it was 18.07 per cent. A study conducted by Druvasree (2013) observed that the starch digestibility was 47.44 per cent in *Karimundga* (47.44 %) and 31.39 per cent in *MAS 946-1* and for the other varieties it ranged between 34.67 per cent to 41.29 per cent. Sathyan (2012) reported the *in vitro* digestibility of starch in raw rice ranged from 77.02 to 88.61 per cent.

*In vitro* availability of calcium for rice flour and rice bran was higher and observed to be 70.81 and 65.41 in the present study. Vijayalakshmi *et al.* (2015) observed the *in vitro* availability of calcium ranged from 25.82 to 45.97 per cent in rice varieties. Xia *et al.* (2017) observed the *in vitro* availability of calcium was found to be 52.17 in brown rice varieties. Liang *et al.* (2009) *in vitro* availability of calcium ranged from 62 to 66 per cent was observed in brown rice varieties. Idris *et al.* (2005) studied the *In vitro* availability of calcium for rice varieties ranged from 28.16 to 32.16 per cent.

# 5.2. Assessment of antioxidant and antiproliferative activities of rice flour and rice bran

### 5.2.1. Antioxidant activities

Plants and their products have been used for medicinal purposes since many years but still not explored. The free radicals are produced in aerobic cells due to consumption of oxygen in cell growth. These free radicals are take part in different disorders like ageing, cancer, cardiovascular diseases, diabetes, rheumatoid arthritis, epilepsy and degradation of essential fatty acids (Barros *et al.*, 2007; Singh *et al.*, 2009). Antioxidants helps in treatment of these diseases and these are the substances whose presence in relatively low concentrations significantly inhibits the rate of oxidation of the target within the biological systems (Lai *et al.*, 2010).

Dipheny lpicryl hydrazyl (DPPH) is a free radical compound which has been extensively used to determine the free radical scavenging activity is considered as a good In vitro model widely used to assess antioxidant efficiency within a very short time (Marxen et al., 2007; Lee et al., 2007). In the present study, DPPH radicals are effectively scavenged by rice flour and rice bran with IC<sub>50</sub> values of 8.50  $\pm$  0.196 µg/ml and 22.5  $\pm$  1.174 µg/ml. The highest DPPH radical scavenging activity is because of the presence of phenolic and flavonoid compounds. Reshmi (2011) indicated Njavara yellow variety had shown highest DPPH scavenging activity with IC<sub>50</sub> value of 31.52 µg/ml compared to Njavara black (33.73 µg/ml) and Harsha (52.39 µg/ml) varieties. A study conducted by Rao et al. (2010) revealed that Njavara had the DPPH sacavenging activity with IC<sub>50</sub> value of 30.85, Vasumathi (87.72 µg/ml), Yamini (70.58 µg/ml) and Jyothi (48.88 µg/ml). According to Smitha et al. (2012) Njavara black variety showed the IC<sub>50</sub> value of 84.66 µg/ml. DPPH radical scavenging activity was higher for Rakthashali compared to other rice varieties. Ghasemzadeh et al. (2018) reported that the DPPH radical scavenging activity in Malasyan black, red and brown rice bran extracts were 39.1, 64.7 and 87.1  $\mu$ g/ml.

The hydroxyl radical is a very reactive oxygen species with a short halflife and is responsible for much of the biological damage inherent to free radical pathology (Hochestein and Atallah, 2008). This radical has the ability to cause the breakage of DNA stands, which is a contributing factor to carcinogenesis, mutagenesis and cytotoxicity. Moreover, hydroxyl radicals have been identified as one of the rapid initiators of the lipid peroxidation process, *via* the abstraction of hydrogen atoms from unsaturated fatty acids (Kappus *et al.*, 2003). In the present investigation, *Rakthashali* rice and rice bran showed the hydroxyl radical scavenging activity with an IC<sub>50</sub> value of  $1.72 \pm 0.030 \,\mu$ g/ml and  $2.47 \pm 0.077 \,\mu$ g/ml respectively. Reshmi (2012) reported the hydroxyl radical scavenging activity in *Njavara* yellow, *Njavara* black and *Harsha* with the IC<sub>50</sub> values of 46.00  $\mu$ g/ml, 55.68  $\mu$ g/ml and 60.78  $\mu$ g/ml respectively.

Over production of superoxide anion radical has long been known as the starting point of reaction oxygen species accumulation in cells, contributing to redox imbalance and other associated deleterious physiological consequences (Pervaiz and Clement, 2007). Result of the present study revealed that *Rakthashali* showed the superoxide radical scavenging with IC<sub>50</sub> values of25.4  $\pm$  2.47 µg/ml and 49.28  $\pm$  2.8µg/ml in rice flour and rice bran respectively. According to Reshmi and Nandhini (2018) the IC<sub>50</sub> value of *Njavara* black was 48.78, whereas *Njavara* yellow has an IC<sub>50</sub> value of 55.43 µg/ml and *Harsha* variety showed the IC<sub>50</sub> value of 60 µg/ml. Rao *et al.* (2010) reported the superoxide radical scavenging activity in *Njavara, Jyothi, Yamini, Vasumathi* with the IC<sub>50</sub> values of 41, 48, 102, 140 µg/ml respectively.

Nitric oxide radicals are more toxic to the tissues and it had the ability to cause inflammatory responses and carcinomas. Excess nitric oxide which is known to accumulate in the acidic environment of stomach reacts with oxygen to form nitrite ions and induce mutagenesis (Yin *et al.*, 2007). Nitric oxide scavenging activity was not detected in the present study. Noh *et al.* (2002) reported that higher phenolic compounds have greater nitric oxide scavenging activity. In the present study the phenol content of rice flour and bran was lower which agreed with the statement. A study conducted in *Njavara* extract showed the nitric oxide scavenging activity with IC<sub>50</sub> value of 55.25 ml. Rao *et al.* (2010) noticed IC<sub>50</sub> values of *Jyothi, Yamini* and *Vasumathi* were 71.41, 107.18 and 102.48 ml respectively. The IC<sub>50</sub> values for nitric oxide scavenging activity of free fractions of black, red and brown rice bran were 32.0, 44.5, 112  $\mu$ g/ml respectively. While, the IC<sub>50</sub> values of bound fractions of black, red and brown

rice bran were 65.7, 78.2 and 150.4  $\mu$ g/ml respectively (Ghasemzadeh *et al.*, 2018).

In the present study the methanolic extract from the samples had shown considerable reducing power activity. The reducing power of rice and rice bran increased with increasing concentration. According to Rao *et al.* (2010) 0.5 mg/ ml of the methanolic rice bran extracts showed the absorbance values of 0.59, 1.04, 1.93 and 2.98 for *Vasumathi, Yamini, Jyothi* and *Njavara*.

Total antioxidant activity is based on the antioxidant potential of pure compounds to quench and inhibit the formation of a coloured radical cation produced by the action of the reagent solution (Evans and Miller, 2004). In the present study, the rice and rice bran showed an increase in total antioxidant activity with the increase in concentration from 10 µg/ml to 100 µg/ml. Rao *et al.* (2010) reported at the concentrations of 0.1 mg/ ml of the methanolic rice bran extracts, the absorbance values of *Vasumati, Yamini, Jyothi* and *Njavara* were 1.65, 2.21, 2.52 and 2.93 µg/ml respectively.

Polyphenols are the major plant compounds with potent antioxidant activity. The redox properties of the polyphenols which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Petti, 2009; Amakura, 2008). In the present study, the phenol content of methanolic extract of rice flour and rice bran was found to be  $3.8 \pm 0.04$  and  $1.9 \pm 0.14$  mg GAE /g extract respectively. Reshmi (2012) reported the lower phenolic content of 0.41 and 0.29 mg in *Njavara* black and *Njavara* yellow variety. *Harsha* variety showed the total phenol content of 0.32 mg/ 100g. The higher levels of phenolic compounds such as protocatechuric acid, syringic acid, ferulic acid, cinnamic acid and coumaric acid are responsible for the total phenol content obtained in the present study. This ferulic and coumaric acids which are mainly present in the rice bran are effective in inhibiting colon cancer (Zhou *et al.*, 2004)

According to Rao *et al.* (2010) the total phenol content was in the range of 3.27 to 12.4 mg GAE/ g bran. The highest total phenol content was observed in *Njavara* (12.72) followed by *Jyothi* (9.44), *Yamini* (4.23) and *Vasumathi* (3.31). Whole rice noticed the phenol content ranged from 0.54 to 0.99 GAE/ g. Ghasemzadeh *et al.* (2018) observed the total phenolic content of black rice ranged from 269.85 to 1214.7 mg GAE/ 100g. They also reported that black rice bran contain high contents of free, bound and total phenolics followed by red rice bran and light brown rice bran.

According to Muntana and Prasong (2010) the total phenolic content were in the range of 0.89 to 0.9884 GAE /g of extract in white rice bran and 1.01 to 1.04 GAE /g of extract in black rice bran and the highest phenol content was observed in red rice bran of 1.0810 to 1.2239 GAE /g.

The flavonoid compounds have good antioxidant and antitumor properties which considerably effects the human health (Havsteen, 2002; Gumul *et al.*, 2007; Petti and Scully, 2009). Catechin and myrecitin are abundant flavonoid compounds. Because of the higher level of flavonoid compoundsit is widely used in cancer treatments, coronary artery diseases, gastrointestinal ulcers and rheumatic diseases (Havsteen, 2002). In the present study, total flavonoid content in the methanolic extract of rice flour and rice bran was found to be  $90.5 \pm 1.4$  mg QTE/g extract for rice flour and  $106.9 \pm 4.0$  mg QTE /g extract for rice bran extract. Flavonoids can scavenge the peroxyl radicals and are effective inhibitors of lipid peroxidation. According to Rao *et al.* (2010) the total flavonoid content was lower to *Vasumathi, Yamini, Jyothi* and *Njavara* varieties which had the values of 1.68, 2.57, 5.33 and 8.5 mg QTE/g.

Higher antioxidant activity shown by the rice flour and rice bran in terms of high DPPH radical scavenging activity, superoxide radical scavenging activity and considerable total antioxidant and reducing power activity might have contributed to the antiproliferative activity. Henderson *et al.* (2012) reported that rice bran may exert beneficial effects against breast, lung, liver and colorectal cancer. The protective chemopreventive agents in the bran are ferulic acid,  $\beta$ -

sitosterol, gamma oryzanol, tocopherols and phytic acid (Barnes *et al.*, 1983). With this line, the present study of the cytotoxicity analysis towards human adenocarcinoma cell line (MCF-7), the rice bran extract showed the loss of cell viability in 50 ug/ml was 26.63 per cent. In rice flour extract the loss in cell viability was observed to be 21.18 per cent in the concentration of 25 ug/ml. The anticancer effects of the rice bran are mediated through the ability of the chemopreventive agents to induce apoptosis, inhibit cell proliferation and alter cell cycle progression in malignant cells. The potent radical scavenging activities observed for both rice flour and rice bran might have contributed to the cytotoxicity in the present study. Phytochemicals like phenols and flavonoids are also high in *Rakthashali* rice, which can be a reason for the observed antiproliferative activity in cancer cell line.

Ghasemzadeh *et al.* (2018) conducted an antiproliferative study in breast cancer cell lines (MCF-7 and MDA-MB-231) in Malaysian black, red and brown rice varieties (*RB*211, *RP*511 and *IR* 402, ). The results showed that black rice bran extract exhibited potent antiproliferative activity with an IC<sub>50</sub> value of 148.6 and 119.2 mg/ ml followed by red bran extract (175 and 151 mg/ ml) and brown rice bran extract (382.3 and 346.1 mg/ ml). It has been reported by the author that the cytotoxic activity of rice bran against breast cancer cell lines is influenced by the variety of rice, growing conditions, cultivation practices and type of cancer cell lines. Another study conducted by Rao *et al.* (2010) noticed the cytotoxic activity in methanolic extracts from bran against  $C_6$  glioma cell line and revealed that *Njavara, Jyothi, Yamini* and *Vasumathi* attained the cytotoxicity with the IC<sub>50</sub> values of 17.53, 25.52, 44.83 and 57.78 µg/ml respectively.

### 5.3. Organoleptic and nutritional qualities of instant soup mixes

Soup mix were prepared with rice flour in the combination with mushroom powder at different levels. Both rice flour and mushroom powder was taken as the control. The mean score for appearance varied from 6.22 to 8.51 with the mean rank score of 1.83 to 6.60. The soup mix prepared with 60 per cent rice flour and 40 per cent mushroom powder ( $T_4$ ) was taken as the best treatment with the maximum mean score of 8.51 for appearance. Among the various treatments, soup mix prepared with 60 per cent rice flour and 40 per cent mushroom powder obtained the highest mean score of 8.54 and mean rank score of 6.63 for colour. The maximum mean scores for flavour, texture, taste and overall acceptability were 8.75, 8.51, 8.68 and 8.75 with the mean rank scores of 6.87, 6.67, 6.77 and 6.90 was obtained for soup mix with treatment  $T_4$ . The sensory parameters of rice flour soup mix obtained a mean score of above 6.22 for all the sensory attributes which shows that rice flour incorporated soup mixes were highly acceptable.

Lakshmy (2011) standardised instant soup mix by incorporating temph flour with various proportion from 50 to 65 per cent. The organoleptic evaluation of soup mixes were carried out and the mean score obtained for overall acceptability of different soup mixes ranged from 7.5 to 8.2. The overall acceptability was lower for the soups prepared by the addition of 65 per cent of temph flour. Up to 60 per cent of incorporation was found to be acceptable.

Barley flaxseed based functional dry soup mix was developed from whole barley flour (46.29 %), roasted flaxseed powder (23.14 %) and the seasonings (30.55 %) (Kaur and Das, 2015).

Singh *et al.* (2017) developed instant soup mix with mushroom pieces and the sensory evaluation was carried out and obtained higher mean scores which varied from 7.95 (aroma) to 8.25 (colour, appearance, texture, taste and overall acceptability).

Abdel-Haleem and Omran (2014) developed different vegetable soup mix by the incorporation of lentils, chick pea and green pea flours. The mean score of lentil, green pea and chick pea soup mixes ranged from 9.10 to 9.25, 7.80 to 8.50 and 8.20 and 8.45 respectively. In the present study, the organoleptic mean scores of above 8 was obtained for the best selected instant soup mix. The supplementation of dried soup mix with lentils, chickpea and green pea as a valuable food and can be nutritionally good.

Thara and Nazni (2018) formulated soup prepared from 80 per cent little millet grits and obtained highest score in sensory parameters like appearance (9.00), taste (8.90), flavour (8.85), texture (8.90) and overall acceptability (8.95).

Joshy (2018) formulated soup mix by the addition of cabbage and raddish leaf powders of various combinations (2.5 %, 3.5 % and 5 %). The result of the sensory evaluation revealed that the cabbage leaf powder incorporated soup mix had the highest overall acceptability with the mean score of 7.75. The mean score of (7.65) was obtained for overall acceptability of instant soup mix prepared with raddish leaf powder. Instant soup mix prepared with cabbage and raddish leaf powders at 5 per cent level was acceptable and can be incorporated in soup mixes.

In the present study the moisture content of 4.55 per cent was observed in rice based soup mix. The moisture content of 3.96 per cent was observed in mushroom soup mix powder (Kumar, 2015). Thara and Nazni (2018) reported that the moisture content of 4.0 per cent in little millet based soup mix. Lakshmy (2011) observed the moisture content of soup mix ranged from 6.53 to 7.53 per cent. Singh *et al.* (2017) observed the moisture content of treated and untreated mushroom soup ranged from 83.69 to 84.04 per cent.

Starch content of 57.56 g/ 100g was obtained in the present study. Thakur *et al.* (2002) found out the starch content of mushroom soup varied from 27.63 to 29.92 per cent. Lakshmy (2011) noticed the starch content of 16.7 to 51.45 g/ 100g in different soup mixes.

Carbohydrate content of 64.56 g/ 100g was observed in rice based soup of the present study. Similar to this, Thara and Nazni (2018) noticed the carbohydrate content of 69.1 per cent in little millet based soup mix. The carbohydrate content of 71.82 per cent was noticed in mushroom soup powder (Kumar, 2015). Total carbohydrate content of 76.28 was noticed in mushroom soup (Singh *et al.*, 2017). Carbohydrate content of 54.70 g/ 100g was noticed in barley flaxseed based dry soup mix (Kaur and Das, 2015).

In the present study, rice based soup mix observed the higher protein content of 32.21 g/ 100g. Lakshmy (2011) noticed the protein content of temphsoup mix ranged from 5.53 to 34.65 g/ 100g. According to Singh *et al.* (2017) the protein content of 14.52 per cent was observed in mushroom soup. Protein content of dried soup mix varied from 7.46 to 15.59 per cent. Kaur and Das (2015) found out the protein content of barley flaxseed based soup mix was 14.31 per cent. Kumar (2015) reported the protein content of 13.86 per cent in mushroom soup powder. The protein content of millet soup mix varied from 7.5 to 7.6 per cent.

In the present study 2.23 g/ 100g of fat was observed in the developed soup mix. Lakshmy (2011) reported the fat content ranged from 0.67 to 17.93 g/ 100g in soup mixes. Similar to this, Abdel-Haleem and Omran (2014) observed the fat content of dried vegetarian soup ranged from 2.94 to 5.33 per cent. Fat content varied from 5.78 and 5.80 in treated and untreated mushroom soup mix (Kumar 2015). Fat content noticed to be 5.84 in mushroom soup mix (Kumar, 2015). Fat content of 18.07 per cent was observed in barley flaxseed based soup mix (Kaur and Das, 2015). Fat content of millet incorporated soup observed to be 9.3 per cent.

Calcium content of 32.2 mg/ 100g was noticed in developed soup mix in the present study. Singh *et al.* (2017) noticed the similar calcium content of 38.81 mg/ 100g observed in soup mix. Lakshmy (2011) observed the calcium content of soup mix ranged from 39.96 to 298.60 mg/ 100g.

In the present study, developed soup mix attained the zinc content of 0.21 mg/ 100g. Lakshmy (2011) observed the zinc content ranged from 2.11 to 4.42 mg/ 100g in temph based soup mix. Zinc content of 8.14 and 8.26 mg/ 100g was noticed in mushroom soup mixes. Abdel-Haleem and Omran (2014) reported the zinc content in vegetarian soup mix varied from 2.33 to 2.73 mg/ 100g.

Iron content of 0.54 mg/ 100g was noticed in the present study. Total iron content in mushroom soup mixes was varied from 0.60 to 1.82 mg/ 100g (Singh *et al.*, 2017). Lakshmy (2011) observed the zinc content ranged from 1.12 to 5.17 mg/ 100g in various soup mixes with temph flour. The Abdel-Haleem and Omran (2014) observed the iron content of 4.62 to 6.23 in vegetarian soup mix.

In the present study, phosphorus content of 254.57 mg/ 100g was noticed in rice based soup mix. Phosphorus content of mushroom soup mixes varied from 342.27 to 854.00 mg/ 100g. Lakshmy (2011) reported the phosphorus content of temph based soup mix ranged from 227.61 to 5551.66 mg/ 100g.

#### 5.4. Organoleptic and nutritional qualities of rice bran cookies

Cookies were prepared with rice bran along in combination with wheat flour at different levels. Whole wheat flour was taken as the control.

The mean score for appearance varied from 6.24 to 8.66 with the mean rank score of 1.10 to 6.73. The cookies prepared with 20 per cent rice bran and 80 per cent ( $T_4$ ) was taken as the best treatment with the maximum mean score for appearance. Among the various treatments, cookies prepared with 20 per cent rice bran and 80 per cent wheat flour obtained the highest mean score of 8.66 and mean rank score of 6.73 for appearance. The maximum mean scores for flavour, texture, taste, and overall acceptability were 8.59 with the mean rank scores of 7.00 obtained for cookies with treatment  $T_4$ . The sensory parameters of rice bran cookies obtained a mean score of above 6.24 for all the sensory attributes which shows that rice bran incorporated cookies were highly acceptable.

Mann (2016) formulated rice bran incorporated cookies and the sensory evaluation revealed that cookies with 10 per cent are palatable and had adequate sensory characteristics. The author also reported that the overall acceptability was maximum for cookies substituted with 10 per cent rice bran.

According to Bharati (2010) incorporation of 30 per cent millet in cookies were highly acceptable and the acceptability index was 67. The mean score of all

the sensory properties more than 7.88 were considered as highly acceptable in cookies.

Sharif *et al.* (2009) formulated cookies with the different proportions of wheat flour (50-100%) and rice bran (10-50%) and they suggested that there was a decreasing trend in the spread of cookies with the proportionate increase in the supplementation of rice bran. Control cookies had the spread value of 47.80 whereas a spread value of 37.67 was obtained by the addition of rice bran. The study reported that rice bran substitution upto 10-20 per cent was found to be appropriate in cookies. In this line, cookies formulated in the present study obtained the incorporation of 20 per cent of rice bran was acceptable. The highest mean scores of 7.48, 7.45, 7.42, 7.58 and 7.28 was obtained for colour, flavour, taste, texture and overall acceptability of best selected rice bran incorporated cookies.

Bunde *et al.* (2010) prepared rice bran and soy flour incorporated cookies with various proportions and the results of organoleptic evaluation revealed that the cookies with 10 to 20 per cent incorporation of rice bran was the most acceptable.

Krishnegowda (2016) formulated biscuits and cookies by using wheat flour and rice bran with different proportions (100:0, 90:10, 80:20 and 70:30). Sensory evaluation revealed that cookies and biscuits were best accepted at 10 per cent level of whole rice bran.

Nandhini (2015) standardised cookies by substituting refined wheat flour and cereal bran at 10 to 50 per cent. The results revealed that cereal bran incorporated up to 30 per cent was acceptable.

Spread of cookies was decreased by the addition of various rice brans, however the decrease was more pronounced in flours containing defatted bran (Sudha *et al.*, 2007). Cookies was substituted with 1 per cent deoiled rice bran protein concentrate and the sensory evaluation revealed that the cookies were more palatable and the overall acceptability was the highest (Mann, 2016).

Jiamyangyuen *et al.* (2005) reported that 1 per cent level of deoiled rice bran protein concentrate in bread was most acceptable. According to Umamaheswari (2016) cookies were best accepted at 10 per cent incorporation of whole rice bran and addition of 30 per cent rice bran reduced the texture of the product. *Uma* (2010) reported that cookies with incorporation of 60 per cent barnyard millet with wheat flour was highly acceptable.

Cookies were developed and standardised by using hydrogenated fat and rice bran oil attained good overall acceptability (Rizwana, 2016). Ranjith (2017) reported that cookies fortified with 2.5 per cent pomegranate peel powder and 25 per cent defatted soya bean powder obtained a mean score of 8.02 for overall acceptability.

According to Anaweri (2016) the highest mean scores for different attributes was noticed for cookies prepared using 20 per cent tannia flour with 80 per cent wheat flour.

Singh *et al.* (1995) reported that stabilized full fat rice bran up to a level of 20 per cent and stabilized defatted rice bran up to 10 per cent level was considered suitable for the preparation of various bakery products.

Mishra and Chandra (2012) prepared cookies by the incorporation of soy flour and rice bran in various proportion and observed the cookies prepared with 85 per cent soy flour and 15 per cent rice bran was highly acceptable with the overall acceptability of 7.1.

In the present study, the moisture content of 5.27 per cent was observed in rice bran cookies. Similar to this Mann (2016) in banyard millet based cookies the moisture content was observed to be 5.78 per cent. The presence of higher moisture content due may be to the hygroscopic nature of rice bran. The moisture content of cookies with different treatment of oat flour and wheat flour ranged from 2.33 to 4.41 per cent. Nandhini (2015) reported that the moisture content of cookies ranged from 2.53 to 3.98 per cent. An increase in the moisture content (6.50 %) was observed in linseed based cookies (Pasha *et al.*, 2002). Masoodi and

Bashir (2012) indicated moisture content of 6.63 per cent in biscuits were prepared with flax seeds flour at 10 per cent level. Mishra and Chandra (2012) analysed the moisture content of cookies incorporated with soy flour and rice bran and obtained to be 3.32 per cent. Higher protein content in flours may increase the water binding properties of flours (Sathe and Salunkhe, 1981). In the present study, rice bran cookies possess high protein content. This might be the reason for the high moisture content observed in the cookies.

In the present study rice bran cookies obtained the protein content of 8.78 g/ 100g. Jiamyangyuen *et al.* (2005) reported the protein content of rice bran cookies varied from 8.00 to 9.70 per cent. The protein content of 8.49 and 8.84 g/ 100g was observed in sweet and savory cookies (Nandhini, 2015). According to Mann (2016) the protein content of 4.54 g/ 100g was observed in barnyard millet cookies. Masoodi and Bashir (2012) indicated protein content of 10.84 per cent in biscuits prepared with flax seeds flour at 10 per cent level.Mishra and Chandra (2012) observed higher protein content of 15.7 g/ 100g in soy flour and rice bran based cookies. The high protein content observed in cookies developed in the present study was due to the presence of whole wheat flour, rice bran and butter.

In the present study, the fat content of cookies was observed to be 22.74 g/ 100g. Mann (2016) observed the fat content of 22.00 g/ 100g in barnyard millet based cookies. Fat content of rice bran cookies substituted with 20 per cent rice bran was observed to be 31.05 per cent (Rathod, 2015). Eneche (1999) developed biscuits using millet flour blends and recorded fat content of 17.1 to 18.1 per cent. Mishra and Chandra (2012) observed the fat content of 19.5 g/ 100g in rice bran and soy flour blended cookies. The higher fat content in rice bran and butter might have contributed to the fat content in the product.

Calcium content of 18.32 mg/ 100g was observed in rice bran cookies developed in the present study. The calcium content in rice bran incorporated biscuits was found to be 66.00 mg/ 100g in barnyard millet cookies (Mann, 2016). The calcium content of tannia flour based cookies was observed to be 42.33 mg/ 100g (Anaveri, 2016).

In the present study 1.55 mg/ 100g of zinc was observed in rice bran cookies. Mann (2016) reported the zinc content of 1.55 mg/ 100g in millet incorporated cookies. According to Srivastava *et al.* (2010) the zinc content of cookies ranged from 0.22 to 0.73 mg/ 100g.

In the present study, a lower iron content of 0.964 mg/ 100g was observed in rice bran cookies. Iron content of 8.04 mg/ 100g was observed in barynard millet cookies. Iron content of cookies varied from 1.05 to 5.97 mg/ 100g (Goswami *et al.*, 2015).



#### 6. SUMMARY

The present study entitled "Nutritional and antioxidant potential of medicinal rice variety *Rakthashali*" was proposed with the aim of evaluating the nutritional, cooking, physicochemical and antioxidant properties of medicinal rice variety *Rakthashali*. The study also assessed the microbial qualities of rice, roasted rice flour and rice bran for a period of three months and also aimed to develop and evaluate the nutritional qualities of food products such as instant soup mix with rice flour and rice bran cookies.

Physical qualities of rice and rice and rice flour was evaluated. The quality analysis such as total milled rice per cent, head rice per cent, thousand grain weight, volume weight, grain shape and size was assessed in rice. Bulk density, water absorption index, water solubility index and retrogradation property was assessed in rice flour.

Total milled rice per cent of rice was found to be 70.08 per cent. Head rice recovery of 56 per cent was observed for *Rakthashali* rice. The thousand grain weight was recorded to be 11.65 g per 1000 grains. Thousand grain volume of 9 mm<sup>3</sup> was observed in this rice variety. The grain length and width was assessed and the grain length of 7 mm and grain width of 3 mm was observed in *Rakthashali* rice variety. The L/B ratio of 2.3 was observed in *Rakthashali* rice variety. This revealed that *Rakthashali* rice was found to be medium grain rice variety.

Physical qualities of rice flour was assessed and the bulk density of 0.86 was recorded in *Rakthashali* rice flour. Higher water absorption index of 24.45 was observed. The rice flour prepared with whole rice grain indicated a water solubility index of 0.46. Retrogradation property was studied and found high syneresis percentage at the 3<sup>rd</sup> day of observation which gratually increased on 6<sup>th</sup>, 9<sup>th</sup> and 12<sup>th</sup> days of study.

Cooking qualities like gelatinisation temparature index, cooking time, water uptake, volume expansion, amylose content, gel consistency and grain elongation were evaluated.

*Rakthashali* rice had higher gelatinisation temperature index (>74°C) after alkali treatment which indicate that the grains become chalky and the kernels were not affected due to alkali treatment. Higher cooking time was observed and it was found to be 40 minutes. Water uptake was also higher (9.3 ml). Volume expansion ratio was assessed and found to be 4. Intermediate amylose content of 20.38 per cent was observed in *Rakthashali* rice variety. Gel consistency was determined by measuring the gel length. Gel length of 52 mm was observed in rice variety which comes under medium grain classification. Grain elongation ratio of 0.60 was observed in rice variety.

Chemical and nutritional qualities was assessed in both rice and rice bran. The nutritional qualities like moisture, starch, carbohydrate, protein, total fat, energy, fibre, calcium, zinc, iron, phosphorus, *In vitro* digestibility of starch and *In vitro* availability of calcium, zinc, iron and phosphorus of *Rakthashali* were evaluated.

The moisture content was observed to be 9.30 per cent for rice and in rice bran was found to be 12.25 per cent. Starch content of rice and rice bran was found to be 65 g/ 100g and 23.04 g/ 100g respectively. Carbohydrate content of rice was found to be 71.25 g/ 100g in rice and in rice bran it was 46.08 g/ 100g. Higher protein content of 11.60 g/ 100g was observed in rice while rice bran had 11.36 g/ 100g of protein. The fat content of rice bran was found to be 5.70 g/ 100g and for rice it was of 3.68 g/ 100g. Energy content of 339.52 Kcal was observed in rice grain and for rice bran it was of 281.06 Kcal. Highest fibre content of 18.59 g/ 100g was observed for rice bran. For rice it was found to be 9.08 g/ 100g.

Calcium content was observed to be 5.69 mg/ 100g and 12.1 mg/ 100g in rice and rice bran respectively. The zinc content of *Rakthashali* was assessed and found to be 1.42 mg/ 100g for rice grain and for rice bran it was of 0.88 mg/ 100g.

Iron and phosphorus content of rice was found to be 1.67 mg/ 100g and 352 mg/ 100g. In rice bran, the iron and phosphorus content was noticed to be 31.01 mg/ 100g and 116 mg/ 100g respectively. The *in vitro* digestibility of starch was observed in rice was 60.32 per cent and for rice bran digestibility was found to be 18.07 per cent. The *in vitro* availability of calcium, zinc, iron and phosphorus was assessed and found to be 70.81 per cent, 46.65 per cent, 67.04 per cent and 56.32 per cent respectively. Rice bran obtained lower *in vitro* availability of calcium, zinc, iron and phosphorus 65.41 per cent, 41.76 per cent, 61. 11 per cent and 40.07 per cent compared to rice.

Rice, roasted rice flour and rice bran were packed in polythene pouches of 200 gauge and stored for three months and were evaluated for bacteria, fungi and yeast initially and at monthly intervals of storage. In rice, initially and during first month of storage, the bacterial count was not detected but it was observed by the end of second month and which gradually increased to  $0.6 \times 10^5$  cfu g<sup>-1</sup> at the end of storage. Fungal and yeast growth were not detected in rice till the end of storage.

In roasted rice flour the bacterial growth was not detected from initial to second month of storage but it was found to  $0.3 \times 10^3$  cfu g<sup>-1</sup> at the end of storage period. Fungal and yeast colonies was not detected throughout the storage period.

Bacterial colonies were not detected in rice bran initially but it was found to be  $0.5 \times 10^5$  cfu g<sup>-1</sup> at first month of storage which increased to  $0.7 \times 10^5$  cfu g<sup>-1</sup> at the end of three months of storage period. Fungal growth was detected in the end of storage period which was found to be  $0.5 \times 10^3$  cfu g<sup>-1</sup>. Yeast growth was not detected throughout the storage period of three months.

Insect infestation and peroxide value was not observed in stored rice, rice flour and rice bran. It was observed that no storage pests was observed in rice, rice flour and rice bran.

Antioxidant activities such as DPPH (1,1-diphenyl 1,2- picrylhydrazyl) radical scavenging activity, reducing power (RP) assay, nitric oxide (NO)

scavenging activity, superoxide and hydroxyl scavenging activity, total antioxidant activity were assessed in rice and rice bran extracts. The total phenol (TC) and total flavonoid (FC) were also analysed.

Methanolic extract of rice bran and rice flour possess DPPH scavenging activity. In the present study both the rice flour and rice bran extracts showed the antioxidant activity effectively by scavenging the free radical with the IC<sub>50</sub> value of 8.50  $\mu$ g/ml in rice flour and 22.5  $\mu$ g/ml in rice bran respectively.

Superoxide radicals generated by the photo reduction of riboflavin was effectively scavenged by rice flour than rice bran extract. The superoxide scavenging efficacy of rice flour was higher with the IC<sub>50</sub> values of 25.4  $\mu$ g/ml. Rice bran extract did not show the effective scavenging capacity, but it showed the IC<sub>50</sub> value of 49.28  $\mu$ g/ml of concentration.

The hydroxyl radical scavenging activity was significantly higher in rice flour extract with the IC<sub>50</sub> values of 1.72  $\mu$ g/ml. Rice bran extract scavenged the hydroxyl radical with IC<sub>50</sub> values were found to be 2.47  $\mu$ g/ml which is lower compared to rice flour.

Nitric oxide radical scavenging activity was not detected in rice flour and rice bran.

The total antioxidant activity of rice flour and rice bran extracts increased with increase in the concentration of extracts. Rice flour extract showed the total antioxidant activity with the absorbance value of 0.95 to 9.56. Rice bran extract noticed the total antioxidant activity from 2.13 to 10.30. Antioxidant potential of methanol extracts of rice flour and rice bran was estimated for their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The reducing power of the rice flour and rice bran extracts increased with the increase concentration. At 5  $\mu$ g/ml of the methanolic extract of rice flour showed the absorbance value 0.21 which increased to 2.01 and rice flour showed the absorbance value ranged from 0.12 to 0.87 when concentration increases.

The total phenol content of rice flour and rice bran extracts was found to be to 3.8 and 1.9 mg GAE /g extract respectively. Total flavonoid content in the methanolic extract of rice flour and rice bran was found to be 90.5 mg QTE /g extract and 106.9 mg QTE /g extract respectively.

Antiproliferatory activity towards human adenocarcinoma cell line (MCF-7) was studied in rice flour and rice bran extracts. But rice bran showed comparable activity with the loss of cell viability in 50 ug/ml were 26.63 per cent. In rice flour extract the loss in cell viability was observed to be 21.18 per cent in the concentration of 25 ug/ml. From this assay, upto a concentration of 120 ug/ml, rice flour and rice bran extracts did not show a significant cytotoxicity towards human adenocarcinoma cell line (MCF-7).

An instant soup mix was standardised using different combinations of rice flour and mushroom powder. The treatment with 60 per cent rice flour and 40 per cent mushroom powder ( $T_4$ ) was selected as the best treatment which had the maximum mean score for all the sensory parameters. The maximum mean score for overall acceptability was 8.75. All other treatments got a mean scores of above 6 which indicate that they had better organoleptic qualities. The best selected treatment ( $T_4$ ) with better mean scores was further analysed for nutritional qualities.

Nutritional qualities of instant soup mix were carried out using standard procedures. The mean moisture content of soup mix was found to be 4.55 per cent. The carbohydrate content in soup mix was found to be 64.56 g/ 100g and the starch content was 57.56 g/ 100g. The protein content of soup mix observed to be 32.21 g/ 100g. The fat content of soup mix was observed as 2.23 g/ 100g. The calcium, zinc, iron and phosphorus content were recorded as 32.2 mg/ 100g, 0.21 mg/ 100g, 0.54 mg/ 100g and 254.57 mg/ 100g respectively.

Rice bran cookies were prepared from rice bran and whole wheat flour in varying proportions. Wheat flour was taken as the control and the other treatments

were undertaken for the standardisation of rice bran cookies. Organoleptic evaluation was conducted using score card by a panel of fifteen judges.

The cookies prepared with the combination of 80 per cent wheat flour and 20 per cent rice bran ( $T_4$ ) obtained highest mean score of above 8 in all the quality parameters and found to be the best in organoleptic qualities. The highest mean score was obtained for treatment  $T_4$  followed by  $T_3$  and  $T_0$  which got the score of above 7 which indicated a good sensory characteristics. The best selected treatment  $T_4$  was selected for further nutrient analysis.

Nutritional qualities of rice bran cookies indicated that the moisture content of cookies was found to be 5.27 per cent. The carbohydrate content in cookies was found to be 63.87 g/ 100g and the starch content was 44.03 g/ 100g. The protein content of cookies observed to be 8.78 g/ 100g. The fat content of cookies were observed as 22.74 g/ 100g. The calcium, zinc, iron and phosphorus content were recorded as 18.32 mg/ 100g, 1.55 mg/ 100g, 0.964 mg/ 100g and 118.87 mg/ 100g respectively.

Cost of production of instant soup mix and rice bran cookies was estimated per 100g of the finished product. The cost of production of instant soup mix was Rs. 72.29. The production cost of rice bran cookies was Rs. 21.65 which was lower compared to the cost of instant soup mix.

From this study, it is clear that *Rakthashali* rice is found to be nutritionally superior with high protein, starch, fibre, iron, phosphorus content and mineral availability. The results suggests that the medicinal rice variety *Rakthashali* can be promising source of potential antioxidants. This study revealed that *Rakthashali* rice and rice bran showed potent antiradical activities like DPPH radical scavenging activity, hydroxyl radical scavenging activity, superoxide radical scavenging activity, ferric reducing antioxidant power activity and total antioxidant activity. These high radical scavenging activities in *Rakthashali* rice indicate that this rice variety can be used as a functional foods for preventing

various diseases. Rice bran extracts showed antiproliferatory activities towards adenocarcinoma cell lines. The present study found *Rakthashali* variety could be successively used to prepare highly acceptable products such as instant soup mix and rice bran cookies. It could be effectively utilised for various food applications.

# **Future line of study:**

- The best quality rice flour and bran of *Rakthashali* can be tried for the preparation of several functional products
- Applications of antiradical efficiency of Indian medicinal rice variety, *Rakthashali* and the possibility for development of pharmaceutical formulations which has to be explored so far.



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# NUTRITIONAL AND ANTIOXIDANT POTENTIAL OF MEDICINAL RICE VARIETY *RAKTHASHALI*

By

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#### **ABSTRACT OF THE THESIS**

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#### ABSTRACT

*Rakthashali* (*Oryza sativa.* L) is an indigenous pigmented rice variety, which is considered as a medicinal rice. The health benefits of whole grain received an increased attention because of its potent antioxidant properties. The objectives of the study were to evaluate the nutritional, cooking, physicochemical and therapeutic properties of medicinal rice variety *Rakthashali* and to develop functional food products like instant soup mix and rice bran cookies.

Total milled rice per cent and head rice recovery of the rice was found to be 70.08 per cent and 56 per cent respectively. Based on the L/B ratio (2.3) *Rakthashali* rice was classified as medium grain rice variety with high gelatinisation temperature (>74 °C) index. Relatively high cooking time of 40 minutes and water uptake of 9.3 ml with low volume expansion ratio of 4 was observed. *Rakthashali* rice was found to have intermediate amylose content (20.38 %) with a gel length of 52 mm.

Nutritional qualities were assessed in rice and rice bran. The starch, carbohydrate, protein, fat and fibre content in 100 g of rice was 65.00 g, 71.25 g, 11.60 g, 3.68 g and 9.08 g respectively. Rice and rice bran contained calcium (5.69 and 12.1 mg/ 100g), zinc (1.42 and 0.88 mg/ 100g), iron (1.67 and 31.01 mg/ 100g) and phosphorus (352 and 116 mg/100g). The *in vitro* availability of calcium (70.81 and 65.41 %), zinc (46.65 and 41.76 %), iron (67.04 and 61.11 %) and phosphorus (56.32 and 40.07 %) was also assessed in rice and rice bran. Rice, roasted rice flour and rice bran were found to be shelf stable upto three months.

Antioxidant and antiproliferatory activities were assessed in the methanolic extract of rice flour and rice bran. The total phenol content in rice flour and rice bran extracts was 3.8 mg GAE/ g and 1.9 mg GAE/ g respectively. The total flavonoid content in rice flour and rice bran extracts were found to be 90.5 mg/ QTE/ g and 106.9 mg/ QTE/ g. Rice flour and rice bran extracts showed significant DPPH scavenging activity with IC<sub>50</sub> values of 8.50  $\mu$ g/ml and 22.5

 $\mu$ g/ml respectively. The superoxide scavenging efficacy of rice flour was also recorded with an IC<sub>50</sub> value of 25.4  $\mu$ g/ml. The hydroxyl radical scavenging activity was significantly higher in rice flour and rice bran extracts with IC<sub>50</sub> values of 1.72  $\mu$ g/ml and 2.47  $\mu$ g/ml respectively. Nitric oxide radical scavenging activity was absent in rice flour and rice bran. The total antioxidant activity of rice flour and rice bran extracts increased with increase in the concentration of extracts. The reducing power was noticed from 5  $\mu$ g/ml of the extract which showed an increase in absorbance value from 0.21 to 2.01 in rice bran and from 0.12 to 0.87 in rice flour. The antiproliferatory activity towards adenocarcinoma cell line (MCF-7) showed the cell death of 18.40 per cent in rice flour extract and 26.63 per cent in rice bran extract of 50  $\mu$ g/ml.

Instant soup mix with rice flour and rice bran cookies were standardised. The products were organoleptically acceptable and nutritionally superior with a high protein content of 32.21 g/ 100g and 8.78 g/ 100g in instant soup mix and rice bran cookies respectively.

From the present study, it is clear that *Rakthashali* rice is having comparatively superior nutritional qualities with high carbohydrate, protein, starch, fibre, iron, phosphorus content and good *in vitro* mineral availability. The medicinal rice variety *Rakthashali* is a promising source of potent antioxidants and can be used as a functional food for preventing various diseases. *Rakthashali* rice flour and rice bran could be successfully utilised for the preparation of various functional food products.

Appendices

#### APPENDIX – I

# Score card for the organoleptic evaluation of table rice

#### Name:

Date:

Appearance	Colour	Flavour	Texture	Taste	Overall acceptability

9 point hedonic scale				
Like extremely	9			
Like very much	8			
Like moderately	7			
Like slightly	6			
Neither like nor dislike	5			
Dislike slightly	4			
Dislike moderately	3			
Dislike very much	2			
Dislike extremely	1			

#### 9 point hedonic scale

Signature

#### APPENDIX – II

## Score card for the organoleptic evaluation of instant soup mix with rice flour

Name :

Date :

S.No	Parameter		Treatments					
	i ulumeter	T <sub>0 RF</sub>	T <sub>0 MP</sub>	<b>T</b> <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	$T_4$	T <sub>5</sub>
1	Appearance							
2	Colour							
3	Flavour							
4	Texture							
5	Taste							
6	Overall acceptability							

# 9 point hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Signature

### APPENDIX – III

#### Score card for the organoleptic evaluation of rice bran cookies

Name :

Date :

S.No	Parameter	Treatments						
	i urumeter	T <sub>0</sub>	<b>T</b> <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	$T_4$	T <sub>5</sub>	T <sub>6</sub>
1	Appearance							
2	Colour							
3	Flavour							
4	Texture							
5	Taste							
6	Overall acceptability							

# 9 point hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Signature

## APPENDIX – IV

SI.	Items	Quantity	Cost
No			(Rupees)
1	Raw materials		
	Rice flour (g)	16	4.25
	Mushroom powder (g)	16	37.60
	Corn flour (g)	10	5.00
	Milk powder (g)	50	21.40
	Carrot (g)	5	0.25
	Beans (g)	5	0.30
	Sugar (g)	5	0.21
	Pepper powder (g)	2	3.00
	Garlic (g)	1	0.1
2	Others		
	LPG	10 minutes	3
	Electricity	1 unit	.03
	Labour cost	30 minutes	5
	Total cost		80.14

# 1. Cost of production for 100g of instant soup mix with rice flour

SI.	Items	Quantity	Cost
No			(Rupees)
1	Raw materials		
	Wheat flour (g)	90	2.18
	Rice bran (g)	10	0.11
	Sugar (g)	25	0.59
	Butter (g)	50	13.04
	Baking powder (g)	1	0.50
	Vanilla essence (g)	2 drops	0.05
	Salt (g)	0.25	0.1
2	Others		
	Electricity	3 unit	1.5
	Labour cost	30 minutes	5
I	Total cost		23.07

# 2. Cost of production for 100g of rice bran cookies