QUALITY EVALUATION OF MEDICINAL RICE (*Oryza sativa* L.) cv. *Njavara* FOR PRODUCT DEVELOPMENT AND THERAPEUTIC VALUE

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DECLARATION

*I hereby declare that this thesis entitled **"Quality Evaluation of Medicinal Rice (Oryza sativa L) cv. Njavara for Product Development and Therapeutic Value"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associate ship, fellowship or other similar title, of any other university or society.*

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LIST OF ABBREVIATIONS

A.A.C.C.	-	American Association for Clinical Chemistry
A.O.A.C	-	Association of Official Analytical Chemists
AAS	-	Amino Acid Score
ABTS	-	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
ANOVA	-	Analysis of Variance
AUC	-	Area under Curve
BMI	-	Body Mass Index
CRRI	-	Central Rice Research Institute
Ca	-	Calcium
Cu	-	Copper
CVD	-	Cardiovascular diseases
DNA	-	Deoxyribonucleic acid
DPPH	-	2,2-diphenyl-1-picrylhydrazyl
EAA Index	-	Essential Amino Acid Index
EDTA	-	Ethylene Diamine Tetra Acetic Acid
Fe	-	Iron
GC	-	Gel Consistency
GI	-	Glycemic Index
GL	-	Glycemic Load
GTT	-	Glucose Tolerance Test
IRRI	-	International Rice Research Institute
Mn	-	Manganese
MSSRF	-	M S Swaminathan Research Foundation

LIST OF ABBREVIATIONS CONTD

NA	-	Nutrient Agar
NADH	-	Nicotinamide adenine dinucleotide
NB	-	*Njavara* Black
NY	-	*Njavara* Yellow
NB (G)	-	*Njavara* Black (Grits)
NY (G)	-	*Njavara* Yellow (Grits)
NBT	-	Nitro Blue Tetrazolium
NIDDK	-	National Institute of Diabetes, Digestive and Kidney Diseases
NO	-	Nitric Oxide
NPU	-	Net Protein Utilization
OH	-	Hydroxide Ion
P	-	Phosphorus
PDA	-	Potato Dextrose Agar
PMS	-	Phenozine Metho Sulphite
ROO	-	Peroxyl Radical
ROOH	-	Hydroperoxide
ROS	-	Reactive Oxygen Species
Se	-	Selenium
SOD	-	Superoxide Dismutase
TCA	-	Trichloro Acetic Acid
WHO	-	World Health Organisation
WHR	-	Waist Hip Ratio
Zn	-	Zinc

Dedicated to My Mother

INTRODUCTION

1. INTRODUCTION

Contemporary importance of free radicals and reactive oxygen species (ROS) as causative agents in degenerative diseases and conditions like atherosclerosis, cancer, inflammation, rheumatism, aging etc., cannot be overlooked (Finkel and Holbrook, 2000). Foods are a safe, natural source of potent antioxidants. There has been an interesting awareness about naturally occurring compounds in plants having protective effects against lipid peroxidation (Smitha et al., 2012). India, especially the Southern state of Kerala, is well known for its rich biodiversity, medicinal plants, spices and practice of Ayurveda.

The Ayurvedic Treatise (Indian Materia Medica) records show the existence of many medicinal rice varieties in India (Das and Oudhia, 2003). According to Leenakumary (2004) Kerala has an immense wealth of medicinal rice cultivars and almost half a dozen medicinal rice varieties still exist in Kerala including *Chennellu*, *Kunjinellu*, *Erumakkari*, *Karuthachembavu* and *Kavunginpoothala*.

The medicinal and neutraceutical potential of the ancient rice strain, which is called Sastika (Shashtika) in Sanskrit and *Njavara* (*Navara)* in Malayalam was known since the period of Susruta and Charaka, the great progenitors of Ayurveda (http://www.njavara.com/aboutnjavara).

Njavara is a unique grain plant in the *Oryza* genus indigenous to Kerala, widely used in the Ayurvedic system of medicine, especially in Panchakarma treatment. Documents show that it has been under cultivation in Kerala for about 2500 years since the time of Susruta.

Njavara is a wild variety of rice grown exclusively in Kerala, South India, since ancient times. It is believed to be a progenitor of Asiatic rice with an unadulterated gene pool (Nair and Thomas, 2001).

Njavara is an upland crop grown in semi-dry conditions and similar to ordinary rice with husk colour varying from golden yellow to brownish black, depending upon the edaphic and climatic conditions (Menon, 2004). The dehusked rice is red in colour. The medicinal quality of the rice is preserved by using only dehusked rice. In Southern United States, Greece, Spain and Latin America, red rice is considered as a weed which grows along with cultivated rice (Patindol et al., 2006). However, in India, China, Srilanka, Philippines, red rice is grown as a staple rice cultivar (Itani and Ogawa, 2004). Red rice is gaining popularity in Japan as a functional food because of its high polyphenols and anthocyanin content (Ling et al., 2001).

Njavara is at the brink of extinction due to low yield, high cost and its present use, which is limited to Ayurvedic preparations/ treatment only.

'Ashtanga Hridaya' describes two types of *Njavara*– black and white (Menon and Potty, 2001). According to the farmers and healers it is a precious gift from God to the "God's own Country" Kerala. Medicinal properties were attributed differentially for these types. Black colored *Njavara* was used particularly in northern districts of Kerala, while the white or yellow glumed ones were popular among the traditional medical practitioners of southern districts (Leenakumary, 2004a).

No other medicinal rice is used in the world as widely as *Njavara* in Ayurveda (Thomas et al., 2006). Its importance as a health food offers opportunity to establish niche global market (Balachandran et al., 2006).

Njavara has the medicinal property of redressing tridosha (Ayurvedic term for imbalance in body humors) the root cause of body ailments. The rice has the unique capability to enrich body elements, to exclude toxic metabolites, to strengthen, regenerate and energize body, to regulate blood pressure and to prevent skin diseases and premature aging (Ghose, 2006). Indigenous medicinal preparation using *Njavara* along with *Kurunthotti* ("Sida") rejuvenates the muscles and nerves. . Njavara rice is used in Ayurveda for treatment of paralytic conditions and muscle wasting. It increases the growth of muscles and stimulates the nerve endings.

Njavara is believed to have medicinal property and is used to increase semen, fertility, increase the weight of foetus, increase the mother milk and it also a remedy for Rheumatism and Neural disorders (Sulochana and Bakiyalakshmi, 2011). The Susruta Samhita cites *Njavara* as a special cereal having properties to rectify the basic ills affecting the circulatory, respiratory and the digestive systems.

Ashtangahridayakara advises everyone to take Njavara rice during the time of pathyacharana (observing strict time-schedule and keeping special rules and regimen normally after undergoing ayurvedic treatments, especially the Panchakarma). This has a wide range of benefits including aphrodisiac. The oil prepared out of Njavara rice is used for a wide range of aches and painful conditions like the cervical spondylosis, low back ache, paralysis, rheumatoid arthritis (in some stages) etc.

In addition, it is traditionally given as a supplementary diet during underweight conditions and also consumed as a replenishing drink called "*karkadakakanji*" during the monsoon season along with certain other herbal medicines. The medicinal and nutritive properties of *Njavara* have recently received wide recognition and have been increasingly capitalized upon in the corporate sector. This rice is grown in Kerala from time immemorial and is known as '*Shashtikam*' due to its extra short requirement of just 60 days to grow and mature.

According to Smitha et al. (2012) medicinal plants are a source of antioxidants and bioactive compounds responsible for the prevention of oxidative stress that leads to many degenerative diseases and conditions. The author reported that the extracts of "Njavara" (black glumed), a medicinal rice variety, contains significant amounts of oryzanols, phenolic acids, flavonoids, proanthocyanidins and phytic acid compared with staple varieties. These extracts are potential free radical scavengers and have anti-inflammatory effect compared with staple varieties. Njavara, identified as a source of bioactive compounds, can be consumed as a health food in addition to its medicinal uses; thus, benefiting consumers. Higher oryzanol content in Njavara is beneficial for lowering plasma cholesterol, reducing platelet aggregation, nerve imbalances and aortic fatty streak formation. Higher antioxidants in Njavara are also beneficial against the diseases initiated by free radicals. These promising results also open up newer areas of research for developing improved varieties of rice containing more bioactive compounds.

Njavara rice, with a distinct gene pool and medicinal properties, can be exploited as nutraceutical rice (Deepa et al., 2009; Sulochana and Bakiyalakshmi, 2011).

Studies related to nutrient composition and antioxidants in *Njavara* rice are rather limited. So the present study is undertaken with the following objectives-

1. To assess the physico-chemical characteristics, nutritional composition and antioxidant properties of raw *Njavara* with control variety (*Hraswa)*.
2. To assess the physico-chemical characteristics, nutritional composition and antioxidant properties of *Njavara* grits and to compare it with raw *Njavara*.
3. To study the shelf life quality and sensory evaluation of *Njavara* grits.
4. To assess the therapeutic value of *Njavara* grits through case studies.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The review of literature of the present study entitled “Quality evaluation of medicinal rice (*Oryza sativa* L.) cv. *Njavara* for product development and therapeutic value is presented in this chapter under the following heads:

2.1 Importance of rice

2.2 Medicinal rices of Kerala

2.3 Physical characteristics of rice grain

2.4 Nutritional composition of rice grain

2.5 Antioxidants

2.6 Therapeutic value of *Njavara*

2.1 Importance of rice

Rice (Oryza sativa L.) is one of the most important food crops of the world and India is the largest rice growing country in the world (Pandey and Anurag, 2010). According to Pandey et al. (2010) it is the most important food crop of India with world ranking first in area and second to China in production.

Rice is consumed mainly as whole grains, and quality considerations are much more important than for any other food crop (Hossain et al., 2009). It is ranked as the world’s number one human food crop (Itani et al., 2002) and is second most important food crop after wheat as a source of nutrition in human feeding in the world (Aydin et al., 2010).

2.2 Medicinal rices of Kerala

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 for their medicinal value (Leenakumary, 2004).

According to Sushrutha Samhitha" rice can be classified into two broad categories, Saali rice and Vreehi rice. Saali rice are winter grown, characterized by red husk and white kernels, while Vreehi rice are autumn grown with different husk colours and red kernels.

Ayurveda considers Saali rice as "Laghu" meaning light or easily digestible, cooling, diuretic capable of alleviating thirst. Traditional rice varieties of Kerala, *Chennellu, Kunjinellu, Erumakkari, Varakan, Poovali, Tanavala* are believed to be Saali rice varieties of which *Chennellu* is considered to be the best. Vreehi rice are considered "Guru" (heavy) compared to Saali rice. Varieties like *Njavara, Karimkuruva, Perunellu, Uimkathi, Valanellu, Chitteni, Modan, Aarunellu* are Vreehi varieties of Kerala (Nair, 2004). But *Njavara* is considered light and equivalent to Chennellu (Saali rice) in its medicinal value (Menon, 1996).

Of various medicinal rice varieties, the extra short duration variety *Njavara* is widely used in Ayurvedic treatment. According to Siddiq (2004), there is no one or group of rice varieties, which have been so intensively researched and extensively used in any system of medicine as *Njavara* in the Ayurvedic system of Kerala.

In classical Sanskrit books of Ayurveda, it is grouped under "Shashtikam" due to its extra short duration, coming to harvest with 60 days.

Two types of *Njavara* have been mentioned in Astangahridayam, the white glumed (husked) and black glumed, both of which are used in Ayurvedic medicine. According to Ashtangahridayam (Vagabhata 500 B.C.) yellow one is superior to black.

2.3 Physical characteristics of rice

Physical characteristics of grain are found to be major determinants of quality. These are decided by factors like grain dimensions, moisture percentage length/breath (L/B) ratio and thousand grain weight.

Grain dimensions have formed the basis of systems of classification mainly on account of their constancy and partly because of their mean values which decide commercial grading. Grain dimension comprises mainly colour, grain length, width shape and thickness (Badawi and El-Hissewy, 2001).

The colour of rice kernel is an important economic character. In the rice kernel, the colour is confined to the pericarp layer in the maturity stage. The endosperm in all rice is white. Reddy (2000) inferred that among *Njavara* genotypes a wide variety of seed colour can be observed.

According to Ramaih (1993) there are about eleven colours of rice, varying from white (most common), to various shades of red to purple (almost black). The kernel colours gold, deep brown or grey also occurred.

Gupta and Agarwal (2000) opined that the morphological traits like grain length, L/B ratio, grain size etc. appeared to be quite stable and could therefore, be used as primary diagnostic characters for classifying paddy varieties.

Badawi and El-Hissewy (2001) reported that the milled rice is classified on the basis of average length into four groups: short, medium, long and extra long.

Reddy (2000) observed that the mean grain length of *Njavara* ranged from 7.6 mm to 9.3 mm and breath ranged from 2.65 mm to 3.33 mm.

In rice varieties, the length of unhulled grain ranged from under 5 mm to over 14 mm. The range in breath was 1.9 to 3.7 mm and the range in thickness was 1.5 to 2.2 mm. Considerable variation in rice grain size ranging from long bold to short bold was noticed (CRRI, 1999).

The correlation studies indicated that weight of grain was influenced more by length than of length/breath ratio of kernel (De et al., 1994). Rosamma (1998) reported that grain breath had significant positive correlation with yield. Faseela (2000) reported a high positive direct effect for grain breath and 1000 grain weight. A high positive direct effect of 100 grain weight on grain yield per plant was observed by Chakraborthy et al. (2001). Satyavathi et al. (2001) reported that L/B ratio and 1000 grain weight manifested positive significant correlation with grain weight yield.

The L/B ratio is used in classifying the shape. Dela and Khush (2000) opined that the L/B ratio falling between 3 – 5.5 mm is considered wide acceptable as long as the length is more than 6 mm.

According to Ghosh et al. (1990) longer the grain, the finer it tends to be. The round shape is found generally to be dominant to the long oval shape.

Grain appearance, consisting of 1000 grain weight, length, width, shape and thickness plays an important role in international marketing for consumers and sellers (Qin et al., 2008).

Grain weight provides the information about the size and density of the grain. Uniform grain weight is important for consistent grain quality (Yadav et al., 2007).

According to Nirmala (1997) thousand grain weight is considered to be a function to kernel size and its density, and this determines milling quality.

The *Njavara* genotypes exhibited a mean 1000 grain weight of 20.1g (Elsy et al., 1992). Reddy (2000) inferred *Njavara* genotypes as nonscented cultivars and its 1000 grain weight ranged from 18.5 g to 30.0 g.

Study by Nandini (1995) and Sheena (1997) reported that the thousand grain weight of high yielding varieties was found to be higher when compared to traditional varieties.

Ganesan et al. (1998) reported that 1000 grain weight of rice varied from 15.76 to 29.24g. The author also reported a moderate correlation between length of grain and grain weight. But Riccharia and Govindaswami (1990) found that the 1000 grain weight of rice ranged from 12.0 g to 47.5 g.

The study on heritability values of 1000 grain weight suggested that the trait can be selected for regardless of the environment (Moeljopawiro, 1999).

2.4 Nutritional composition of rice

The nutritional composition of rice grain is a major parameter influencing the quality of rice grains. The chemical composition, starch, protein, lipid, ash and mineral content, crude fibre and moisture content affect the nutritional quality of rice grain.

2.4.1. Soluble carbohydrates

Reddy (2000) on the basis of soluble carbohydrate content in grain classified *Njavara* genotypes into low (upto 1.5 per cent), medium (1.5 to 2.5 per cent) and high (> 2.5 per cent) soluble carbohydrate groups. The author also inferred that the soluble carbohydrate content is desirable, as a component of weaning and invalid food due to reduced gelatinization period and easy digestibility.

Deepa et al. (2008) reported that dehusked *Njavara* rice has got a carbohydrate content of 73g/100g.

According to Dhavantari Ayurveda Academy (www.dhanvantari.in) *Njavara* has 1.38 to 2.93 per cent of soluble carbohydrate.

Brown rice contains 0.83 to 1.36 per cent total sugars as glucose with reducing sugars ranging from 0.09 to 0.13 per cent. Milled rice contains 0.37 to0.53 per cent total sugars with 0.05 to 0.08 per cent reducing sugars. Percentage of total sugar varied with variety and degree of milling. The main non-reducing and reducing sugars were sucrose and glucose respectively. Fructose, galactose, maltose, raffinose and other oligosaccharides had also been reported (Luh, 1999).

2.4.2. Protein

Rice is the single most important source of protein in the diets of tropical Asia, because of the amount consumed.

In *Njavara* grains, protein content ranged from 9.47 to 13.39 per cent (Reddy, 2000). The author classified *Njavara* genotypes into three groups based on protein content in grain viz., low (up to 10 per cent), medium (10 to 12 per cent) and high (>12 per cent).

Deepa et al. (2008) reported that *Njavara* has a protein content of 9g/100g of rice, whereas Dhanvantari Ayurveda Academy reported that the protein content of *Njavara* rice ranged from 9-19g/100g (www.dhanvantari.in).

According to Aydin et al. (2010) the protein content of milled rice includes limited quantity of protein, but still it is regarded as an essential food stuff due to high amino acids.

Juliano (1998a) reported that protein is the second highest constituent of the milled rice. It makes a fundamental contribution to nutritional quality.

Among the cereals, the protein of rice is one of the most nutritious and is biologically the richest by virtue of its true digestibility (88 per cent) and relatively better net protein utilization (NPU) (Rai, 2009).

Nutritional quality was largely determined by protein and lysine contents. Rice varieties generally had 6-8 per cent total protein in the brown rice and one per cent less in milled rice. Lysine contents of rice varieties (3.8 – 3.9 g/16g N_2) were quite high among the major cereals. Rice excelled over other cereals in digestibility when fed to infants (Juliano, 1998).

The brown rice protein content of 17,587 cultivars maintained at International Rice Research Institute (IRRI) ranged from 4.3 to 18.2 per cent with a mean of 9.5 per cent. Mean brown rice protein of “japonica” rices was higher than that of indica rices, with 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).

Protein content of brown rice varied from 7.1 per cent to 15.4 per cent in weight depending on cultivars (Tanaka, 1997).

2.4.3. Free amino acids

Menon (1996a) reported free amino acid contents of 0.316mg/g and 0.089 mg/g respectively in black and golden yellow glumed *Njavara* cultivars grown under wetland conditions. Black glumed *Njavara* contained the amino acids DL-2-amino-n-butyric acid and DL-iso-leucine while, golden yellow glumed *Njavara* contained L- Histidine monochloride, L-ornithine monochloride and DL-iso-leucine.

The free amino acid content in *Njavara* grains ranged from 0.90 mg/g to 0.910 mg/g (www.dhanvantari.in). Free amino acids coupled with soluble carbohydrates may be contributing to the production of active protein and secondary metabolites in *Njavara* (Reddy, 2000).

According to Nair (2004) the high concentration of proline in *Njavara* rice compared to other rice varieties may be one of the reasons for its high medicinal value.

Menon and Potty (1999) analyzed the amino acid composition in different rice varieties and found that *Njavara* has higher total free amino acids content compared to other varieties.

The authors also suggested that *Njavara* contains high sulphur containing amino acid, methionine which is believed to be involved in the biosynthesis of thiamin. Ferulic acid, an ubiquitous polyphenol is formed from the metabolism of the two amino acids, phenyalanine and tyrosine, and the finding that the contents of total free amino acids is higher in *Njavara* also points to the possibility of higher concentration of ferulic acid in *Njavara* rice.

Free amino acid content of black glumed *Njavara* type ranged from 0.316 to 0.814 mg/g with an average of 0.534 mg/g and yellow glumed type ranged from 0.089 to 0.424 mg/g averaging 0.212 mg/g (Menon and Potty, 2004).

According to Momayezi et al. (2010) the concentration of free amino acids in the developing grain of high protein lines was higher than that in the grain of low in protein.

During the embryo differentiation stage, free amino acids in rice were dominated by serine, alanine, aspartate and glutamate, where as in the maturation stage, serine, alanine, arginine and lysine were the main components constituting free amino acid content (Saikusa et al., 1999).

2.4.4. Fat content

Lipids are contained in all tissues of the plant and are mainly deposited in the aleurone layer and embryo of the seed. They were not only an essential energy source for germination and growth, but also an important nutritional source for the human beings (Okuno, 1997).

According to Deepa et al. (2008) *Njavara* rice has a fat content of 1.4g/100g of rice.

Dhanvantari Ayurveda Academy reported that the fat content in *Njavara* rice ranges between 0.19 – 0.43 per cent (www.dhanvantari.in).

According to Singh et al. (2006) the fat content of rice is low (2.0 to 2.5 per cent) and much of the fat is lost during milling.

Milled rice contains a lipid level of approximately 1.1 g/100g or 5 per cent dry basic (Zhou et al., 2003; Kitta et al., 2005). In addition, most lipids in rice consists of long chain fatty acids such as linoleic (18:2) and linolenic (18:3) acids (Khongseri, 2003; Kitta et al., 2005). These lipids are absorbed on the surface of starch granules and bound inside (Zhou et al., 2003).

2.4.5. Amylose content

Starch, the nutritional reservoir in rice exists in two different forms: amylose, the unbranched type of starch with glucose residues with 1-4 linkage and amylopectin, the branched form with 1-4 and 1-6 cross linkage makes up the remaining part of the starch (Aberg, 1994).

Simi and Abraham (2008) evaluated the proportions of *Njavara* rice starch and revealed that it had bigger granules and showed high gelatinization temperature and higher thermal stability. The authors infer that the inherent high thermal and pasting properties make *Njavara* rice suitable as poultice for the body massage in the Panchakarma treatment.

Deepa et al. (2008) also reported that *Njavara* needed longer time to cook (38 min.) when compared to Jyothi and IR 64 (30 min.).

Amylose content is most important parameter to assess eating and cooking quality of rice (Das et al., 2005; Shi et al., 2005).

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

Dhanvantari Ayurveda Academy (www.dhanvantari.in) reported that starch grains of *Njavara* are multifaceted and compound with a grain size of 4.79 to 5.62 mm. The amylose content is 16.99 to 23.27 per cent and the amylase activity is 16.33 mg to 46.67 mg.

The amylose content determines flakiness/stickiness of cooked rice (Dey and Hussain, 2009). Rice with a high amylose content (25-30 per cent) (non-waxy-glutinous rice) tends to cook firm and dry, where as rice with an intermediate amylose content (20-25 per cent) tends to be safer and sticker and rice with a low amylose content (<20 per cent) is generally quite soft and sticky (Yadav et al., 2007).

2.5 Antioxidants

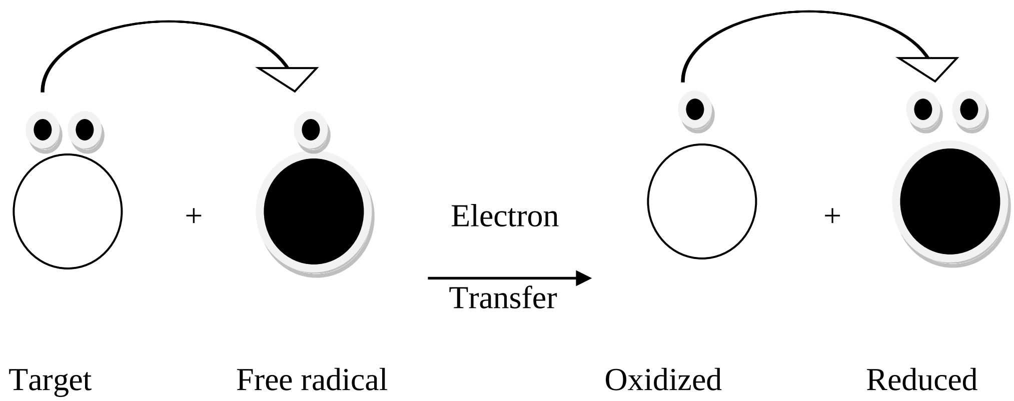
It is widely recognized that many of the today’s diseases are due to the oxidative stress that results from an imbalance between formation and neutralization of pro-oxidants (Hazra et al., 2008; Braca et al., 2002).

Cells have developed antioxidant mechanisms to quench the free radicals but when the generation of free radicals exceeds the scavenging capacity of the cell, the excess free radicals seek stability through electron paring with biological macro molecules such as proteins, lipids and DNA in healthy human cells resulting the induction of lipid peroxidation which leads to cancer, atherosclerosis, cardiovascular diseases (CVD), ageing and inflammatory diseases

(Hodzic et al., 2009; Lai et al., 2009; Aswatha et al., 2008;Devi and Arumughan, 2007).

2.5.1. Free radicals

In the structure of atoms and molecules, electrons usually associate in pairs, with each pair moving within a defined region of space around the nucleus. This space is referred to as an atomic or molecular orbital. One electron in each pair has a spin quantum number of +1/2, the other -1/2. A free radical is capable of independent existence (hence the term ‘free’) that contains one or more unpaired electrons, that is, one that is alone in the orbital (Halliwell, 1996). This situation is energetically unstable, making such species highly reactive and short lived. Stability is achieved by the removal of electrons from (i.e. oxidation of surrounding molecules to produce an electron pair:



However, the remainder of the attacked molecule then possesses an unpaired electron and has therefore become a free radical. In this way, the presence of a single radical is an atom of the element hydrogen, with one proton and a single electron. Other examples of free radicals are as follows

Name	**Formula**	**Description**
Hydrogen	$H^\bullet$	The simplest free radical.
Singlet O_2	$^1O^\bullet_2$	High energy and mutagenic form of O_2, can be produced b transfer from light, respiratory burst of neutrophil or lipid peroxidation.
Superoxide	$O^{-\bullet}_2$	An O_2 centered radical. Has limited reactivity.
Conjugated acid of superoxide	$HOO^\bullet$	Simplest form of peroxyl radical.
Hydroxide	$HO^\bullet$	Highly reactive O_2 centered radical. Very reactive indeed, attack all molecules of human body.
Hydroxyl radical	$^\bullet OH$	It is the neutral form of the hydroxide ion (OH^-). They are highly reactive and consequently short-lived (Montzka et al., 2011)

$^{\bullet}$Superscript dot is used to denote free radical species.

Free radicals disrupt the equilibrium of biological systems by damaging their major constituents molecules, leading eventually to cell death (Chotimarkkom et al., 2008) i.e.

1) Lipid peroxidation causing severe cell membrane function.
2) Protein oxidation causing fragmentation, cross-linking or aggregation, with the consequences of interference of ion channels, failure of cell receptors and failure of oxidative phosphorylation.
3) Oxidation of bases and/or deoxy sugars of DNA causing single or double strand breaks which have been implicated as a cause of mutagenesis, carcinogenesis and cell death.

2.5.2. Free radicals reaction

Free radicals are formed as a result of Lipid peroxidation (or autoxidation) (Dekkers et al., 2006). It is a chain reaction that proceeds in three stages (Halliwell and Gutteridge, 1995):

In the initiation phase (Reaction 1), carbon centered lipid radicals, $R^{\bullet}$, are produced by the abstraction from or addition to a polyunsaturated fatty acid (PUFA) RH of an oxy radical generated elsewhere.

I^{st} Stage: Initiation: Production of $R^{\bullet}$ (Carbon centered radical)……………..1

In the propagation phase (Reaction 2 and 3), the carbon centered radical reacts rapidly with molecular O_2 to form a peroxyl radical ($ROO^{\bullet}$), a chain carrying radical that is able to attack another polyunsaturated lipid molecule. Although the initial peroxyl radical is converted to a hydroperoxide (ROOH), this process produces a new carbon centered radical, which is rapidly converted by Reaction 2 into another peroxyl radical (Halliwell and Gutteridge, 1995).

II[nd] Stage: Propagation:

$R^{\bullet} + O2 \longrightarrow ROO^{\bullet}$ (fast)………………………………..2

$ROO^{\bullet} + RH \longrightarrow ROOH + R^{\bullet}$ (H atom abstraction)………..3

The propagative process continues and can become a runway process, consuming valuable polyunsaturated fat and producing a corresponding quantity of hydroperoxide (ROOH). The chain reaction does not stop until the chain-carrying peroxyl (ROO) radical meets and combines with another peroxyl radical to form inactive products (Reaction 4).

III[rd] Stage: Termination:

$ROO^{\bullet} + ROO^{\bullet} \longrightarrow$ inactive products………………………4

Ideally, this undesirable process can be prevented altogether by stopping the initial production of radicals. Compounds that do this are called antioxidants.

2.5.2. Significance of antioxidants

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. They are generally found in fruits and vegetables. They act as a defense system against oxidative damage in our bodies and may be helpful in avoiding chronic diseases and the effects of aging (Oliveri, 2000).

There are two types of antioxidants – preventive antioxidants and chain breaking antioxidants (Valko et al., 2006).

Preventive antioxidants will inhibit the initial production of free radicals. Free radicals scavenger enzyme system includes superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, catalase (Valko et al., 2006).

It reduces the rate of initiation of free radicals producing hydroperoxide products and chain breaking antioxidants scavenge free radicals (ROO, RO etc) to prevent continued H_2 abstraction. The most important antioxidant enzymes in the human cell are SOD, catalase and glutathionine peroxidase eliminates species involved in the initiation of free radical chain reaction (Sies, 1997)

Chain breaking antioxidants can inhibit the propagative phase. They include SOD, uric acid and Vit. E (Halliwell and Gutteridge, 1999).

Choi et al. (2007) and Adeolu et al. (2009) is of the opinion that free radicals are known to be scavenged by synthetic antioxidants, but due to their adverse side effects leading to carcinogenicity, search for effective and natural antioxidants has become crucial.

Geethakutty et al. (2004) described *Njavara* as a good antioxidant having high nutritive value.

The results of the study done by Rao et al. (2010) showed that *Njavara* rice bran contains significantly high polyphenolic compounds with superior antioxidant activity as evidenced by scavenging of free radicals including 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Nitric Oxide (NO).

The study also found that *Njavara* extracts showed highest reducing power activity, antiproliferative property in C6 glioma cells when compared with two commercially available basmati rice varieties; Vasumathi,Yamini and a non-medicinal variety, Jyothi.

Studies have shown that rice bran, which is a byproduct of rice milling contains a significant amount of natural phytochemicals including sterols, higher alcohols, γ- oryzanol, tocopherols, tocotrienols and phenolic compounds (Devi and Arumughan, 2007; Nam et al., 2006; Isao et al., 2004). These bioactive molecules have known to reduce serum cholesterol, decrease the incidence of atherosclerosis and also have antitumor properties (Deepa et al., 2008; Simi and Abraham, 2008; Itani and Ogawa, 2004; Halliwell, 1992).

A study conducted by Hong et al. (2009) found that "takju" a Korean traditional rice wine possesses a powerful radical scavenging activity against a variety of oxidative systems. The author also pointed out that "takju" should be considered a useful antioxidant, and its functional compound reduces oxidative stress.

2.6 Therapeutic value of *Njavara*

Rice varieties of therapeutic value have been known since ages from both ancient scriptures and personal experiences of people in the traditional rice growing regions of the world that exclusive rice varieties of health value existed and were in use (Siddiq, 2004).

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

Rice is used in the Ayurveda system of medicine for the treatment of a wide range of ailments, which includes indigestion, diabetes, arthritis, epilepsy, paralysis etc. In this system of medicine widely practiced in Kerala, a group of rice varieties known as *"Njavara"* finds an important place (Siddiq, 2004).

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.

The most important use of *Njavara* is in '*Njavarakizhi"* in which *Njavara* rice is the base. *'Njavarakizhi'* is used in the Ayurvedic treatment of various skeletal and muscular diseases, paralysis and sciatica (Nair et al., 2004).

Oleation to head and body using special cloth pieces containing a smooth paste of *Njavara* rice cooked in Sida (Sidarectusa. Lin) decoction and milk (*Njavarakizhi)*, makes the body supple, removes stiffness of joints due to various vitiated vatha dominant conditions, cleans the body channels, and brings about better blood circulation (Nair et al., 2004).

It also improves complexion, increases appetite, improves digestion and corrects mental irregularities. This makes body strong and steady, rejuvenated with well developed musculature. Judicious application of this is very effective in hypertension, skin diseases and prevents premature ageing (Nair et al., 2004).

Thus *Njavarakizhi* is both restorative and curative due to the nourishing quality of the ingredients. Though various fomentations belong to pre operative measures of Panchakarmas, this particular treatment has palliative quality and hence done as independent treatment (Vasudevan and Binitha, 2004).

Thomas et al. (2004) is of the opinion that *Njavara* is traditionally given as a supplementary diet to the underweight and also consumed as a replenishing drink called '*Karkadakakanji'* during the monsoon season along with certain other herbal medicines.

Studies by Menon and Potty (1999) revealed that this is a unique cereal having high content of free amino acids. It is assumed that sulphur containing methionine (a free amino acid) may be the constituent impairing medicinal property to this cultivar.

*'Shastikathilaka',*a preparation from the bran of *Njavara* is used to give glowing colour and brightness to the skin (Jayakuamr, 2003).

According to Swaminathan (2004) it is also useful in nervous diseases, body aches, numbness and wasted muscles due to polio myletes and motor neuron diseases.

Moos (2004) is of the opinion that *Njavara* can be used for diabetes, fever, cough, respiratory problems. It can also be used for de-worming. Porridge of *Njavara* grains in milk is traditionally given as a special food for invalids.

Some other special uses of *Njavara* reported from the experience of Ayurvedic practitioners are the following – psoriasis can be effectively controlled by the '*Lepanam'* or the application of *Njavara* rice paste. This rice paste is an excellent remedy to remove skin lesions (Geethakutty et al., 2004).

Another very potential use of *Njavara* rice reported is the use as a 'weight gainer' in the treatment of low weight babies. *Njavara* rice gruel prepared in meat soup is reported to increase the body weight of foetus and the general health of the expectant mothers.

According to ancient Ayurvedic records, indigenous preparation using *Njavara* along with "*Kurunthotty" (Sida rhombifolla var. retusa)* rejuvenates the muscles and nerves. Local healers claim that *Njavara* could solve urinary problems of children. *Njavara* rice increases mother's milk. *Njavara* flakes powdered with roots of Aswagandha (Withania somnifera) and sugar increases vigour, body weight and act as an aphrodisiac. *Njavara* is recommended as a safe food to diabetic patients (Hali, 2006).

Hali (2006) reported that local healers use *Njavara* for curing stomach ulcer and generate blood to wipe off anaemia. Mothers in families in some parts of Alappuzha prepare a special rub out of coconut and gingelly oil mix together with powdered *Njavara* grain and herbals like piper Longum boiled with equal quantities of milk from cow and buffalo to combat polio in children. Regular application of this rub help in building muscles in polio affected parts.

Njavara rice paste applications in pustules formed due to the biting of viper snake, reduce the pain. The pain is said to severe and lesions normally do not cure easily due to modern medicine. Application of warm rice paste twice a week cures burning sensation of foot.

Molecular studies conducted by a team of scientists from the KAU on *Njavara* have indicated the presence of a gene fragment encoding a protein which is reported to have anti-carcinogenic (chemo-preventive) property, especially against breast cancer (Shareesh, 2007). This protein, Christened Bowman-Birk, Trypsin Inhibitor protein, i.e. also known to possess anti inflammatory and anti allergic properties in animals.

Njavara rice is said to be safe food and thus recommended to feed new born babies in the form of a dish locally called '*Angri'* made of *Njavara* flour and dried powder made out of a banana variety called '*kunnan'* much before their first feeding ritual (MSSRF, 2005).

Regular cleansing of head with washed away water of *Njavara* rice one hour before bath is helpful in preventing hair fall. The ash made from *Njavara* husk is good for healing of small burns and cuts (Anilkumar, 2004).

MATERIALS & METHODS

3. MATERIALS AND METHODS

The present study entitled "Quality evaluation of medicinal rice (Oryza sativa L) cv. *Njavara* for product development and therapeutic value" was conducted in three experiments. First experiment encompasses an assessment of various parameters like physico-chemical characteristics, nutritional composition and antioxidant activity of two ecotypes of *Njavara* rice and *Hraswa* (KAU short duration variety - which was taken as control), evolved by Agricultural Research Stations located at Mannuthy, Thrissur of Kerala Agricultural University.

In the second experiment, a product was developed using both the ecotype of *Njavara* and its physico-chemical, nutritional composition, sensory and shelf life quality was also assessed. In the third experiment the developed product was tested for its therapeutic value viz. glycemic index and antioxidant properties.

3.1 Ist Experiment – Quality evaluation of selected rice varieties

3.1.1. Materials selected

Three rice varieties were selected for the study. The selected varieties are given in Table 1. (Plate 1)

Table 1. Rice varieties selected

Sl. No.	Varieties
1.	*Njavara* Black (NB)
2.	*Njavara* Yellow (NY)
3.	*Hraswa* (control)

Rice varieties were collected from Rice Research Station of KAU, Moncompu and ARS, Mannuthy, Thrissur and were processed into raw milled.

Njavara Black (NB)

Njavara Yellow (NY)

Hraswa (control variety)

Plate 1. Rice varieties selected

The raw milled rice of the three varieties was stored in airtight containers for undertaking various laboratory analyses.

3.1.2. Quality parameters selected

Rice grain quality is multi dimensional. It can be influenced by many parameters like physico-chemical characteristics, nutritional composition and antioxidant properties.

Different quality parameters studied on the materials were:

1. Physico-chemical characteristics
2. Nutritional composition
3. Antioxidant properties

Under each parameter, a number of indicators are reported to influence the quality of rice and are listed below.

3.1.2. (1). Physico-chemical characteristics

Physico-chemical characteristics of the rice grains were found to be a major determinant of quality and acceptability of rice. The indicators ascertained for evaluation of physical characteristics are

a. Moisture

Moisture content was estimated by the method of A.O.A.C (1990).

b. Size

For determining size, the rice samples were grouped into three classes i.e. extra long, long, medium and short according to the method given by IRRI (1996).

Extra long	-	> 7.5 mm
Long	-	6.6 – 7.5 mm
Medium	-	5.51 – 6.6 mm
Short	-	≤ 5.5 mm

c. Shape

For determining shape, the rice samples were classified into three groups i.e., slender, medium, bold and round grain according to the method suggested by IRRI (1996).

Slender	-	L/B ratio > 3.0
Medium	-	L/B ratio 2.1 – 3.0
Bold	-	L/B ratio 1.1 – 2.0
Round	-	L/B ratio < 1.0

d. Length and Width

Grain length has long been used in most rice breeding programmes as a characteristic for classifying rice varieties. Grain width is an important factor in determining the grain shape and weight. The grain length and width were determined by taking the length and width of ten grains randomly using a measuring scale/screw gauge.

e. Length- Breadth ratio (L/B ratio)

Length of the grain is measured in its greatest dimension, width along the ventral side. Length-breath (L/B) ratio of rice varieties were calculated as per the method outlined by Pillaiyar and Mohandoss (1981).

f. Colour

Colour of the rice varieties were ascertained by direct observation.

g. Thousand grain weight

Thousand grain weight of rice varieties were determined by weighing one thousand rice grains randomly selected (Sindhu et al., 1975).

h. Gel consistency

100mg of rice flour was taken in a test tube (2x19.5 cm), 0.2 ml of ethanol containing 0.25 per cent thymol blue and 2.0 ml of 0.2 N KOH were added and kept in boiling water bath for 8 minutes, cooled, mixed well and kept in ice bath for 20 minutes. Later the tubes were laid horizontally for one hour and measurements were made using graph paper (Cagampang et al., 1973).

i. Bulk density

Bulk density is the ratio of the mass to the volume of the sample or mass per unit volume, expressed as g/cc or Kg/L. Bulk density is used as an index for comparing the volume of different foods. The sample was taken to a height of 20 cm in a 50 ml beaker. It was levelled without compressing. The weight of the sample with the beaker and water was filled to the same level (20 ml). The weight of the water with beaker was recorded and calculated using the formula.

$$Bulk\ Density = \frac{Weight\ of\ the\ sample}{Weight\ of\ equivalent\ volume\ of\ water}$$

j. Gelatinization temperature

Gelatinization temperature was estimated following the method of Patindol et al. (2005).

k. Chalkiness index

A visual rating of the chalky proportion of the grain is used to measure chalkiness based on the Standard Evaluation System.

l. Viscography

Viscography of the samples were analysed using Brookfield Viscometer.

m. Hardness

Hardness of rice was measured by an Instrom Universal Food Testing Machine using an Ottawa Texture Measurement System Cell, with a cooking time of 20 minutes according to Rousset et al. (1995).

The hardness of rice was measured at the breakdown and crashing points by a tensiron meter.

3.1.2. (2). Nutritional Composition

The major nutrients analysed in the samples were listed below:

a. Energy

Energy or calorific value was estimated using Bomb Calorimeter as per the method described by Swaminathan (2004a).

b. Protein

The nitrogen content of samples was estimated by micro Kjeldahl's wet digestion method. The nitrogen values were multiplied by the factor 6.25 to get the crude protein content (A.O.A.C. 1970).

c. Evaluation of protein quality

The quality of proteinaceous food depends on its amino acid composition in relation to the protein content and digestibility (Ghosh and Chakravarthy, 1990). Amino acid composition may also serve as a good relative measure to compare with other food stuffs of established nutritive value.

The amino acid content of *Njavara* was estimated using through HPLC method. Total of 18 amino acids (including 8 essential amino acids) have been evaluated from the samples. Based on the essential amino content, Amino Acid Score (AAS), Essential Amino Acid Index (EAA Index), nutritional index were also computed.

AAS also known as chemical score is based on the amount of limiting amino acid present in the test protein in relation to its presence in reference protein. EAA calculations were done following the method as advanced by Oser (1959). AAS was calculated using the following formula:

$$\text{Amino Acid Score} = \frac{\text{mg of amino acid in 1 gm of test protien}}{\text{mg of amino acid in 1 gm of reference protein}} \times 100$$

EAA index is the ratio of essential amino acids contained in a food to the essential amino acid content in reference protein (Ghosh and Chakravarthy, 1990). The EAA index was computed using the formula given below

$$\text{E.A.A Index} = \frac{\text{Geometric mean of amino acid in the } \textit{Njavara}}{\text{Geometric mean of amino acid in reference protein}} \times 100$$

d. Crude fibre

For determination of fibre content, rice samples were digested with 1.25 per cent H_2SO_4 followed by 1.25 per cent NaOH solution and crude fibre content was determined according to A.A.C.C. method (2009).

e. Soluble fibre

Soluble fibre content was estimated by the method described by Raghuramalu et al. (1983).

f. Total starch

Starch content of the samples was estimated using anthrone reagent described by Sadasivam and Manickam (1992).

g. Amylose

The amylose content in the rice samples was determined using colorimetry method with iodine suggested by Juliano (1971).

h. Amylose-amylopectin ratio

Amylose-amylopectin ratio is the main factor for classifying rice into waxy and non-waxy. Amylose-amylopectin ratio of rice samples were estimated as per the method suggested by Mc Cready and Hassid (1943).

i. Total ash

Total ash content was estimated as per the method described by Raghuramalu et al. (1983).

j. Calcium (Ca)

Calcium was estimated by the method of Sadasivam and Manickam (1992).

k. Phosphorus (P)

Phosphorus was estimated by the method of Sadasivam and Manickam (1992).

l. B- Vitamins

B- Vitamins like thiamine and riboflavin content was estimated using Flurimetry method.

m. Total phenols

Method suggested by Sadasivam and Manickam (1992) were used for estimating total phenols.

n. Trace elements

A.O.A.C. (1990) methods were used for estimating trace elements like zinc (Zn), iron (Fe), manganese (Mn) and copper (Cu)

o. Selenium (Se)

The samples were powdered and digested in concentrated nitric acid in a microwave digester. Se content of the digest was estimated in AAS using hydride vapour generator.

3.1.2. (3). Antioxidant property

Antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. They act as a defense system against oxidative damage in our bodies and are helpful in avoiding chronic diseases and the effects of aging (Oliveri, 2000).

Njavara rice is regarded as a special rice variety with beneficial properties for the circulatory, respiratory, digestive and nervous systems according to the Indian indigenous system of medicine or Ayurveda (Goffman and Bergman, 2004). Antioxidant property was determined by the following methods.

a. Diphenyl Picryl Hydrazyl (DPPH) radical scavenging activity

Free radical scavenging activity of sample to characterize antioxidant activity was estimated as suggested by Blois (1958). Different amount of the methanolic extract of the sample was taken and DPPH (0.1 mM dissolved in methanol) was mixed together and the reaction mixture was left in dark room for 20 minutes. The absorbance was measured at 517 nm against the blank prepared by mixing DPPH and methanol. The antioxidant activity was expressed in terms of per cent inhibition of DPPH free radicals using the following equation:

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

Where, $Abs_{control}$ = absorbance of DPPH solution (blank) and Abs_{sample} = absorbance of sample. The IC_{50} of each sample (concentration in µg/ml required to inhibit DPPH radical formation by 50 per cent) has also been calculated.

b. Hydroxyl radical scavenging activity

In order to assess the hydroxyl free radical scavenging activity of the methanolic extracts of the rice samples, the deoxyribose method was used, as described by Halliwell et al. (1987), with some slight modifications. The reaction mixture contained phosphate buffer (20 mM, pH 7.4), 60 mM deoxyribose, 10mM Hydrogen peroxide, 1 mM ferric chloride, 1.04 mM EDTA, different amount of powdered samples and the final 2mM ascorbic acid. The reaction mixtures were incubated for 1hr. at 37°C, after which 17 mM trichloro acetic acid (TCA) was added. The mixture was then boiled for 15 minutes, ice cooled and measured for absorbance at 532 nm. Distilled water in lieu of extract was utilized as blank and the sample solution without added deoxyribose was used as a sample blank.

c. Superoxide anion radical scavenging activity

Superoxide anion scavenging activity was measured based on the method described by Robak and Gryglewski (2001). Superoxide radicals were generated in a PMS-NADH system via the oxidation of NADH and then assayed by the reduction of nitro blue tetrazolium (NBT). The superoxide radicals were generated in reaction mixture containing sodium phosphate buffer (100 mM, pH 7.4) containing 150µM NBT, 468 µM NADH solution in sodium phosphate buffer and different concentrations of methanolic extracts of the samples. To this 60 µM phenozine metho sulphite (PMS) solution was added. The reaction mixture was incubated for 5 minutes at 25°C and the absorbance was measured at 560 nm.

3.2 IInd Experiment: Quality evaluation of processed Njavara grits

The two ecotypes of *Njavara* was processed and converted into grits using pulveriser (Plate 2).

Plate 2. ***Njavara*** **grits**

The grits was powdered and then the parameters like

3.2.1 Physico-chemical characteristics

3.2.2. Nutritional composition

3.2.3. Antioxidant activity

The above parameters were assessed as per the procedures as done in first phase. Shelf life quality and sensory evaluation of Njavara grits was also done.

3.2.4. Shelf life quality

To assess the shelf-life stability, the product was kept sealed in a poly propylene cover and kept at ambient conditions. The sealed product was kept for six months and at the end of each month, the following parameters were assessed-

3.2.1.a. Moisture

Moisture content was estimated by the method of A.O.A.C (1990).

3.2.1.b. Peroxide value

Method suggested by Sadasivam and Manickam (1992) was used for estimating peroxide value.

3.2.1.c. Insect count

Insects are responsible for the quality deterioration of the product by spoilage and contamination with their faeces during the storage period. Insect infestation was examined at monthly intervals.

3.2.1.d. Microbial infestation

The stored *Njavara* rice grits was assessed for the presence of various micro-organisms that included bacteria, fungus and yeast after the storage period of three months. Serial dilutions of the samples followed by spread plating were employed to estimate the population of viable micro-organisms in the developed product (Johnson and Curl, 1972).

One gram of the *Njavara* grits was transferred aseptically to 9 ml sterile water blank and suspended thoroughly by mixing. Further progressive serial dilution up to 1000 fold of the suspension was done in sterile water by serially transferring 1ml samples each into 9 ml water blanks.

Nutrient agar (NA) and potato-dextrose agar (PDA) medium were used for culturing of bacteria and fungi respectively. Plates were poured and allowed for solidification. 0.1 ml of the suspension from each dilution was then transferred on to the solidified agar medium using a sterile pipette and spread evenly with a sterile glass spreader. The whole procedure was done aseptically in a laminar air flow chamber. Plates were then kept for incubation at room temperature. Colonies appearing in the plates were recorded after 2 days in the case of bacteria and after 4 days for fungi. The microbial load of the samples was then expressed as cfu/g.

3.2.5. Selection of Superior variety

The selection of superior rice variety was done based on nutritional qualities viz., energy, protein, crude fibre, soluble fibre, total starch, amylose, amylopectin, amylose-amlyopectin ratio, total ash, calcium, phosphorus, thiamine, riboflavin, total phenols and trace elements using discriminant function analysis.

3.3 IIIrd Experiment – Assessment of therapeutic value of *Njavara* grits through case studies

In order to assess the therapeutic value of *Njavara* grits, supplementation study was carried in which *Njavara* rice in grits and powered form was prepared in the laboratory and was given to selected human volunteers with diabetes mellitus.

3.3.1. Yield ratio and processing loss of *Njavara* grits

Yield ratio was calculated using the formula

$$Yield\,Ratio = \frac{Weight\ of\ husked\ rice\ (Kg)}{Weight\ of\ grits\ (Kg)}$$

Processing loss was calculated using the formula

$$\text{Processing Loss} = \frac{Weight\ of\ husked\ rice\ (Kg) - Weight\ of\ grits\ (Kg)}{Weight\ of\ husked\ rice\ (Kg)}$$

3.3.2. Formulation of recipes with *Njavara* grits

Various recipes were formulated in the laboratory incorporating the developed product in order to ensure proper inclusion of the developed product in the diet of selected human subjects.

Recipes like uppuma, kozukatta, oratti and porridge were formulated with *Njavara* grits (Plate 3).

3.3.3. Sensory evaluation

Sensory evaluation plays an important role in the quality of food products. Quality refers to degree of excellence and suitability for specific utility of the product. For judging consumer acceptability, sensory evaluation is essential.

Manay and Swamy (2002) opined that sensory evaluation plays a vital role in the food industry, because it represents a very unique technique that harness human behavioural instincts of perception, learning, cognition, psychophysics and psychometrics for the evaluation of food quality.

For conducting sensory evaluation, a panel of judges has to be selected, so as to ascertain consistent and accurate results.

a. Selection of judges

The panel members for sensory analysis at the laboratory level were selected from a group of teachers. These judges were selected through triangle test as suggested by Mahony (1985). Details are given in Appendix I.

b. Preparation of score card

The score card used for the evaluation of developed product is given in Appendix II.

Plate 3. Formulated recipes using *Njavara* grits

3.3.4. Conduct of case studies

For the conduct of the case studies, five human subjects who were diabetic but not on medication in the age group of 40-50 years and who were willing to participate were purposively selected through personal interview.

After the selection process, preliminary information regarding their socio-economic profile, health status, dietary and life style pattern and nutritional status were collected through a suitably structured questionnaire (Appendix III).

3.3.3.1) Socio-economic profile

In order to elicit information on socio-economic profile of the respondents details regarding age of the subjects, family income, type and size of the family, religion, educational status, money spent on food and health care etc. were collected using the questionnaire.

- Age

It refers to the number of calendar years completed by the respondents at the time of interview. This variable measured directly by asking the respondent the number of years he/she completed at the time of investigation (Sindhudevi, 1994).

- Family Income

Monthly family income from all sources was taken into account for measuring this variable.

- Family size

In the present study family size was measured by taking into consideration the specific number of members in the family of the respondents.

- Type of family

In this study family type means nuclear family or joint type family. Nuclear family consists of husband, wife and their unmarried children whereas joint family is composed of grand parents and their married children and married sons and daughters with their spouse.

- Educational status

It is defined as the formal education attained by the respondent (Jayalekshmi, 2001).

3.3.3.2) Dietary habit and life style pattern

Using the pre-tested questionnaire, the subjects' food habits and dietary pattern were collected.

In life style pattern, the subjects' personal habits like consumption of alcohol, smoking, stress and strain in the daily life, habit of doing exercise etc. were also collected.

3.3.3.3) Nutritional status

According to Kamath (1986) nutritional status is defined as the state of health enjoyed as a result of nutrition. It is one of the critical indicators of health, therefore regular nutritional assessment is important to maintain the health of respondents (Mourya and Jaya, 1997).

Nutritional status of the selected respondents was assessed through anthropometry. Anthropometry provides the single most universally applicable, inexpensive technique for assessing the size, proportions and composition of the human body. Anthropometry has been accepted as an important tool for the assessment of nutritional status (Vijayaraghavan, 1987).

Anthropometric measurements relevant to the study include height, weight and BMI.

- Height

Height or the total length apart from nutritional and environmental factors is influenced by hereditary factors. The extend of height deficit in relation to age as compared to regional standards is regarded as a measure of the duration of malnutrition (Gopaldas and Sheshadri, 1987).

To determine height, an anthropometric rod was used. The rod was fixed vertically on a smooth wall, perpendicular to the ground taking care to see that the floor was smooth.

The subjects were asked to remove their slippers and to stand with feet paralleled and heels, buttocks, shoulder and back of head touching the wall. The head was held comfortably erect, the arms hanging close by the side. A smooth, thin ruler was held on the top of the head in the centre crushing the hair at angles to the wall and the height read off from the lower edge of the ruler to the nearest 0.1 cms. An average of the three measurements was taken as final measurement of height of the respondents.

- Weight

Weight is the measurement of body mass (Rao and Vijayaraghavan, 1986). According to Kaul and Nyamongo (1990) a change in body weight may be the result of changes in the health of an individual, changes in food consumed or even changes in one's physical activity.

For weighing, platform weighing balance was used as it is portable and is convenient to use in the field. The weighing scale was checked periodically for accuracy. The scale was adjusted to zero before each measurement. The subjects having minimum clothing were asked to stand on the platform of the scale, without touching anything and looking straight ahead. The weight was recorded to the nearest of 0.5 Kg. Each reading was taken thrice to ensure correctness of the measurement.

- Body Mass Index (BMI)

BMI is expressed as the ratio of weight to height square i.e. Weight (Kg) / Height (m^2) was used as a good parameter to grade chronic energy deficiency (James et al., 1988).

$$BMI = \frac{Weight(Kg)}{Height(m^2)}$$

- Waist-Hip ratio

The Waist Hip Ratio (WHR) reflects the proportion of body fat located intradominally as opposed to that in the subcutaneous region (Lean et al., 1995). Waist was measured above the umbilicus meaning the narrowest circumference and hip was measured in the broadest area of hip. After documenting the waist and hip measurements of the respondents their WHR was calculated by dividing the circumference of the waist by the circumference of the hip (Chadha et al., 1995).

3.3.5. Conduct of feeding trail

The selected subjects were supplied the developed product for a period of three months based on their individual calorie requirement and disease condition (Appendix IV).

3.3.6. Assessment of the efficacy of the *Njavara* grits

The feeding trail was conducted for a period of 3 months to assess the efficacy of the *Njavara* grits on the blood glucose levels of the subjects. Blood glucose level of the subjects were taken initially (before the feeding trial), intermittently (after 45 years) and finally (after the feeding trial).

Along with the blood glucose level, glycemic index and antioxidant properties were also ascertained.

3.3.6.1. Glycemic index

3.3.6.1. a) Conduct of Glucose Tolerance Test (GTT) among the subjects

For the conduct of GTT, the subjects were asked to fast overnight. Blood samples were collected from the finger tips for determining their fasting blood glucose level (Plate 4). In order to get the glucose tolerance, the subsequent blood glucose was recorded at 15 minutes, 30 minutes, 45 minutes, 60 minutes, 90 minutes and 120 minutes. Procedure followed is appended (Appendix V).

3.3.6.1.b) Computation of glycemic index

Glycemic index of the test food (*Njavara* grits) was calculated as per the method suggested by Jenkins (1982).

Plate 4. Collection of blood sample

Each subject was given portions of test food (*Njavara* grits) and glucose containing 50g available carbohydrate on separate occasions after an overnight fasting.

Blood samples were taken from the finger tips before the administration of test food and glucose to access the fasting blood sugar level of the subjects. After the administration of test meal, blood glucose level of the subjects were analysed independently at half hour interval up to two hours (1/2 hr, 1 hr, 11/2 hr and 2 hr).

Blood glucose concentration of the subjects was determined by using a glucometer.

The response of the reference food mainly glucose and also that of the test food administered on the subjects were plotted, against time ‘t’.

The area under curve (AUC) thus obtained was found out from test food as well as for reference food (glucose). The Glycemic Index (GI) of test food is computed as the ration of AUC of test food and AUC of reference food.

$$G.I. = \frac{AUC(Test\ food)}{AUC(\text{Re}ference\ food)} x100$$

Using the glycemic index, glycemic load was also calculated. The formula used for finding out the glycemic load was

$$G.L. = \frac{G.I.X\ Available\ CHOH\ in\ the\ portion\ size}{AUC(\text{Re}ference\ food)} x100$$

3.3.6.2. Antioxidant properties

The following antioxidant properties of the blood samples (serum) were analysed;

a. DPPH radical scavenging activity: Method as followed in 3.1.2. (3) a (serum was taken as such instead of methanolic extract)

b. Hydroxyl radical scavenging activity: Method as followed in 3.1.2. (3) b (serum was taken as such instead of methanolic extract)

c. Superoxide anion radical scavenging activity: Method as followed in 3.1.2. (3) c (serum was taken as such instead of methanolic extract)

3.3.7. Statistical Analysis

In order to obtain meaningful results, the data generated was subjected to the following statistical analysis:

- ANOVA

It was used for determining the variances of treatment in their effect on the dependent variables.

- Discriminant Function Analysis

Discriminant function of Fisher (1936) is used to define the merit of rice varieties used in this study on the basis of nutritional composition, in terms of an index which is defined as selection index by Smith (1937) who used this index to indicate the genetic worthiness of a plant based on observable characters.

The genetic worthiness of variety based on a characters is linearly expressed as

$$H = a_1G_1 + a_1G_1 + \ldots\ldots\ldots\ldots.a_nG_n$$

Where a1+a2 ………an are the economic weights assigned to each variables. This merit cannot be evaluated directly because only the phenotypic or observable values are measured. The phenotypic value is linearly expressed as

$$I = b_1r_1 + \ldots\ldots\ldots b_n\ r_n$$

if n characters are involved.

The superiority of varieties using I as a discriminant function will ensure maximum gain provided the 'b' values are estimated in such a way that the correlation between I and H is maximum. The maximization of this correlation will yield to the simultaneous solution of a system of 'n' equations of the form.

$$\underline{X}\ \underline{b} = \underline{G}\ \underline{a}$$

Where $\underline{X}$ and $\underline{G}$ stands respectively for the phenotypic and genotic variance-co-variance matrices and $\underline{b} = \underline{X}^{-1}\ \underline{G}\ \underline{a}$

The mathematical description of I is known as selection index, this function will help to discriminate among the superior and inferior varieties. Those varieties with highest values are considered as superior ones.

RESULTS

4. RESULTS

The results of the present study entitled "Quality evaluation of medicinal rice (*Oryza sativa* L.) cv. *Njavara* for product development and therapeutic value" is presented under the following heads:-

4.1. Ist Experiment – Quality evaluation of selected rice varieties

4.1.1. Physico-chemical characteristics

4.1.2. Nutritional composition

4.1.3. Antioxidant properties

4.2. IInd Experiment – Quality evaluation of processed *Njavara* grits

4.1.1. Physico-chemical characteristics

4.1.2. Nutritional composition

4.1.3. Antioxidant properties

4.1.4. Shelf-life quality

4.1.5. Selection of superior variety

4.3. IIIrd Experiment – Assessment of the therapeutic value of *Njavara* grits through case studies

4.3.1. Formulation, sensory evaluation and suitability of *Njavara* grits

4.3.2. Results of case study

4.1. Ist Experiment – Quality evaluation of selected rice varieties

Three rice varieties viz. *Njavara* Black (NB), *Njavara* Yellow (NY) and *Hraswa* (control) were raw milled and their physico-chemical, nutritional and antioxidant properties were studied.

4.1.1. Physico-chemical characteristics

To determine the physico-chemical characteristics, colour, size, shape, length, breadth, L/B ratio, thousand grain weight, moisture percentage, gelatinization temperature, bulk density, gel consistency, viscography, chalkiness index and hardness of the rice varieties were studied.

Table 2 shows the colour, size and shape of the selected rice varieties.

Among the three varieties, all the varieties were found to be red in colour and medium in shape. Regarding the size, NB and *Hraswa* (control) variety was found to be medium and NY was found to be short.

Table 2. Colour, size and shape of selected rice varieties

Variety	Colour	Size	Shape
NB	Red	Medium	Medium
NY	Red	Short	Medium
Hraswa (Control)	Red	Medium	Medium

Table 3 presents the length, breadth and L/B ratio of the selected rice varieties. Length of the grain is measured in its greatest dimension, width along the ventral side and thickness across the dorsal side.

Table 3. Length, breadth and L/B ratio of selected rice varieties

Variety	Length (mm)	Breadth (mm)	L/B ratio
NB	5.60	2.12	2.65
NY	5.30	1.88	2.84
Hraswa (Control)	6.48	2.42	2.68
F-value	98.99**	26.78**	2.59
CD-value	0.019	0.016	0.190

** Significant at 1 % level

The result of the above table revealed that there was significant difference (at 1 per cent level) among the varieties for length and breadth but not for L/B ratio.

The length of *Njavara* varieties was found to be 5.60 mm and 5.30 mm where as length of *Hraswa* was found to be 6.48 mm.

The breadth of NB, NY and *Hraswa* was found to be 2.12 mm, 1.88 mm and 2.42 mm respectively.

The L/B ratio of *Njavara* varieties was 2.65 for NB and 2.83 for NY. The L/B ratio of control variety *Hraswa* was 2.68. Statistical analysis of the data revealed that the L/B ratio was not significant among the varieties.

The data on moisture percentage, thousand grain weight and gelatinization temperature is depicted in Table 4.

Table 4. Moisture percentage, thousand grain weight and gelatinization temperature of selected rice varieties

Variety	Moisture (%)	Thousand grain weight (g)	Gelatinization temperature (°C)
NB	11.60	16.40	85.60
NY	13.00	13.00	83.00
Hraswa (Control)	11.90	20.80	89.40
F-value	2.99	57.35**	74.02**
CD-value	1.313	1.591	1.153

** Significant at 1 % level

Moisture percentage of the rice varieties NB, NY and *Hraswa* (control) were 11.60, 13.00 and 11.90 respectively. Statistically there was no significant difference among the varieties.

Value for thousand grain weight was recorded highest for control variety i.e. 20.80 g, while thousand grain weight of *Njavara* varieties was 16.40 g for NB and 13.00 g for NY. Statistical analysis showed that there was a significant difference among the varieties regarding the thousand grain weight.

The gelatinization temperature for NB and NY were found to be 85.60 °C and 83.00 °C respectively while gelatinization temperature of control (*Hraswa*) was 89.40 °C. It was found that there was a significant difference at 1 per cent level among the varieties with respect to gelatinization temperature.

Table 5. Bulk density of selected rice varieties

Variety	Bulk density (g/ml)
NB	1.00
NY	0.99
Hraswa (Control)	0.96
F-value	15.84**
CD-value	0.016

** Significant at 1 % level

Data on bulk density (Table 5) revealed that there was significant difference among the varieties at 1 per cent level. The bulk density of NB and NY were 1.00 g/ml and 0.99 g/ml respectively where as the values of control variety *Hraswa* was 0.96 g/ml.

Table 6 depicts the data on gel consistency.

Table 6. Gel consistency of selected rice varieties

Variety	Gel consistency (mm)	
	30 min	**60 min**
NB	54.40	56.60
NY	54.00	55.60
Hraswa (Control)	49.40	51.40
F-value	55.16**	33.58**
CD-value	1.152	1.467

** Significant at 1 % level

The gel consistency for 30 minutes and 60 minutes in NB and NY were 54.40 mm and 56.60 mm and 54.00 mm and 55.60 mm respectively. The control variety *Hraswa* had 49.40 mm and 51.40 mm for 30 minutes and 60 minutes respectively. The data revealed that there was a significant difference at 1 per cent level.

Table 7 shows the data of chalkiness index, viscography and hardness of selected rice varieties.

Table 7. Chalkiness index, viscography and hardness of selected rice varieties

Variety	Chalkiness index	Viscography (pb)	Hardness (Kg)
NB	0.05	16.80	3.72
NY	5.02	11.60	3.08
Hraswa (Control)	3.21	10.00	4.68

Table 7 depicts the chalkiness index of the selected rice varieties. NB had the lowest chalkiness index of 0.05, while NY had the highest chalkiness index of 5.02. Control variety *Hraswa* had a chalkiness index of 3.21.

Data on values of viscography of rice varieties showed that NB was having highest value of viscography (16.80 pb), while NY was having 11.60 pb and control variety *Hraswa* was having 10.00 pb.

In the present study, it was found that, *Hraswa* (control variety) got the highest hardness, with a value of 4.68 Kg, while NB and NY had 3.72 Kg and 3.08 Kg respectively (Table 7).

4.1.2. Nutritional composition

To assess the chemical/nutritional composition of the selected rice varieties, the following parameters were determined i.e. energy, protein, crude fibre, soluble fibre, total starch, amylose, amylopectin, amylose-amlyopectin ratio, total ash, calcium, phosphorus, thiamine, riboflavin, total phenols and trace elements.

Table 8 presents the energy content of selected rice varieties.

Table 8. Energy content of selected rice varieties

Variety	Energy content (Kcal)
NB	323.00
NY	357.00
Hraswa (Control)	336.00

It can be understood from table 8 that energy value was highest for NY (357.00 Kcal) and lowest for NB (323.00 Kcal), where as *Hraswa* had an energy content of 336.00 Kcal/100 g of rice.

Protein content of the rice varieties are depicted in Table 9.

Table 9. Protein content of selected rice varieties

Variety	Protein (g)
NB	12.15
NY	11.80
Hraswa (Control)	8.77
F-value	82.64**
CD-value	0.630

** Significant at 1 % level

Table 9 showed that there was a significant difference between the protein content of three rice varieties. NB had a protein content of 12.15 g, while NY and *Hraswa* had 11.80 g and 8.77 g protein per 100 g of rice respectively.

Evaluation of protein quality

The nutritive value of a protein depends to an important degree on the relation of amino acids in its molecules to those required for building new tissues. If the amino acid composition of a food substance meets the amino acid composition of a tissue, the food protein is of a high quality.

In the present study, to evaluate the protein quality of *Njavara* rice, 18 amino acids were estimated and the results are depicted in Fig. 1.

Fig. 1 shows the comparison of essential amino acids of raw *Njavara* rice samples with reference protein i.e. egg. The figure depicts that for *Njavara* rice varieties, except methionine and lysine, all other amino acid contents were on par with the reference protein. In the case of phenylalanine, it was above the value of reference protein.

The figure also depicted that values of amino acids like threonine, valine, methionine and isoleucine of *Hraswa* (control variety) was lower than the values of raw *Njavara* rice samples. Whereas, leucine, phenylalanine, lysine and tryptophan values of *Hraswa* were higher than *Njavara* black and yellow varieties.

Apart from the essential amino acid content, non essential amino acid content were also determined and are given in table 10.

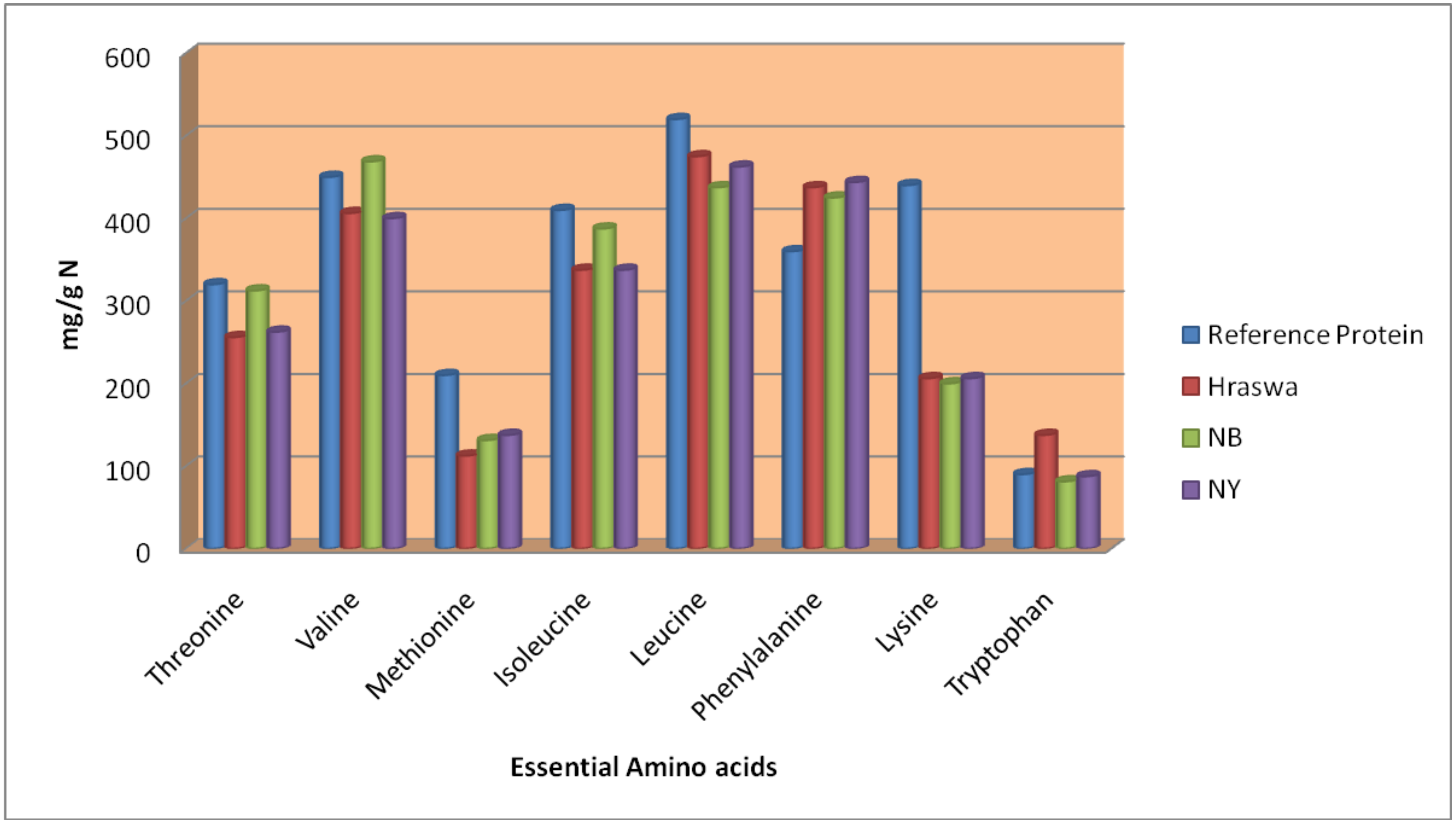


Fig. 1. Comparison of essential amino acids of raw *Njavara* rice samples with control (*Hraswa)* and reference (egg)

Table 10. Non-essential amino acid content of selected rice varieties

Sl.No.	Non-essential amino acids	mg/g N		
		NB	**NY**	***Hraswa***
1.	Aspartic acid	625.00	625.00	625.00
2.	Serine	337.50	375.00	381.25
3.	Glutamic acid	1181.25	1056.25	1056.25
4.	Proline	418.75	475.00	475.00
5.	Glycine	206.25	156.25	150.00
6.	Alanine	275.00	400.00	387.50
7.	Cysteine	-	-	-
8.	Tyrosine	175.55	206.25	212.50
9.	Histidine	425.00	406.25	406.25
10.	Arginine	406.25	418.75	418.75

Table 10 revealed that highest amount of non-essential amino acids present in the rice samples were glutamic acid i.e. 1181.25 mg/g N in NB, 1056.25 mg/g N in NY and *Hraswa* (control variety), followed by aspartic acid (625.00 mg/g N) and proline (418.75 mg/g N in NB and 475.00 mg/g N in NY and *Hraswa* control variety).

To further investigate the protein quality of the rice samples, Amino Acid Score (AAS) was also determined and the values are given in table 11.

Table 11. Amino Acid Score of selected rice varieties

Sl.No.	Amino acids	Amino Acid Score (AAS)		
		NB	**NY**	***Hraswa***
1.	Lysine	45.45	46.88	46.88
2.	Tryptophan	90.28	97.22	152.78
3.	Phenylalanine	117.50	123.26	121.53
4.	Methionine	62.50	65.48	53.57
5.	Threonine	97.65	82.03	80.00
6.	Leucine	84.13	88.94	91.35
7.	Isoleucine	94.51	82.32	82.32
8.	Valine	104.16	88.89	90.28
Limiting amino acids		Lysine and Methionine	Lysine and Methionine	Lysine and Methionine

The above table revealed that among the three rice varieties the amino acid score of phenylalanine (117.50), valine (104.16) and threonine (97.65) were highest for NB, whereas Amino Acid Score of phenylalanine (123.26), tryptophan (97.22) and leucine (88.94) were highest for NY.

In *Hraswa*, the highest Amino Acid Scores were found in tryptophan (152.78), phenylalanine (121.53) and leucine (91.35).

Among the *Njavara* rice varieties, NY had more individual number of amino acid score than NB.

The Essential Amino Acid Index (EAA Index) of selected rice varieties was also computed and depicted in table 12.

Table 12. EAA Index of selected rice varieties

Variety	E.A.A Index
NB	26.81
NY	26.12
Hraswa	26.95

The EAA index of control variety *Hraswa* was found to be high, when compared to NB and NY.

The values of crude fibre and soluble fibre are given in table 13.

Table 13. Crude fibre and soluble fibre content of selected rice varieties

Variety	Crude fibre (g)	Soluble fibre (g)
NB	0.206	0.114
NY	0.198	0.096
Hraswa(Control)	0.190	0.056
F- values	3.84*	55.08**
CD values	0.013	0.012

** Significant at 1 % level * Significant at 5 % level

It was evident from Table 13 that the crude fibre content of the rice varieties was significantly different. Among the three rice varieties, NB had the highest value of crude fibre (0.206g) and variety *Hraswa* (control) had the lowest value (0.190 g).

Soluble fibre content of the rice varieties varied significantly (Table 13). The soluble fibre content of NB was 0.114 g and for NY it was 0.09 g and for *Hraswa* it was 0.056 g.

Data on total starch and amylose content are presented in Table 14.

Table 14. Total starch and amylose content of selected rice varieties

Variety	**Starch (g)**	**Amylose (g)**
NB	76.14	21.64
NY	74.45	24.37
Hraswa(Control)	75.81	24.81
F- values	4.24*	24.12**
CD values	1.340	1.077

** Significant at 1 % level

In this study, the highest starch content was noticed in control variety NB (76.14 per cent), where as NY and *Hraswa* (control variety) contained 74.45 per cent and 75.81 per cent respectively. The data when analysed statistically revealed a significant difference among the varieties for starch content (F=4.24*).

Amylose content varied significantly among the selected rice varieties (Table 14). The highest amylose content was found in control variety *Hraswa* (24.81), while NB (21.64 per cent) was found to have the lowest amylose content.

Table 15. Amylopectin and amylose-amylopectin ratio of selected rice varieties

Variety	Amylopectin (%)	Amylose-amylopectin ratio
NB	54.50	0.39
NY	50.08	0.49
Hraswa(Control)	51.00	0.49
F- values	16.43**	22.38**
CD values	1.771	0.033

** Significant at 1 % level

The amylopectin content of the rice varieties of NB, NY and *Hraswa* were 54.50 per cent, 50.08 per cent and 51.00 per cent respectively. Statistically there was a significant difference at 1 per cent level between the varieties (Table 15).

The amylose-amylopectin ratio of the rice varieties also had a significant difference at 1 per cent level (F=22.38 **). The mean values of amylose-amylopectin ratio are 0.39 in NB and 0.49 in NY and *Hraswa* (control variety).

Total ash, calcium and phosphorus content of the rice varieties were estimated and are depicted in Table 16.

Table 16. Total ash, Calcium and Phosphorus content of selected rice varieties

Variety	Total ash (%)	Calcium (mg/100g)	Phosphorus(mg/100g)
NB	1.52	12.80	352.40
NY	1.26	12.20	351.40
Hraswa(Control)	1.26	10.80	135.40
F- values	12.07**	6.08*	12732.9*
CD values	0.133	1.283	3.413

** Significant at 1 % level * Significant at 5 % level

The statistical analysis of the data revealed that there exists a significant difference in total ash content (F=12.07**). Total ash content in *Hraswa* and NY was found to be same i.e. 1.26 per cent but in NB the ash content was found to be higher than the other two varieties i.e. 1.52 per cent.

Calcium content of the rice varieties had significant difference at 1 per cent level. Calcium content was found lowest in control variety *Hraswa* (10.80 mg/100 g) and highest in NB (12.80 mg/100 g).

There was a significant difference in phosphorus content of rice varieties. NB had highest value of 352.40 mg/100g where as control variety *Hraswa* had the lowest value of phosphorus i.e. 135.40 mg/100 g of rice.

Table 17 shows the thiamine and riboflavin content of rice varieties.

Table 17. Thiamine and riboflavin content of selected rice varieties

Variety	Thiamine (mg/100g)	Riboflavin(mg/100g)
NB	0.98	0.051
NY	0.58	0.048
Hraswa(Control)	0.40	0.047

Table 17 depicts the B complex vitamin content of the rice varieties. The figure revealed that NB had highest thiamine content (0.98 mg/100g) and *Hraswa* had the lowest thiamine content of 0.40 mg/100 g of rice.

Riboflavin content of the rice varieties showed that *Njavara* Black (NB) had the highest value (0.051 mg/100g), where as NY and control variety *Hraswa* contained 0.048 mg and 0.047 mg of riboflavin respectively per 100 g of rice.

Total phenol content of the rice varieties was estimated and the results are presented in Table 18.

Table 18. Total Phenol content of selected rice varieties

Variety	Total phenol content (mg/100g)
NB	0.41
NY	0.29
Hraswa(Control)	0.31
F- values	17.55**
CD values	0.079

** Significant at 1 % level

The mean total phenol content of the rice varieties NB, NY and control variety *Hraswa* were 0.41 mg, 0.29 mg and 0.31 mg/100g of rice respectively.

When statistically analysed, it was noticed that there was a significant difference among the rice varieties (F=17.55**).

Trace elements such as Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu) and Selenium (Se) were determined (Table 19) and the results revealed that control variety *Hraswa* had the highest value among all the trace elements.

Table 19. Trace element content of selected rice varieties

Variety	Fe (mg/L)	Mn (mg/L)	Zn (mg/L)	Cu (mg/L)	Se (µg/ml)
NB	0.36	0.75	0.88	1.53	4.73
NY	0.36	0.83	0.90	1.30	2.22
Hraswa (Control)	0.36	1.22	0.90	1.55	7.41

In the case of NB and NY, Fe content was equal in both the varieties, but in NY, Mn and Zn content was slightly higher than NB and in the case of Cu. NB had higher value compared to NY.

From table 19 it can be observed that *Hraswa* (control variety) had the highest Se content of 7.41µg/ml, while NB had 4.73 µg/ml and NY 2.22 µg/ml which had the lowest Se content among the three rice varieties.

4.1.3. Antioxidant properties

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions (Sies, 1997).

4.1.3. a) DPPH radical scavenging activity

The DPPH radical scavenging activities among the three rice varieties are shown in Fig. 2. The IC_{50} value was calculated from the graph (it was noted as how much is the concentration of sample needed to scavenge the free radicals at 50 per cent inhibition).

The results revealed that *Njavara* Yellow had the highest DPPH activity with an IC_{50} value of 31.62 µg/ml (i.e. 31.62 µg/ml of Njavara yellow is needed to scavenge the free radicals of DPPH), followed by NB (IC_{50} value 33.73 µg/ml) and *Hraswa* (control) variety (IC_{50} value 52.39 µg/ml). IC_{50} value of positive control ascorbate was 3.90 µg/ml.

4.1.3. b) Hydroxyl radical scavenging activity

The assay is based on quantification of the degradation product of 2-deoxyribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-ascorbate-EDTA-H_2O_2 system (the Fenton reaction) (Elizabeth and Rao, 1990).

The hydroxyl radical scavenging activities of *Njavara* and *Hraswa* are shown in Fig. 3. In this study, the hydroxyl radical scavenging activities of *Njavara* Yellow was found to be the highest with an IC_{50} value of 46 µg/ml, followed by NB with an IC_{50} value of 55.68 µg/ml and *Hraswa* (control) variety with an IC_{50} value of 60.78 µg/ml. Ascorbic acid was used as reference compound and its IC_{50} value was 3.90 µg/ml.

4.1.3. c) Super oxide anion-radical scavenging activity

The super oxide anion-radical scavenging activity of the selected rice varieties are shown in Fig. 4.

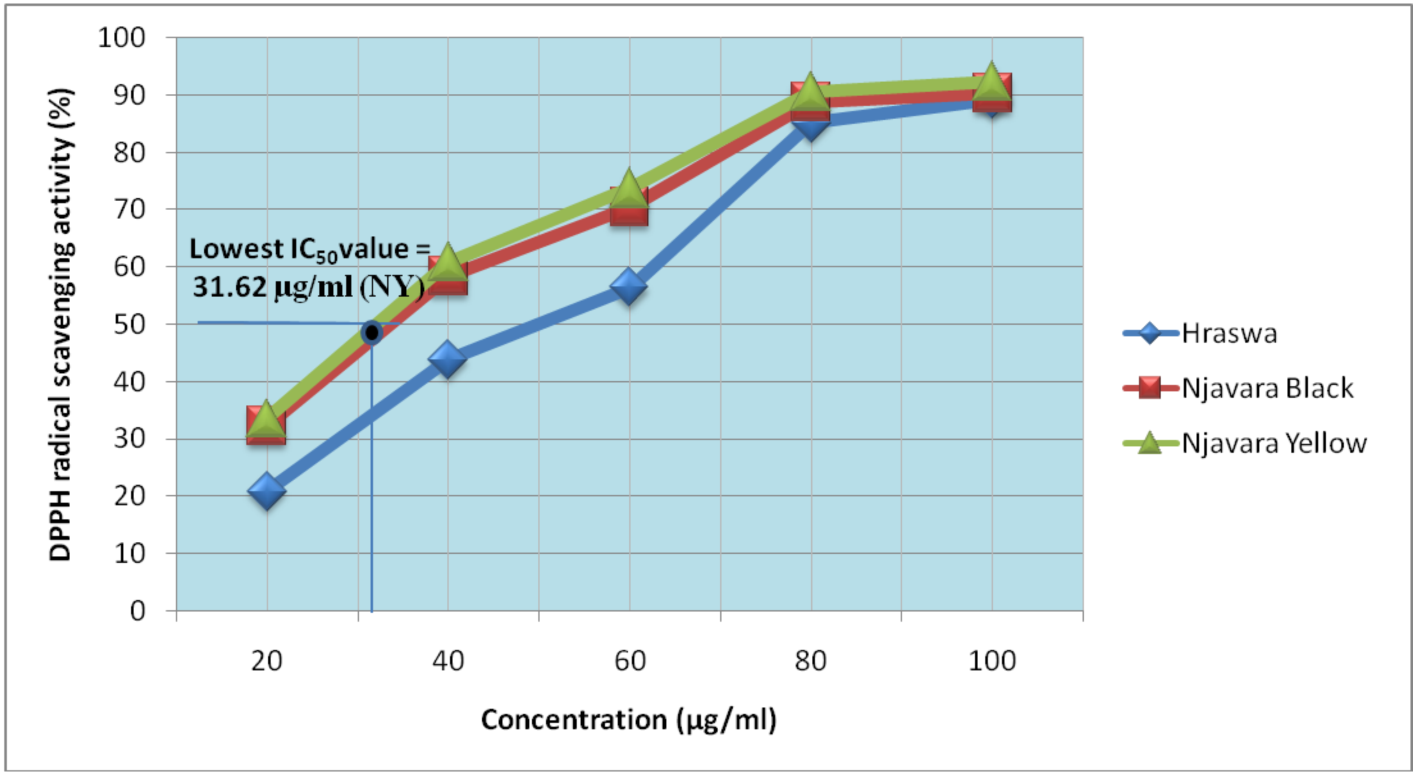


Fig. 2. DPPH radical scavenging activity of selected rice varieties

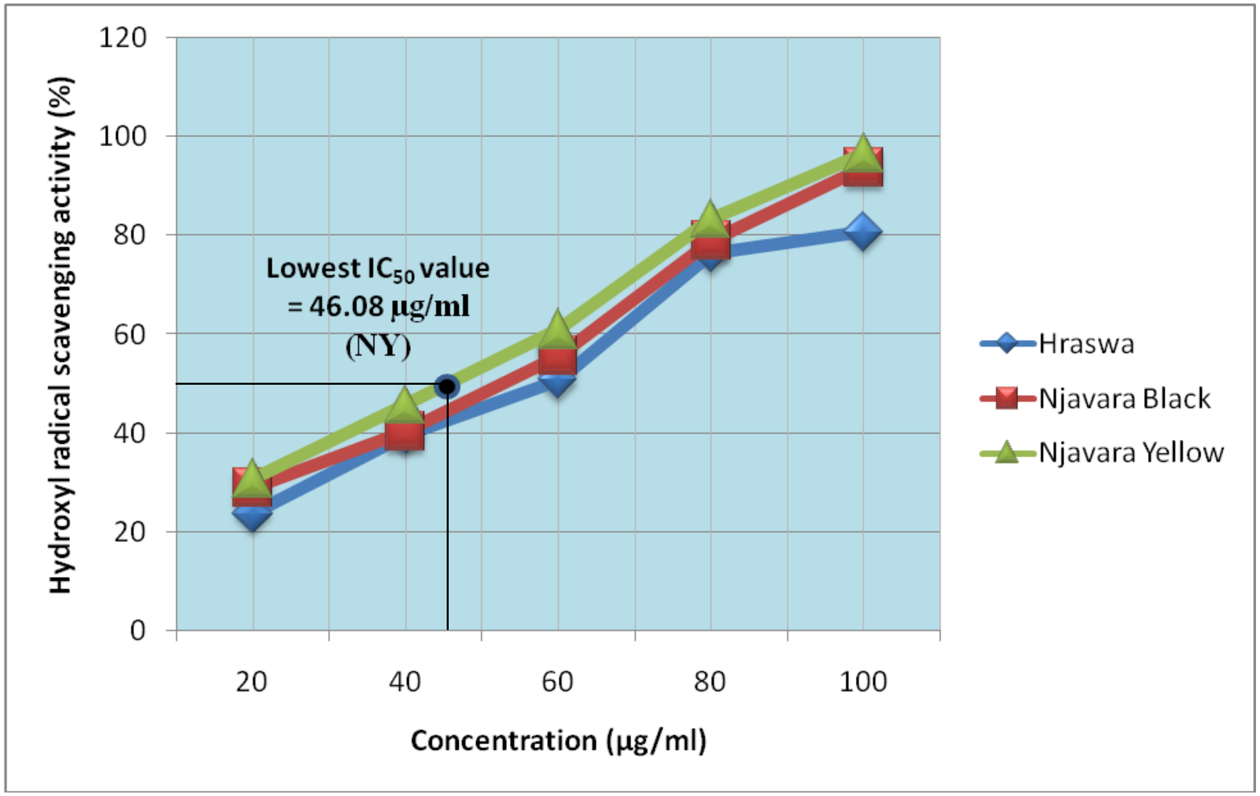


Fig. 3 Hydroxyl radical scavenging activity of selected rice varieties

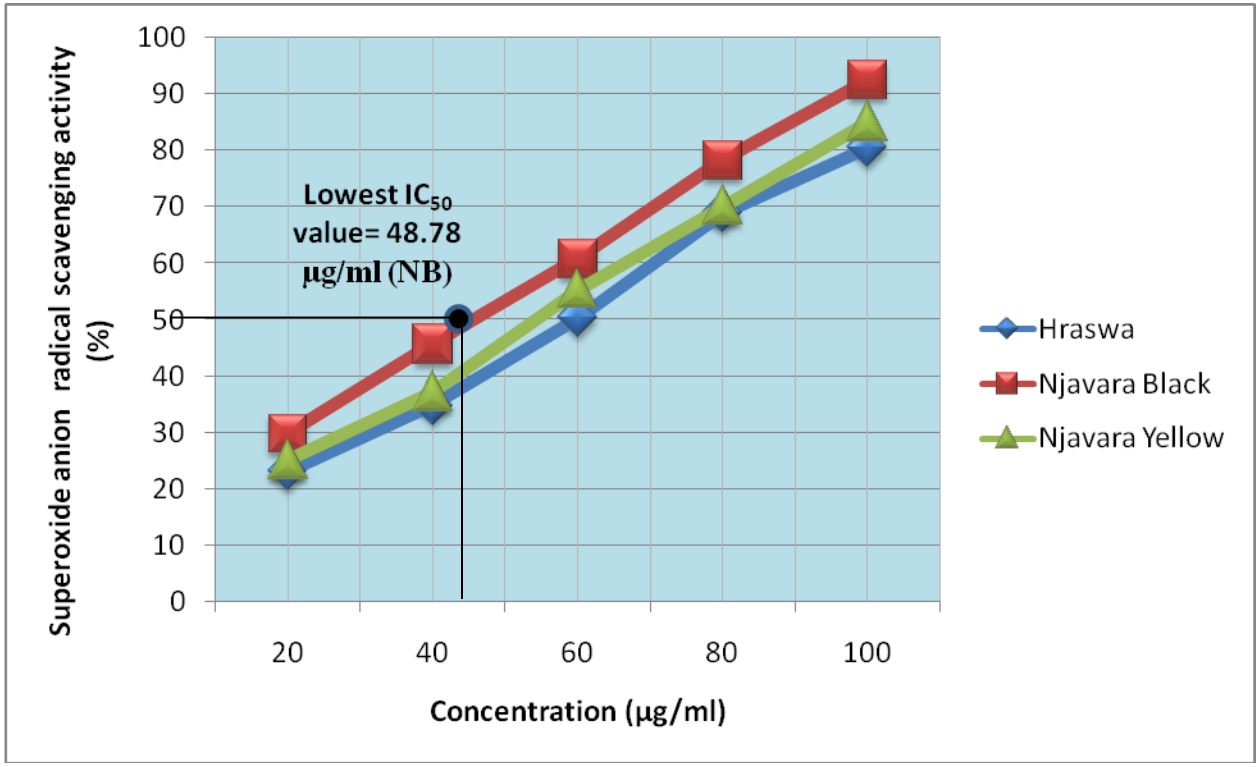


Fig.4 Superoxide anion radical scavenging activity of selected rice varieties

The results revealed that *Njavara* black (IC_{50} value = 48.78 µg/ml) showed a higher superoxide radical activity compared to *Njavara* yellow (IC_{50} value = 55.43 µg/ml). Among the three rice varieties, *Hraswa* (control) showed lowest superoxide activity (IC_{50} value = 60 µg/ml). IC_{50} value of control was 3.90 µg/ml.

4.2. IInd Experiment – Quality evaluation of *Njavara* grits

In the second experiment, the two ecotypes of *Njavara* varieties i.e. *Njavara* Black (NB) and *Njavara* Yellow (NY) was parboiled and converted to grits form using a pulveriser. The grits was powdered and was used for various analyses.

The grits from NB was designated as *Njavara* Black grits [NB (G)] and from NY designated as *Njavara* Yellow grits [NY (G)].

The grits were assessed for the following quality parameters:- physical, chemical/nutritional composition, antioxidant activity, shelf-life quality and sensory evaluation and the results were compared with raw *Njavara* varieties.

4.1.1. Physico-chemical characteristics

To determine the physical characteristics; moisture percentage, gelatinization temperature, bulk density, gel consistency and viscography of the grits were studied.

Table 20 shows the moisture percentage and gelatinization temperature of the grits.

Table 20. Moisture percentage and gelatinization temperature of *Njavara* rice varieties and *Njavara* grits

Variety	**Moisture (%)**			**Gelatinization temperature (°C)**		
	Raw	**Grits**	**Mean**	**Raw**	**Grits**	**Mean**
NB	11.60	15.36	13.48	85.60	88.00	86.60
NY	13.00	15.12	14.06	83.00	88.40	85.70
Mean	12.30	15.24		84.30	88.20	

	CD-Values	F-Values	CD-Values	F-Values
Varieties	0.777	2.51	1.071	4.74*
Treatments	0.777	64.38**	1.071	59.63**
VxT	1.098	5.01*	1.514	8.82**

** Significant at 1 % level * Significant at 5 % level

Table 20 revealed that *Njavara* grits had a higher moisture percentage compared to raw *Njavara* form. The moisture percentage increased from 11.60 to 15.36 in NB, whereas in NY it has increased from 13.00 to 15.12 per cent. Statistical analysis revealed that there was a significant increase in the moisture percentage at 1 per cent level after it has converted into grits form.

A significant increase in the gelatinization temperature was also noticed in the case of *Njavara* grits (F=59.626**).

Table 21 shows the data on bulk density of *Njavara* grits.

Table 21. Bulk density of *Njavara* rice varieties and *Njavara* grits

Variety	Bulk density (g/ml)		
	Raw	**Grits**	**Mean**
NB	1.00	0.99	0.99
NY	0.99	0.98	0.99
Mean	0.99	0.99	

	CD- Values	F-Values
Varieties (V)	0.015	1.51
Treatments (T)	0.015	1.64
VxT	0.022	0.24

Results of bulk density showed that there was a slight decrease in the bulk density values compared to raw form in both the varieties, but statistically it was not found to be significant.

Table 22 depicts the data on gel consistency of *Njavara* rice varieties and *Njavara* grits.

Table 22. Gel consistency of *Njavara* rice varieties and *Njavara* grits

Variety	Gel consistency (mm)					
	Raw	**Grits**		**Raw**	**Grits**	
	30 min	30 min	Mean	60 min	60 min	Mean
NB	54.40	70.20	62.30	56.60	71.40	64.00
NY	54.00	56.40	55.20	55.60	58.20	56.90
Mean	54.20	63.30		56.10	64.80	
	CD –Values	F-Values		CD –Values	F-Values	
Varieties (V)	2.027	55.09**		2.178	47.78**	
Treatments (T)	2.027	90.49**		2.178	71.75**	
VxT	2.868	49.06**		3.079	35.27**	

** Significant at 1 % level

Gel consistency values showed that there was a significant increase for both *Njavara* varieties in 30 minutes and 60 minutes. The value for NB in 30 minutes was 54.40 mm in raw form whereas in grits form, it increased to 70.20 mm and in NY the value increased from 54.00 mm to 56.40 mm in 30 minutes.

For 60 minutes, the values were 56.60 mm for raw NB and it increased to 71.40 mm in grits form. In NY, the raw value was 55.60 mm and for grits form it was 58.20 mm.

Table 23 shows the data on chalkiness index of *Njavara* rice varieties and *Njavara* grits

Table 23. Viscography of *Njavara* rice varieties and *Njavara* grits

Variety	Viscography (pb)	
	Raw	Grits
NB	16.80	24.00
NY	11.60	23.60

The results proved that NB had highest value for viscography in both raw and grits form.

4.2.2. Nutritional composition

To assess the chemical/nutritional composition of the grits, the same parameters as in the case of 1st experiment was determined.

Fig.5 depicts the energy content of both raw *Njavara* rice varieties and *Njavara* grits. The figure revealed that energy content of NY (G) was higher than NB (G) with a value of 364.00 Kcal for NY (G) and 342.00 for NB (G).

Processing of *Njavara* rice into grit form was found to increase the calorific value of rice varieties. The value for *Njavara* Yellow was increased from 357.00 Kcal to 364.00 Kcal and for *Njavara* Black from 323.00 Kcal to 342.00 Kcal.

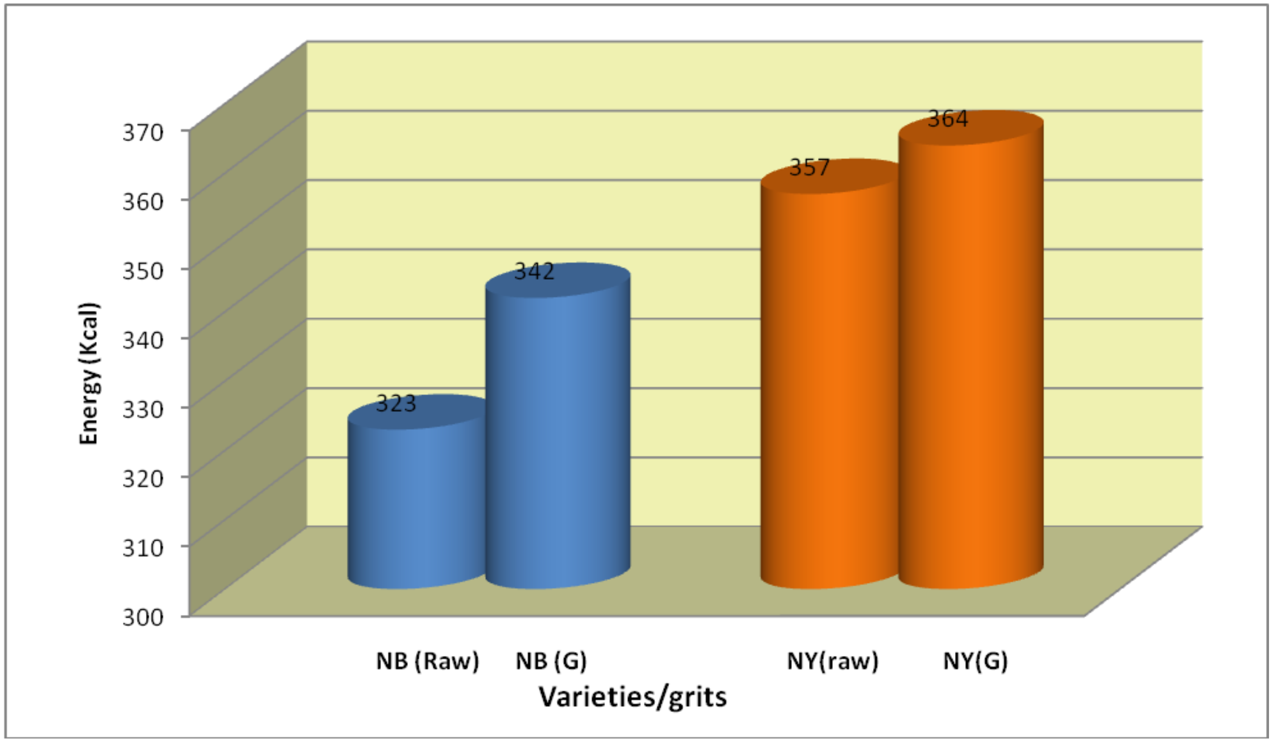


Fig.5 Comparison of energy content of raw *Njavara* rice varieties and *Njavara* grit

Table 24 depicts the protein content of raw *Njavara* rice varieties and processed *Njavara* grits. The results revealed that there was a significant decrease in the protein values in both the samples (F-value of 83.126**).

The mean protein value of raw NB was 12.15g/100g rice, whereas after it was converted into grits the value decreased to 8.05g. In the case of raw NY, the mean value was 11.8 g but in grits form it was 8.75 g/100 g of rice.

Table 24. Protein content of raw *Njavara* rice varieties and *Njavara* grits

Variety	**Protein (g)**		
	Raw	**Processed (grits)**	**Mean**
Njavara Black (NB)	12.15	8.05	10.10
Njavara Yellow (NY)	11.80	8.75	10.27
Mean	11.97	8.40	

	CD- Values	F-Values
Varieties (V)	0.831	0.19
Treatments (T)	0.831	83.13**
VxT	1.176	1.79

** Significant at 1 % level

Evaluation of protein quality

The protein quality of processed *Njavara* grits was evaluated by estimating both essential and non-essential amino acids.

Fig. 6 and Fig. 7 show the comparison of essential amino acid content of raw *Njavara* rice varieties and *Njavara* grits.

Both the figures depicted that values of essential amino acids of *Njavara* grits were found to be lower than that of raw *Njavara* rice varieties.

Table 25. Non-essential amino acid content of *Njavara* grits

Sl.No.	Amino acids	mg/g N	
		NB (G)	NY (G)
1.	Aspartic acid	637.50	593.75
2.	Serine	356.25	325.00
3.	Glutamic acid	1381.25	126.25
4.	Proline	131.25	81.25
5.	Glycine	137.50	125.00
6.	Alanine	325.00	293.75
7.	Cysteine	-	-
8.	Tyrosine	106.25	50.00
9.	Histidine	350.00	325.00
10.	Arginine	412.50	931.25

Table 25 revealed that NB (G) had high amount of non-essential amino acids except arginine in comparison with NY (G).

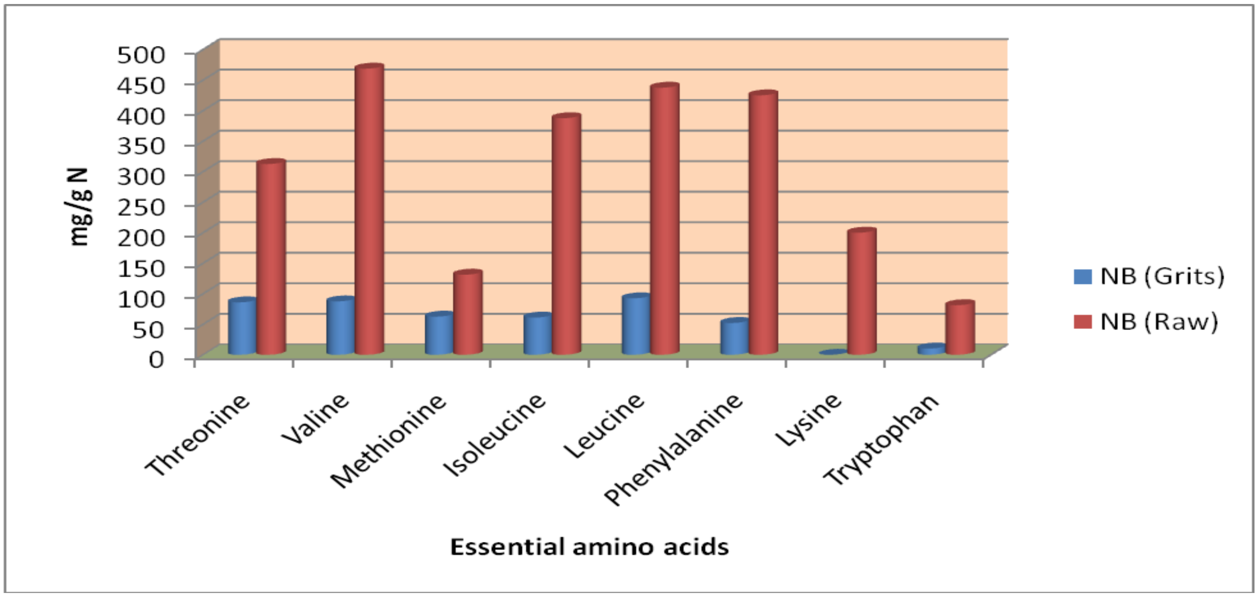


Fig. 6 Comparison of essential amino acid contents of *Njavara* Black (grits) with *Njavara* Black (raw)

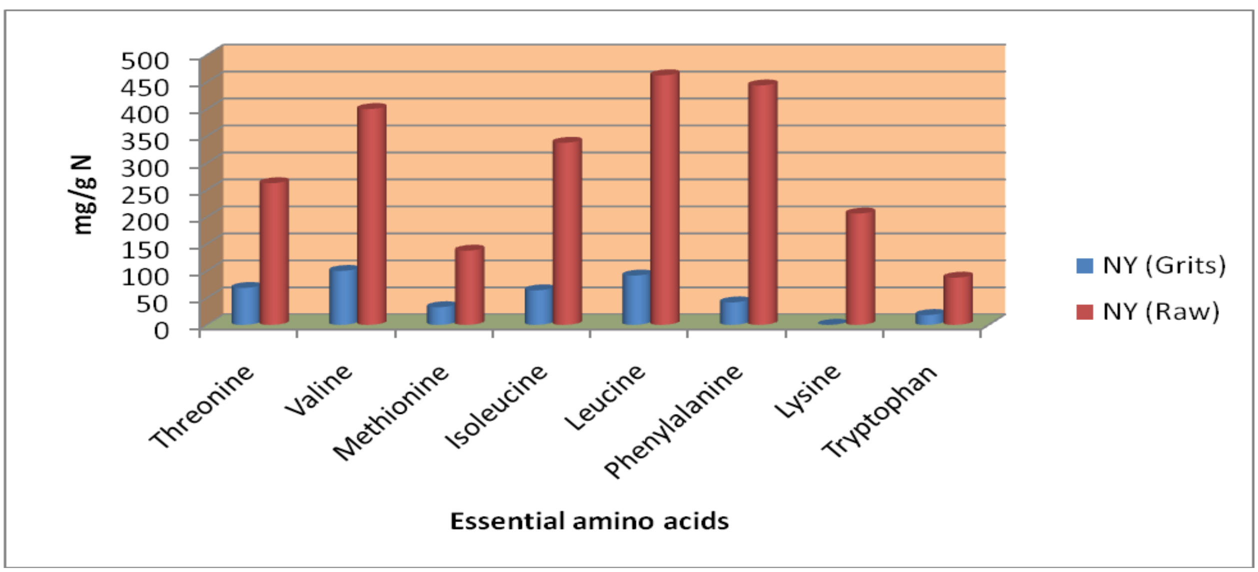


Fig. 7 Comparison of essential amino acid contents of *Njavara* Yellow (grits) with *Njavara* Yellow (raw)

The Amino Acid Score (AAS) of the processed *Njavara* grits varieties are presented in table 26.

Table 26. Amino Acid Score of *Njavara* grits

Sl.No.	Amino acids	Amino acid score (AAS)	
		NB (G)	NY (G)
1.	Lysine	-	-
2.	Tryptophan	10.28	18.26
3.	Phenylalanine	52.08	41.67
4.	Methionine	62.50	32.74
5.	Threonine	85.94	68.36
6.	Leucine	92.55	91.35
7.	Isoleucine	60.98	64.02
8.	Valine	87.50	100.00
Limiting amino acids		Lysine and Tryptophan	Lysine and Tryptophan

The above table revealed that in *Njavara* grits of black variety, the amino acid score for phenylalanine, methionine, threonine and leucine were higher than *Njavara* grits of yellow variety. The amino acid score of tryptophan, isoleucine and valine were higher for *Njavara* grits of yellow variety.

The essential amino acid index (EAA Index) of *Njavara* grits are given in table 27.

Table 27. EAA Index of raw *Njavara* rice varieties and *Njavara* grits

Variety	**E.A.A Index**	
	Raw	**Grits**
NB	26.81	17.44
NY	26.12	16.56

The EAA index of grits was found to be lower when compared to that of raw values. The table showed that in grits form NB (G) (17.44) was found to be higher than NY (G) (16.56).

Table 28 reveals the values of crude fibre and soluble fibre content of raw *Njavara* rice varieties and *Njavara* grits

Table 28. Crude fibre and soluble fibre content of raw *Njavara* rice varieties and *Njavara* grits.

Variety	Crude fibre (g)			Soluble fibre (g)		
	Raw	**Grits**	**Mean**	**Raw**	**Grits**	**Mean**
NB	0.206	0.236	0.221	0.114	0.056	0.085
NY	0.198	0.254	0.226	0.096	0.090	0.093
Mean	0.202	0.245		0.105	0.073	

	CD-Values	F-Values	CD-Values	F-Values
Varieties	0.013	0.62	0.008	7.47*
Treatments	0.013	45.65**	0.008	53.22**
VxT	0.019	4.17	0.011	52.49**

** Significant at 1 % level * Significant at 5 % level

The crude fibre content of NB (G) was found to be 0.236 g and that of NY (G) was 0.254 g/100g of rice. In the present study, it was noted that the crude fibre content increased from 0.206 g (raw NB) to 0.236 g in NB(G) grits and from 0.198 g (raw NY) to 0.254 g in NY(G). Statistically the increase in crude fibre was found to be significant at 1 per cent level (F = 45.65**).

A significant decrease was also noted with respect to soluble fibre content. The mean value of soluble fibre content of NB (G) was found to be 0.056 g and that of NY (G) was 0.096g. The data revealed that the conversion into grits form had decreased the soluble fibre content of the rice varieties.

Values of total starch and amylose content of processed *Njavara* grits are shown in Table 29.

Table 29. Total starch and amylose content of raw *Njavara* rice varieties and *Njavara* grits.

Variety	Total starch (%)			Amylose (%)		
	Raw	**Grits**	**Mean**	**Raw**	**Grits**	**Mean**
NB	76.14	57.98	67.06	21.64	18.74	20.19
NY	74.45	56.84	65.65	24.36	16.76	20.56
Mean	75.29	57.41		23.00	17.75	

	CD-Values	F-Values	CD-Values	F-Values
Varieties	0.889	72.94**	0.680	1.32
Treatments	0.889	942.39**	0.680	267.45**
VxT	1.258	157.96**	0.962	53.63**

** Significant at 1 % level

The mean values of starch for *Njavara* grits were 57.98 per cent for black variety and 56.84 per cent for yellow variety. In the present study, conversion into grit form significantly reduced the total starch content in both the varieties.

Statistically, significant interaction was observed between varieties, treatments and varieties and treatments at 1 per cent level.

Data on amylose content (Table 29) revealed a significant decrease at 1 per cent among rice varieties with respect to treatments. The amylose content was decreased from 21.64 per cent to 18.74 per cent in NB and from 24.36 per cent to 16.76 per cent in NY.

Table 30 depicts the values of amylopectin and amylose-amylopectin ratio.

Table 30. Amylopectin and amylose-amylopectin ratio of raw *Njavara* rice varieties and *Njavara* grits.

Variety	Amylopectin (%)			Amylose-amylopectin ratio		
	Raw	**Grits**	**Mean**	**Raw**	**Grits**	**Mean**
NB	54.50	39.24	46.87	0.40	0.48	0.44
NY	50.08	40.08	45.08	0.49	0.42	0.46
Mean	52.29	39.66		0.45	0.45	

	CD-Values	F-Values	CD-Values	F-Values
Varieties	1.131	36.32**	0.021	7.25*
Treatments	1.131	204.95**	0.021	13.62**
VxT	1.599	204.32**	0.030	131.61**

** Significant at 1 % level

Amylopectin value of NB (G) was 39.24 per cent and NY (G) was 40.08 per cent. The data was found to be statistically significant at 1 per cent level.

Amylose-amylopectin ratio of processed NB (G) was 0.48 and that of processed NY (G) was 0.42. Statistical analysis revealed that there was a significant difference between treatments and varieties and treatments at 1 per cent level.

Values of total ash, calcium and phosphorus content of rice varieties are depicted in Table 31.

The data on total ash revealed that converting into grits from had increased the total ash content of rice varieties from 1.52 per cent to 1.64 per cent in NB and from 1.26 per cent to 1.52 per cent in NY. Statistically, there was an increase in the values of ash content in both the varieties (F=20.628**).

Calcium content of the rice varieties decreased when converted to grits form. The raw mean value of NB was 12.80 mg but it reduced to 8.60 mg in grits form and for NY it was 12.20 mg in raw form and it reduced to 9.00 mg/100 gm of rice in grits form.

The phosphorus content of the rice varieties also decreased as a result of conversion into grits. The mean value of raw NB was 352.40 mg and 351.40 mg for raw NY. After conversion the values reduced to 325.40 mg and 326.00 mg for NB and NY respectively. The data was statistically analysed and found that there was a significant decrease in the values at 1 per cent level (F=690.767**).

The values of thiamine content of rice varieties are shown in Fig.8.

It was observed that thiamine content of the rice varieties had increased after conversion into grits form. For NB, the values increased from 0.098 mg/100 g of rice to 1.018 mg/100 g of rice, where as for NY the values increased from 0.058 mg to 0.143 mg/100 g of rice.

Riboflavin content of the rice varieties in raw and grits form are depicted in Fig. 9. In this parameter also there was an increase in riboflavin content after

Table 31. Total ash, Calcium and Phosphorus content of raw *Njavara* rice varieties and *Njavara* grits.

Variety	**Total ash (%)**			**Calcium (mg)**			**Phosphorus (mg)**		
	Raw	**Grits**	**Mean**	**Raw**	**Grits**	**Mean**	**Raw**	**Grits**	**Mean**
NB	1.52	1.64	1.58	12.80	8.60	10.70	352.40	325.40	338.90
NY	1.26	1.52	1.39	12.20	9.00	10.60	351.40	326.00	338.70
Mean	1.39	1.58		12.50	8.80		351.90	325.7	

	Total ash CD-Values	Total ash F-Values	Calcium CD-Values	Calcium F-Values	Phosphorus CD-Values	Phosphorus F-Values
Varieties	0.088	20.63**	0.911	0.05	2.113	0.05
Treatments	0.088	20.63**	0.911	73.99**	2.113	690.77**
VxT	0.125	2.79	1.289	1.35	2.988	0.65

** Significant at 1 % level

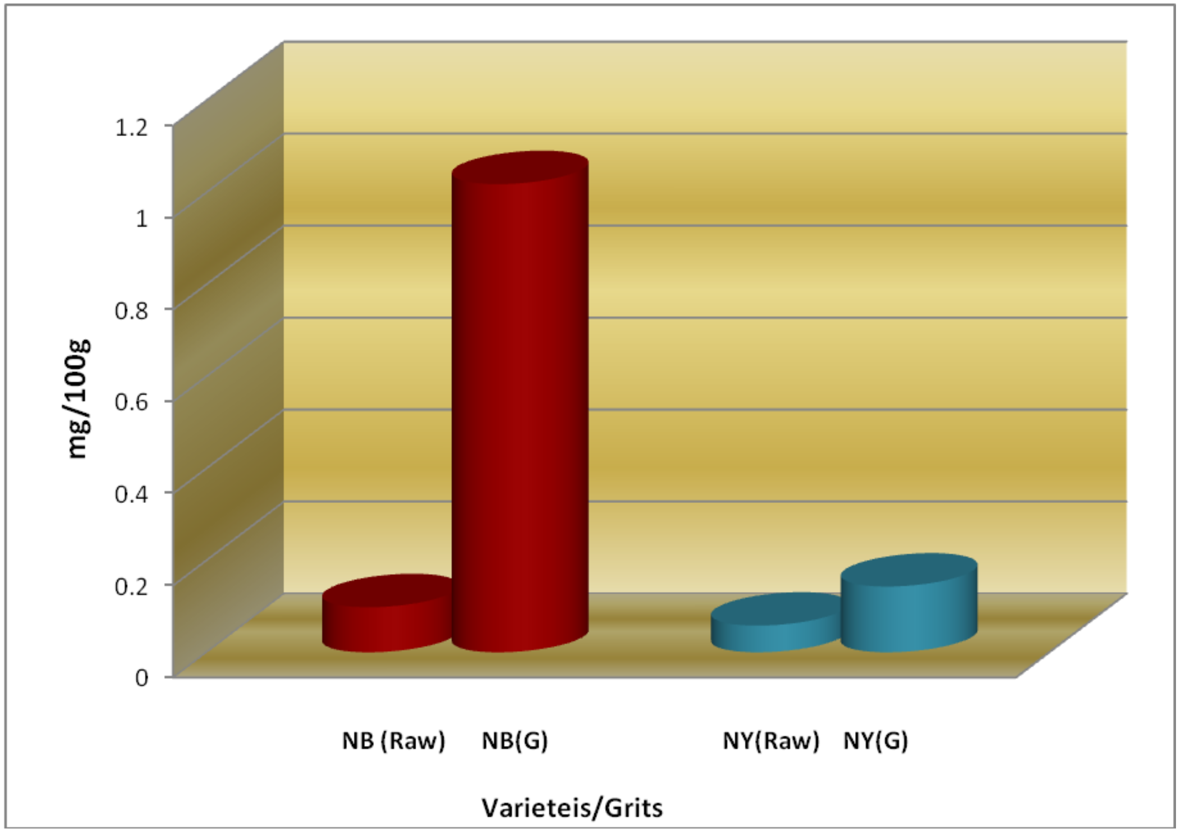


Fig. 8 Comparison of thiamine content of raw *Njavara* varieties and *Njavara* grits

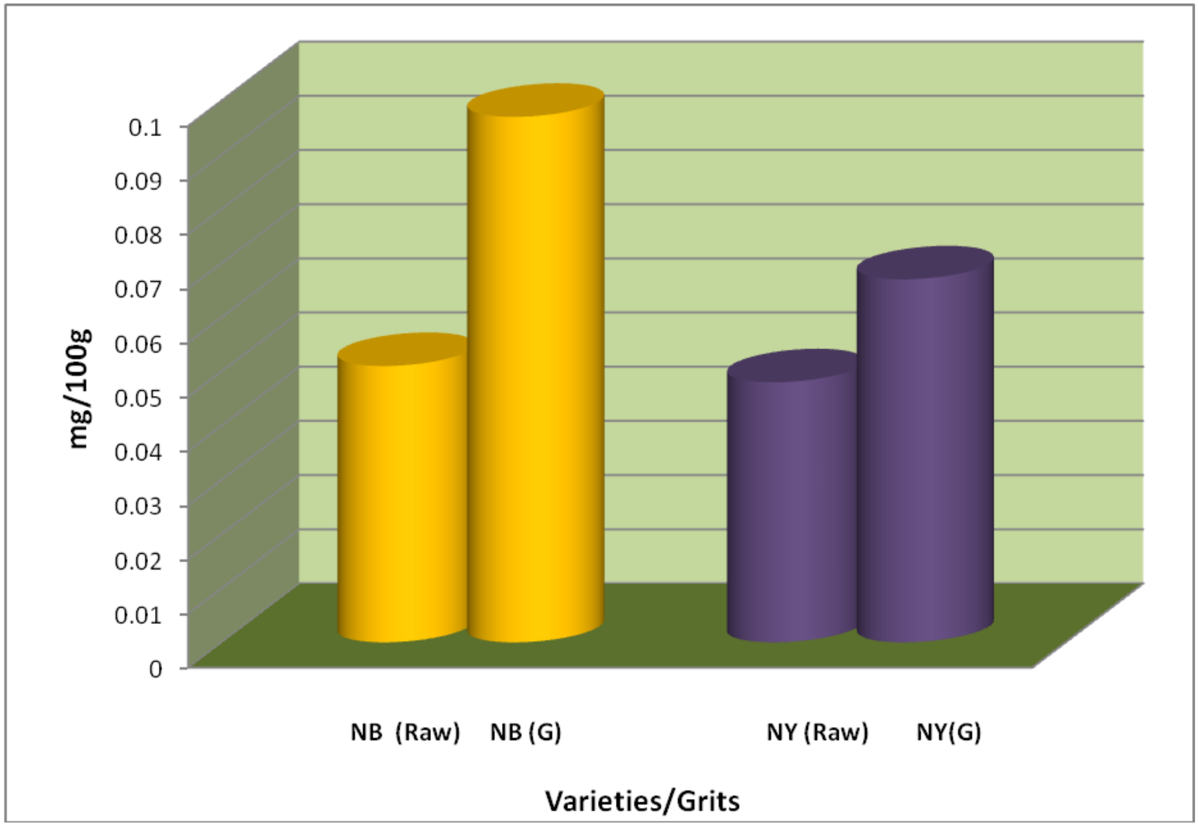


Fig. 9 Comparison of riboflavin content of raw *Njavara* varieties and *Njavara* grits

conversion into grits form. The riboflavin content of NB in raw form was 0.051 mg/100g of rice and it increased to 0.097mg in grits form.

In the case of NY the value was 0.048 mg/100g of rice and in grits form it has increased to 0.067mg/100 g of rice.

Table 32 shows the total phenol content of the rice samples.

Table 32. Total phenol content of raw *Njavara* rice varieties and *Njavara* grits.

Variety	Total Phenol content (mg)		
	Raw	**Grits**	**Mean**
NB	0.41	0.42	0.41
NY	0.29	0.33	0.31
Mean	0.35	0.37	
	CD-Values	F-Values	
Varieties	0.056	32.51**	
Treatments	0.056	8.12*	
VxT	0.079	5.99*	

** Significant at 1 % level * Significant at 5 % level

In the present study, the total phenol content of the rice had increased from 0.41 mg to 0.42 mg in NB. The same effect was observed in the case of NY. The mean value of raw NY was 0.29 mg and the value changed to 0.33 mg in grits form. Statistically, the data showed a significant increase in the values (F=8.12*).

Trace element content (Fig. 10) of the rice varieties revealed that Fe content had increased in both the varieties after the conversion of grits form, from 0.36 mg/L to 1.56 mg/L in NB and from 0.36 mg/L to 1.46 mg/L in NY.

Regarding other three elements i.e. Mn, Zn and Cu, there was only a slight increase in their content in grits form. The raw values of NB for Mn, Zn and Cu was 0.75 mg/L, 0.88 mg/L and 1.53 mg/L respectively and it increased to 0.85 mg/L, 0.64 mg/L and 0.89 mg/L respectively in grits form.

In the case of raw NY, the values for Mn, Zn and Cu were 0.83 mg/L, 0.90 mg/L, 1.30 mg/L respectively and after processing the values were 0.94 mg/L, 0.91 mg/L and 1.32 mg/L for Mn, Zn and Cu respectively.

Se content of the rice varieties showed an increase in this heavy metal content after processing (Fig. 11). After conversion into grits form the Se content of NB was 9.79 µg/ml and 9.36 µg/ml for NY, whereas in raw form the Se content of NB and NY was 4.73 µg/ml and 2.22 µg/ml respectively.

4.2.2. Antioxidant properties

4.2.2. a) DPPH radical scavenging activity

Free radical scavenging activities of the *Njavara* grits were studied by the DPPH assay. Fig. 12 illustrates the results of the DPPH activity among the *Njavara* rice varieties. The findings revealed that *Njavara* yellow (IC_{50} value = 35.89 µg/ml) has more DPPH scavenging activity compared to *Njavara* black (IC_{50} value = 47.80µg/ml). The results also revealed that the DPPH activity of both the rice varieties have fallen down compared to the raw form in the 1st phase.

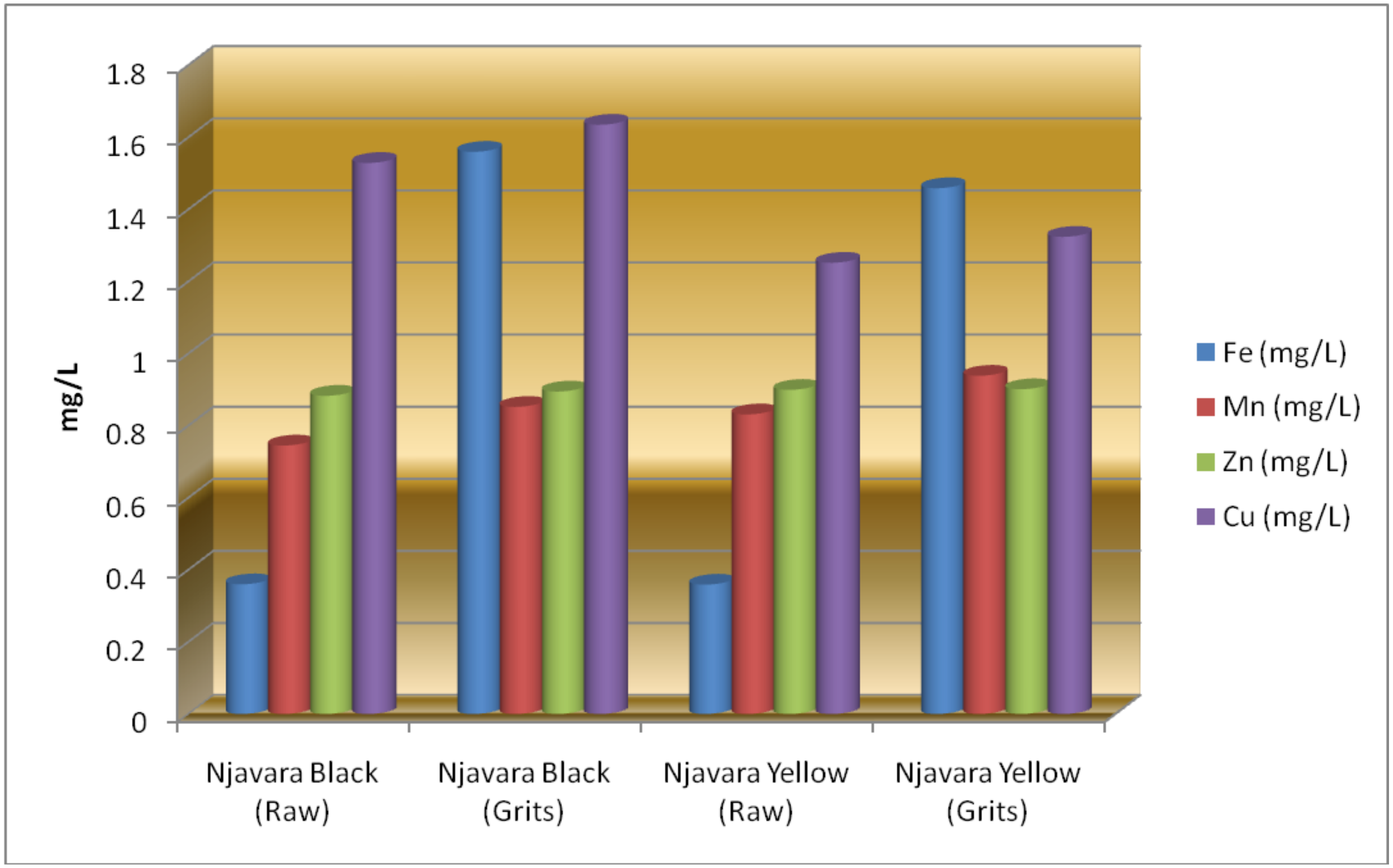


Fig. 10 Comparison of trace element content of raw *Njavara* rice varieties and *Njavara* grits

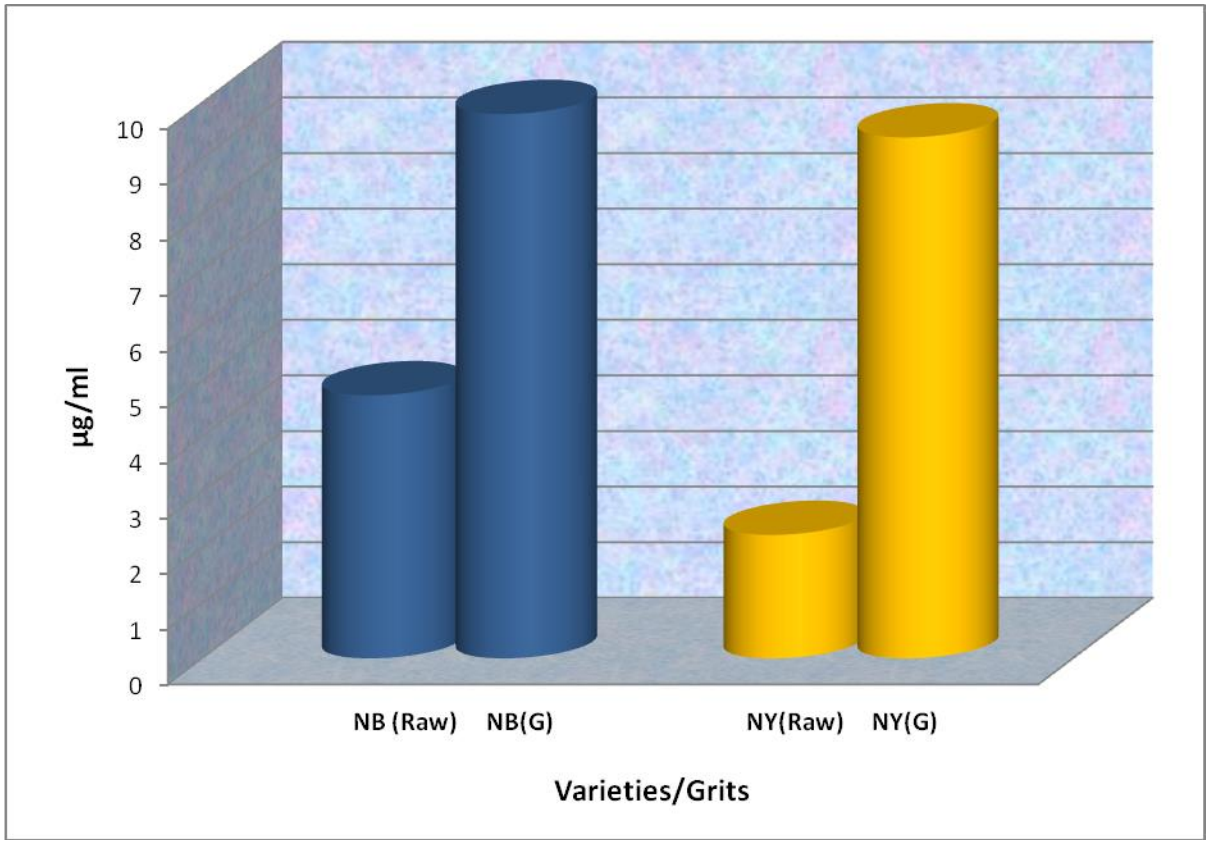


Fig. 11 Comparison of Se content of raw *Njavara* rice varieties and *Njavara* grits

Table 33 showed that there is a significant difference between the varieties, treatments at 1 per cent level.

4.2.2. b) Hydroxyl radical scavenging activity

Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity. This method involves in-vitro generation of hydroxyl radicals using Fe3+/ascorbate/EDTA/H_2O_2 system using Fenton reaction. Scavenging of this hydroxyl radical in presence of antioxidant is measured (Elizabeth and Rao, 1990).

The results of the hydroxyl radical scavenging activity revealed that NY (G) had more hydroxyl radical scavenging activity (IC_{50} value = 53.78 µg/ml) compared to NB(G) (IC_{50} value = 60.4 µg/ml) (Fig. 13). Statistical analysis revealed that there was a significant difference between varieties, treatments and varieties and treatments (Table 33).

4.2.2. c) Superoxide anion-radical scavenging activity

Superoxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non-enzymatic reaction such as autoxidation by catecholamines. In the present study, superoxide radical reduces NBT to a blue coloured formazan that is measured at 560 nm (Panda et al., 2011).

Njavara black grits showed higher superoxide anion radical scavenging activity when compared to *Njavara* yellow grits. The IC_{50} value for NB (G) was found to be 50.93 µg/ml, while that of NY (G) was 60.00 µg/ml and of Vitamin C (standard) = 3.90 µg/ml (Fig. 14). Statistically the results were found to be significant.

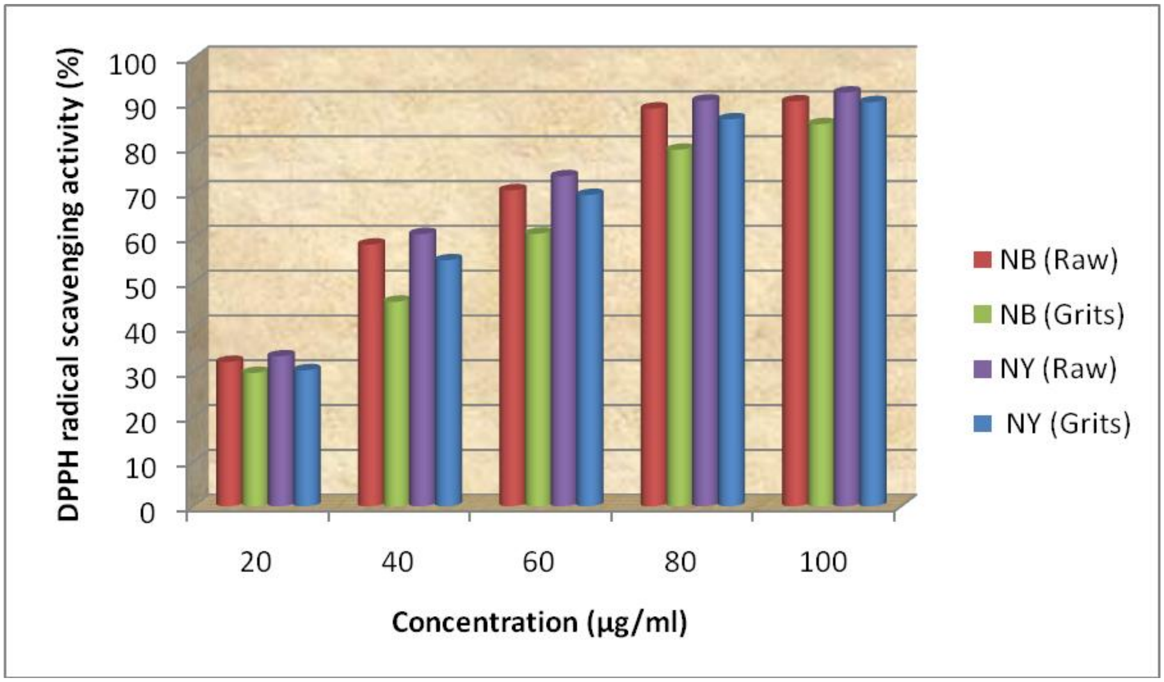


Fig. 12 Comparison of DPPH radical scavenging activity of *Njavara* rice varieties and *Njavara* grits

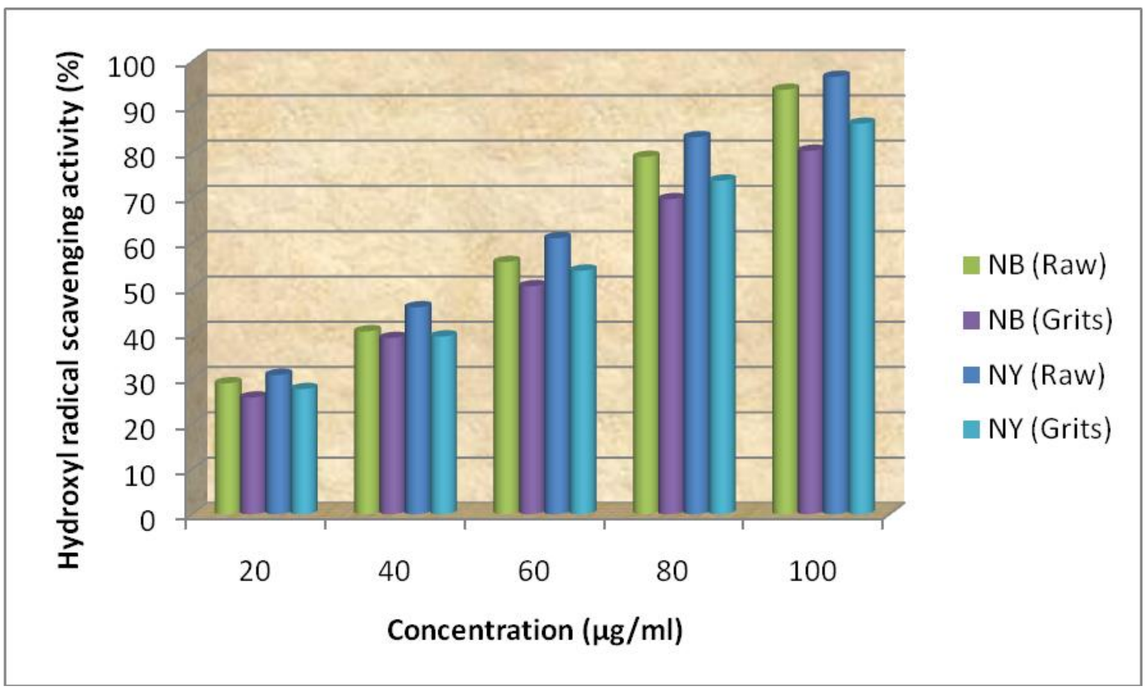


Fig. 13 Comparison of hydroxyl radical scavenging activity of *Njavara* rice varieties and *Njavara* grits

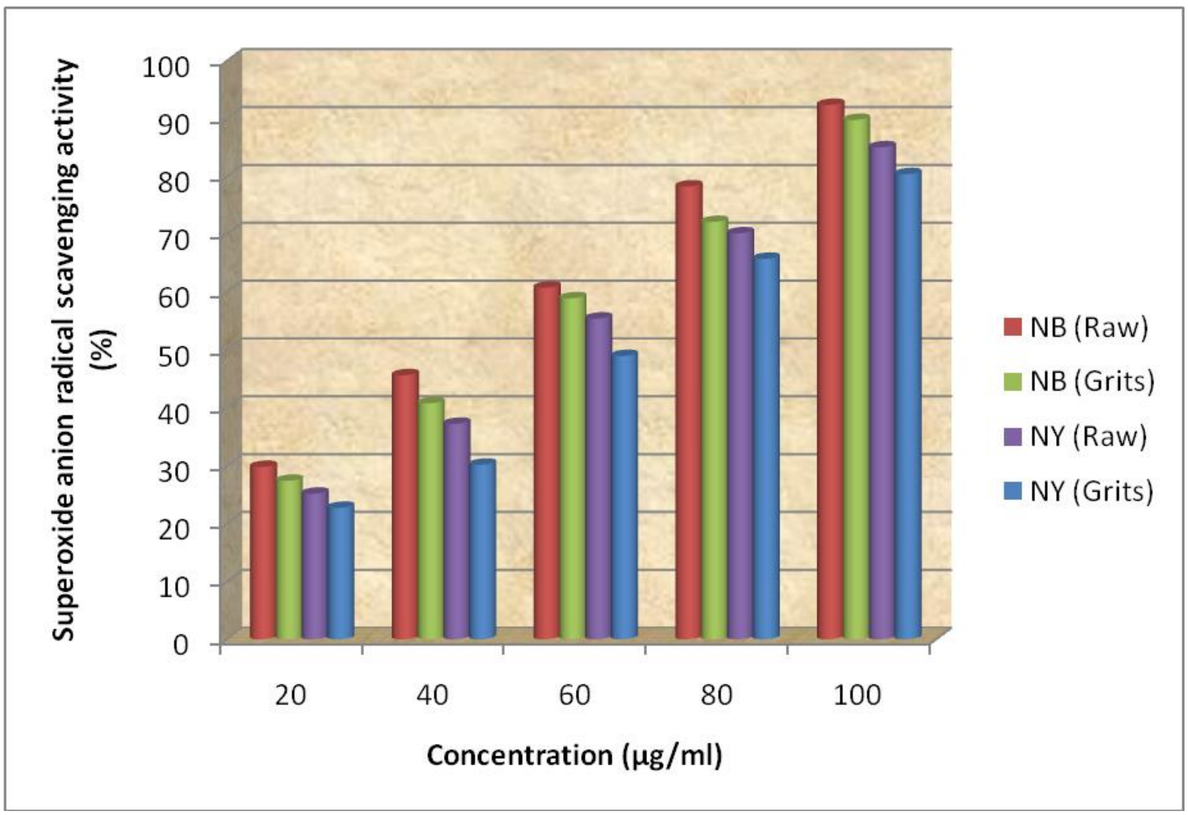


Fig. 14 Comparison of superoxide anion radical scavenging activity of *Njavara* rice varieties and *Njavara* grits

Table 33. Antioxidant activities of raw *Njavara* rice varieties and *Njavara* grits.

Variety	DPPH radical scavenging activity (µg/ml)			Hydroxyl radical scavenging activity (µg/ml)			Superoxide anion-radical scavenging activity (µg/ml)		
	Raw	**Grits**	**Mean**	**Raw**	**Grits**	**Mean**	**Raw**	**Grits**	**Mean**
NB	33.73	47.80	40.76	55.68	60.40	58.04	48.77	50.96	49.87
NY	31.62	35.89	33.75	46.08	53.78	49.93	55.49	60.36	57.93
Mean	32.67	41.84		50.88	57.09		52.13	55.66	

	DPPH CD-Values	DPPH F-Values	Hydroxyl CD-Values	Hydroxyl F-Values	Superoxide CD-Values	Superoxide F-Values
Varieties	0.183	6638.00**	0.274	4008.82**	0.449	1447.37**
Treatments	0.183	11312.58**	0.274	2324.89**	0.449	277.87**
VxT	0.258	3254.89	0.388	118.16**	0.635	40.30**

** Significant at 1 % level

4.2.3. Shelf-life quality

Shelf life quality of *Njavara* grits was ascertained by storing the product in a polypropylene cover for a period of 6 months at ambient condition. The parameters like moisture, peroxide value, insect count and microbial infestation were analysed at the end of each month.

4.2.3. a) Moisture Percentage

The results of the moisture content analysed are presented in Table 34.

Table 34. Moisture percentage of stored *Njavara* grits

Particulars	Moisture (%)						
	1st month	2nd month	3rd month	4th month	5th month	6th month	Mean
NB (G)	15.36	15.49	15.64	15.83	15.97	16.10	15.73
NY (G)	15.12	15.25	15.25	15.99	16.09	16.23	15.72
F-Values 0.02				CD- Values 0.200			

From the above table, it was observed that there was no significant difference of moisture content between the varieties. The mean moisture level of NB (G) after six months storage was 15.73 per cent and that of NY (G) was 15.72 per cent.

4.2.3. b) Peroxide value

Peroxide value gives an indication about the extent of peroxidation taking place in the stored food materials. The extent of peroxidation in the stored items was estimated and the values obtained are depicted in the Table 35.

Table. 35 Peroxide value of stored *Njavara* grits

Particulars	Peroxide value (meq/100g)						
	1st month	2nd month	3rd month	4th month	5th month	6th month	Mean
NB (G)	0.45	0.47	0.49	0.51	0.52	0.55	0.49
NY (G)	0.42	0.43	0.46	0.49	0.53	0.56	0.48
F-Values 3.57				CD- Values 0.040			

Peroxide value could not detect in both the *Njavara* grits since they have low fat content. There was no significant difference between the rice varieties, with respect to peroxide value. The mean peroxide value for NB (G) was found to be 0.49 meq/100g and that of NY (G) was found to be 0.48 meq/100g.

4.2.3. c) Insect count

The incidence of insect attack in stored grits was assessed monthly for a period of six months. The developed *Njavara* grits stored in polypropylene covers were completely free of insect infestation.

Table 36. Microbial growth in the developed *Njavara* grits

Particulars	Microbial growth (cfu/g)											
	1st month		2nd month		3rd month		4th month		5th month		6th month	
	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi	Bacteria	Fungi
NB	ND	ND	$0.5x10^3$	ND	$1.45\ x10^3$	ND	$2.75x10^3$	$0.8x10^3$	$3.35x10^3$	$1.9x10^3$	$3.5x10^3$	$3.4x10^3$
NY	ND	ND	$0.2\ x10^3$	ND	$1.75\ x10^3$	ND	$2.5x10^3$	$0.9x10^3$	$3.11x10^3$	$1.18x10^3$	$3.25x10^3$	$1.66x10^3$

ND – Not Detected

4.2.3. d) Assessment of microbial growth in developed *Njavara* grits

Microbial growth in the stored grits was assessed for a period of six months. Media used for the assessment of bacteria and fungi were nutrient agar (NA) and potato dextrose agar (PDA) respectively. It was observed that in all the media, fresh samples were free of any of the micro-organisms. Among the stored samples, microbial growth was observed. Table 36 depicts the details regarding the microbial growth present in the stored *Njavara* grits.

From the table, it was noticed that microbial growth was comparatively high in NB (G). For the first month, there was no microbial growth. In the 2nd and 3rd month there was no fungal attack in both the samples

4.2.4 Selection of Superior Variety

The superior variety was selected based on the following parameters viz., energy, protein, crude fibre, soluble fibre, total starch, amylose, amylopectin, amylose-amlyopectin ratio, total ash, calcium, phosphorus, thiamine, riboflavin, total phenols and trace elements viz. Fe, Mn, Zn, Cu and Se using the discriminant function analysis.

Table 37 shows the selection of superior variety.

Table 37. Selection of superior variety of *Njavara*

Variety	Index score	
	Raw	Grits
NB	4907.75	4647.21
NY	4861.13	4553.33

The above table showed that *Njavara* black raw was found to be the superior variety in both raw and processed forms.

But there was not much difference in the values of both the rice varieties statistically, hence both the varieties of rice were given randomly to check its therapeutic value.

4.3. IIIrd Experiment – Assessment of the therapeutic value of *Njavara* grits through case studies

In the present study, to assess the therapeutic value of *Njavara* grits, it was given to selected subjects for a period of 3 months.

When the rice was converted into grits it was noted that from 1Kg (weight of husked rice) of husked rice around 700 g (weight of grits) of grits was obtained. The yield ratio was found to be 1.43

Processing loss was also calculated and the results revealed that the processing loss was found to be 0.30 per cent.

Along with yield ratio and processing loss, cost was also calculated (based on the price of paddy, milling charge, charge for converting into grits etc.) and it was found that for 1 Kg grits , the cost would be around Rs.96/-.

Recipes were formulated in the laboratory using *Njavara* grits and sensory evaluation tests were carried out.

4.3.1. Formulation, sensory evaluation and suitability of *Njavara* grits

Njavara grits (both NB and NY) were first parboiled and sun dried. It was milled and converted to grits form using a pulveriser.

Different recipes like uppuma, kozhukatta, oratti and porridge were tried using the *Njavara* grits for checking its suitability and the recipes were assessed for sensory evaluation by a panel of judges.

According to Broody (2003) sensory evaluation is the key factor for determining the shelf-life of many food products. Sensory evaluation is designed to reflect common preference, to maintain the quality of food at a given standard, for the assessment of process variation, cost reduction, product improvement, new market development and market analysis (Manay and Swamy, 2002). The attributes selected in this study were colour, appearance, flavor, taste and doneness. The score obtained for these attributes are presented in Table 37.

From Table 38, it can be seen that the recipe uppuma was having highest score for overall acceptability (22.80) followed by porridge (19.80), kozhukatta (17.00) and oratti (15.40).

These recipes were recommended to the subjects for proper and daily inclusion in their diet.

Table 38. Organoleptic evaluation of recipes

Particulars	Attributes (scores)					
	Colour	Appearance	Flavour	Taste	Doneness	Overall acceptability
Uppuma	4.20	4.00	4.80	4.80	5.00	22.80
Kozhukatta	2.80	2.50	3.40	3.80	4.50	17.00
Porridge	4.50	4.00	3.80	3.50	4.00	19.80
Oratti	2.80	2.00	4.00	3.60	3.00	15.40
F- Values 7.11**				CD-Values 0.30		

4.3.2. Results of case study

Five human subjects in the age group of 40-50 years were selected for the case study (Subject A to E).

Subject A

1) **Socio economic profile**

Age	:	47 yrs.
Gender	:	Male
Religion	:	Hindu
Type of family	:	Nuclear
Family income (monthly) (Rs.)	:	Rs.30, 000- 40, 000
Educational status	:	Degree
Occupation	:	Business

2) **Duration of diseases & other related problems**

The patient is having diabetes for the past two years. In his family no one was having diabetes. There was no diabetes related diseases found in him. He is a non-smoker.

3) **Exercise pattern**

The subject is having a moderate activity level, with no exercise habit.

4) Dietary habit

The subject is a non-vegetarian. He includes cereals, pulses, vegetables, milk and milk products, tea and fish daily in his diet, where as meat was included once in a week. His frequency of meals was three meals per day and the oil used for cooking is coconut oil. He has an average calorie intake of 1563 Kcal/day.

5) Anthropometric data

Height (cms)	:	165
Weight (Kg)	:	73
BMI	:	26.81
Category	:	Obese grade I
Waist circumference (cms)	:	105
Hip circumference (cms)	:	100
W/H ratio	:	1.05

Interpretation

The subject is an obese grade I, with a high waist hip ratio of 1.05. The subject was given 58.5 g of NY grits for 3 months.

6) Effect of *Njavara* grits on blood sugar level

Table 39 shows the fasting blood sugar levels of the subject before and after the supplementation.

Table 39. Changes in blood glucose levels of subject A

Monitoring intervals	Blood sugar levels (mg/dl)
Initial	193
Intermittent (after 45 days)	200
Final	173

The table showed that the initial value of fasting blood sugar of subject A was 193 mg/dl. The blood sugar level was increased after 45 days, but it has finally come down to 173 mg/dl after a feeding period of 3 months (Fig. 15).

7) Glucose Tolerance Test (GTT)

Fig. 16 shows the GTT of the subject, and the result revealed that for *Njavara* grits the values were lower when compared to reference food (glucose).

8) Antioxidant properties

The antioxidant activities in blood was also assessed and the findings revealed that DPPH radical scavenging activity, hydroxyl radical scavenging activity and superoxide anion radical scavenging activity were increased after supplementation for a period of three months. Fig. 17, 18 and 19 shows the changes in the antioxidant activities before and after the supplementation.

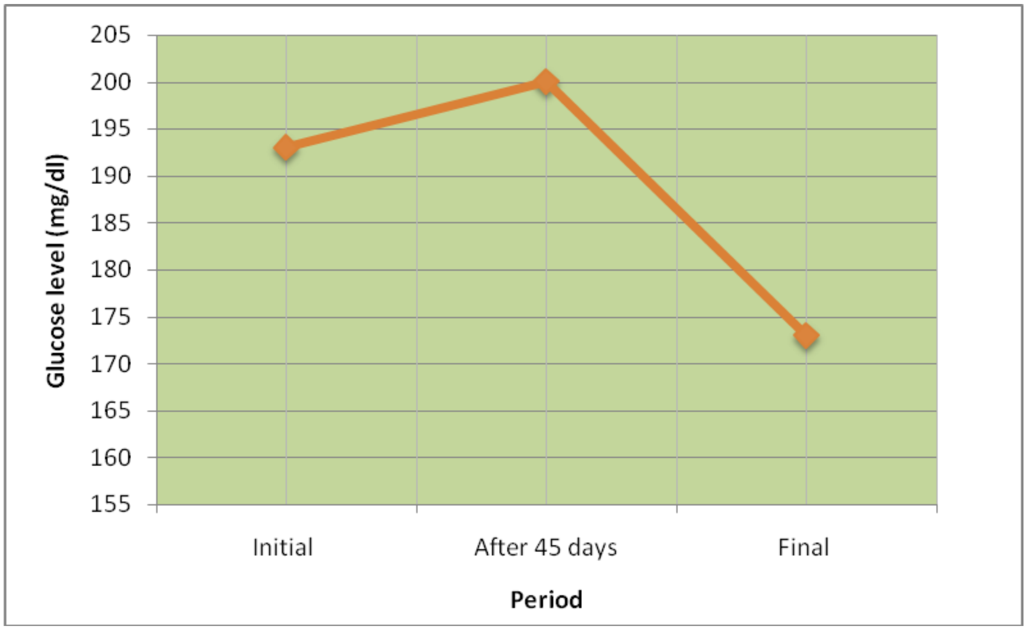


Fig. 15 Changes in blood sugar levels of subject A

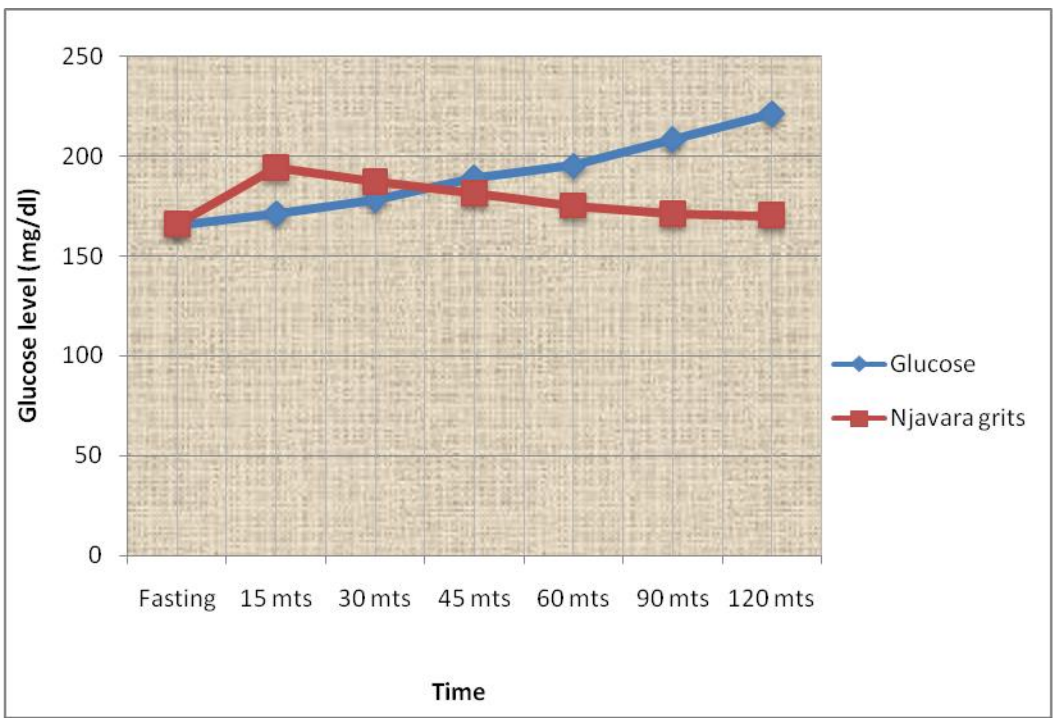


Fig. 16 GTT of subject A

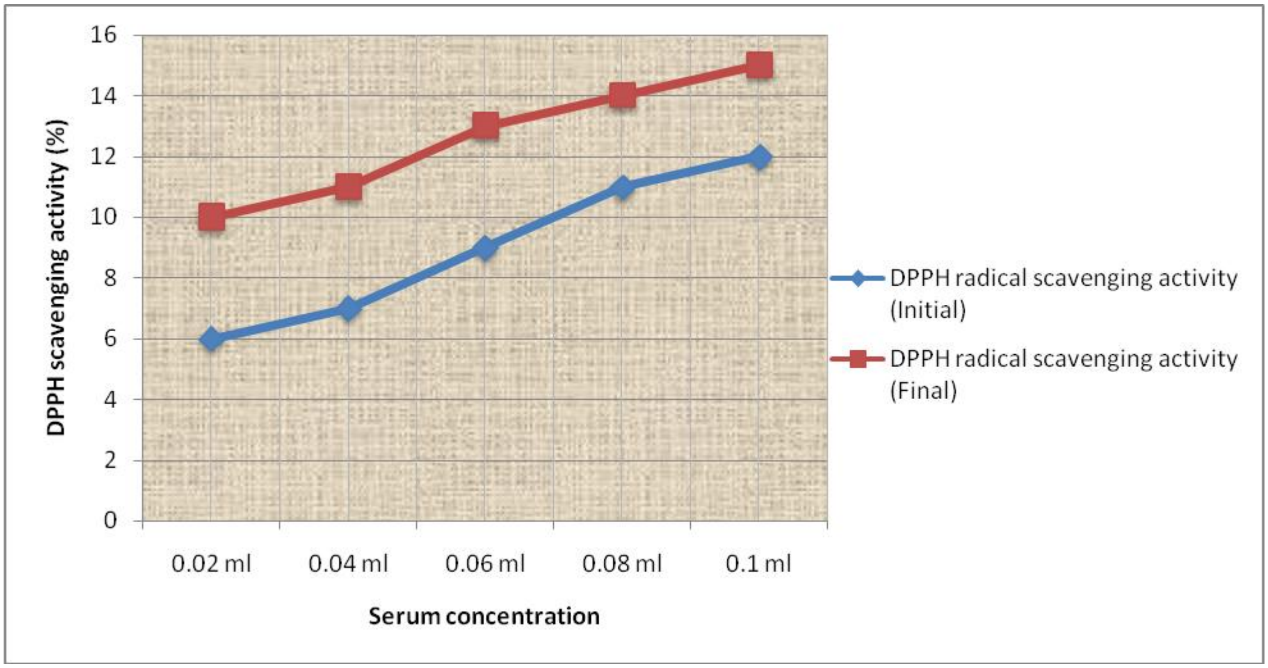


Fig. 17 DPPH radical scavenging activity of subject A (before and after supplementation)

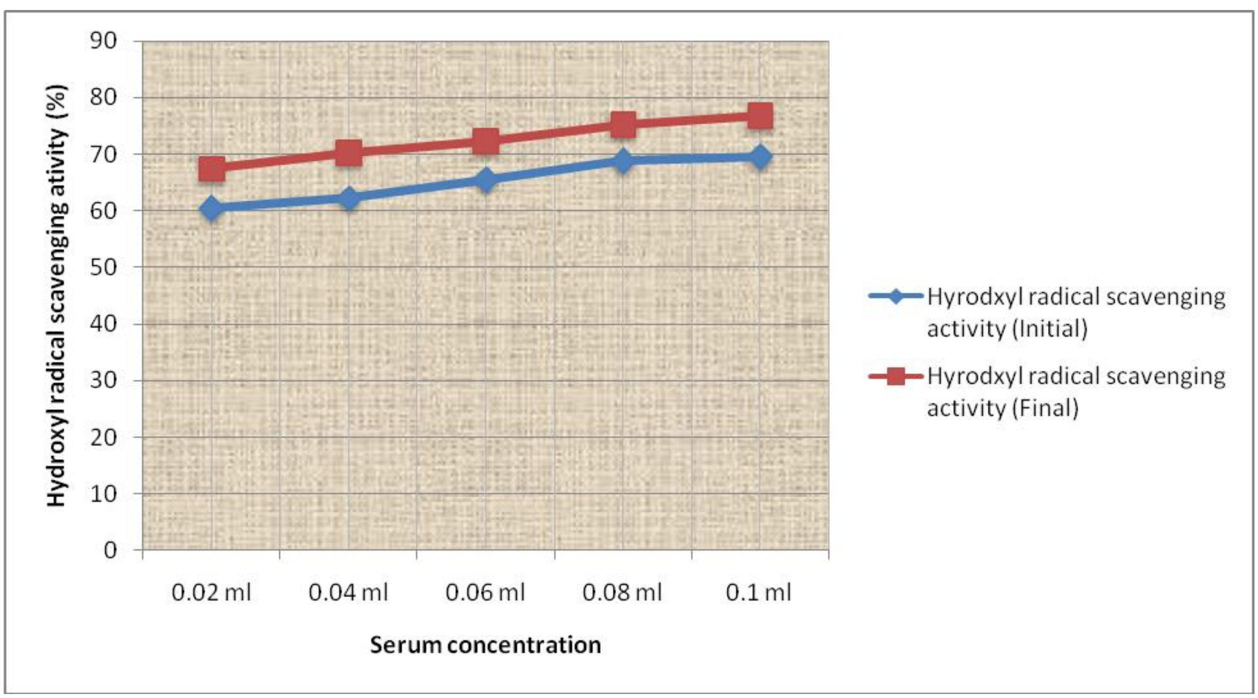


Fig.18 Hydroxyl radical scavenging activity of subject A (before and after supplementation)

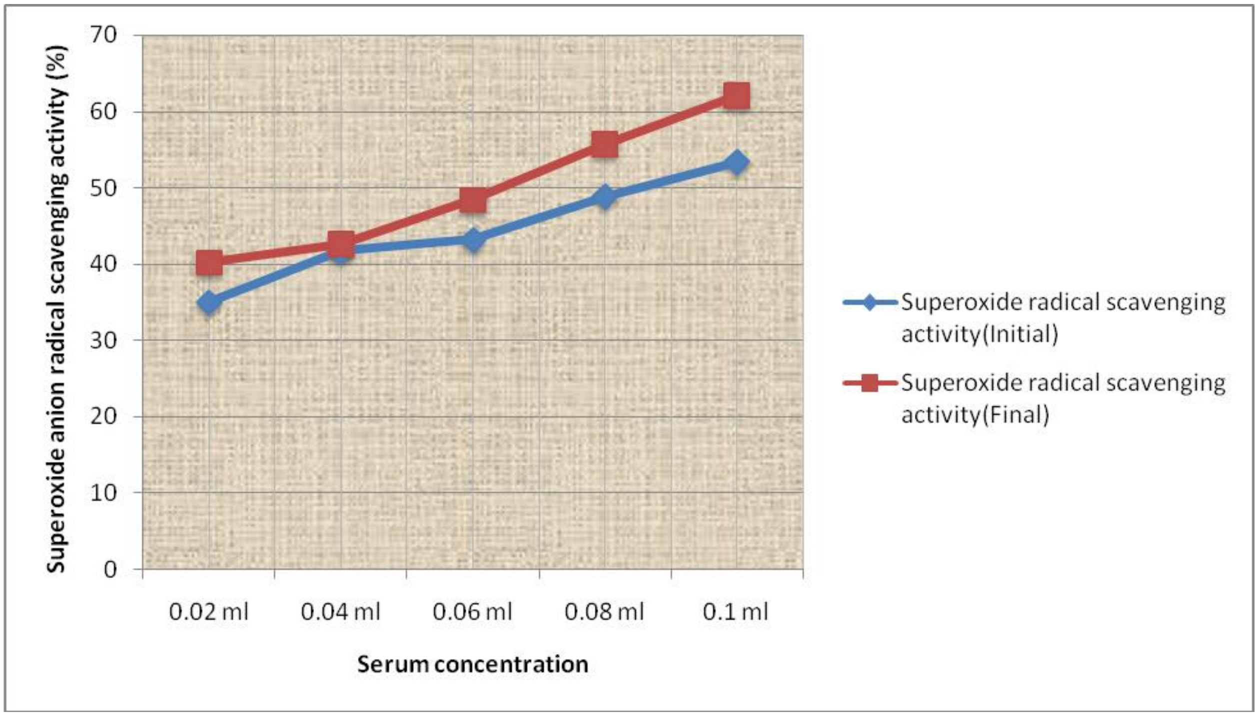


Fig.19 Superoxide anion radical scavenging activity of subject A (before and after supplementation

Subject B

1) **Socio economic profile**

Age	:	43 yrs.
Gender	:	Male
Religion	:	Hindu
Type of family	:	Nuclear
Family income (monthly) (Rs.)	:	Rs.40, 000-45,000
Educational status	:	Degree
Occupation	:	Govt. employee

2) **Duration of diseases & other related problems**

The subject was diagnosed with diabetes only 9 months before the feeding trial. He has a family history of diabetes and both his parents were diabetic. There was no diabetes related diseases found in him. He is a non-smoker.

3) **Exercise pattern**

The subject was having a sedentary activity level, with no exercise habit.

4) **Dietary pattern**

The subject is a non-vegetarian. He includes cereals, pulses, vegetables, milk and milk products, tea and fish daily in his diet, where as meat was included once in two week. His frequency of meals was three meals per day and he uses coconut oil and sunflower oil for cooking purpose. The average calorie intake of was 1617 Kcal/day.

5) Anthropometric data

Height (cms)	:	170
Weight (Kg)	:	65
BMI	:	22.49
Category	:	Normal
Waist circumference (cms)	:	95
Hip circumference (cms)	:	99
W/H ratio	:	0.96

Interpretation

The subject comes under normal category of BMI, with a high waist hip ratio of 0.96.The subject was given 86.6 g of NY grits for 3 months.

6) Effect of *Njavara* grits on blood sugar level

Table 40 shows the fasting blood sugar levels of the subject before and after the supplementation.

Table 40. Changes in blood glucose levels of subject B

Monitoring intervals	Blood sugar levels (mg/dl)
Initial	140
Intermittent (after 45 days)	110
Final	90

The table shows that the initial value of fasting blood sugar of subject B was 140 mg/dl, after 45 days of supplementation it comes down to 110 mg/dl, and finally the blood sugar level decreased to 90 mg/dl after a feeding trail of 3 months (Fig. 20).

7) Glucose Tolerance Test (GTT)

Fig. 21 shows the GTT of the subject, and the result revealed that for *Njavara* grits the values were lower when compared to reference food glucose.

8) Antioxidant properties

Fig. 22, 23 and 24 shows the antioxidant activities of subject B. The findings revealed that DPPH radical scavenging activity, hydroxyl radical scavenging activity and superoxide anion radical scavenging activity were increased after three months supplementation.

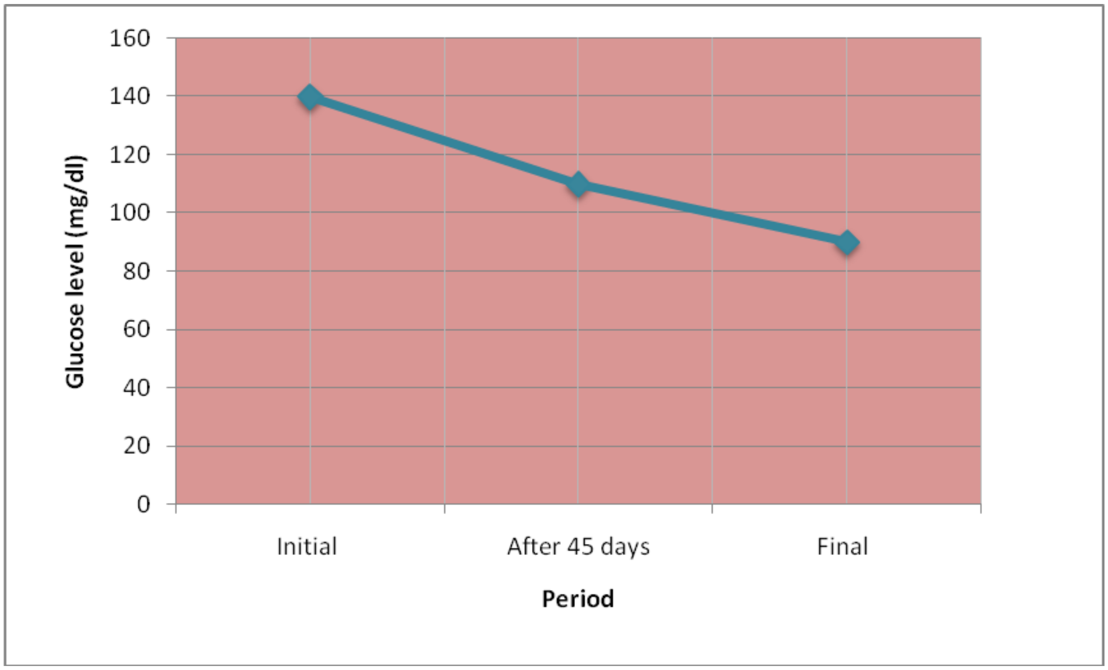


Fig. 20 Changes in blood sugar levels of subject B

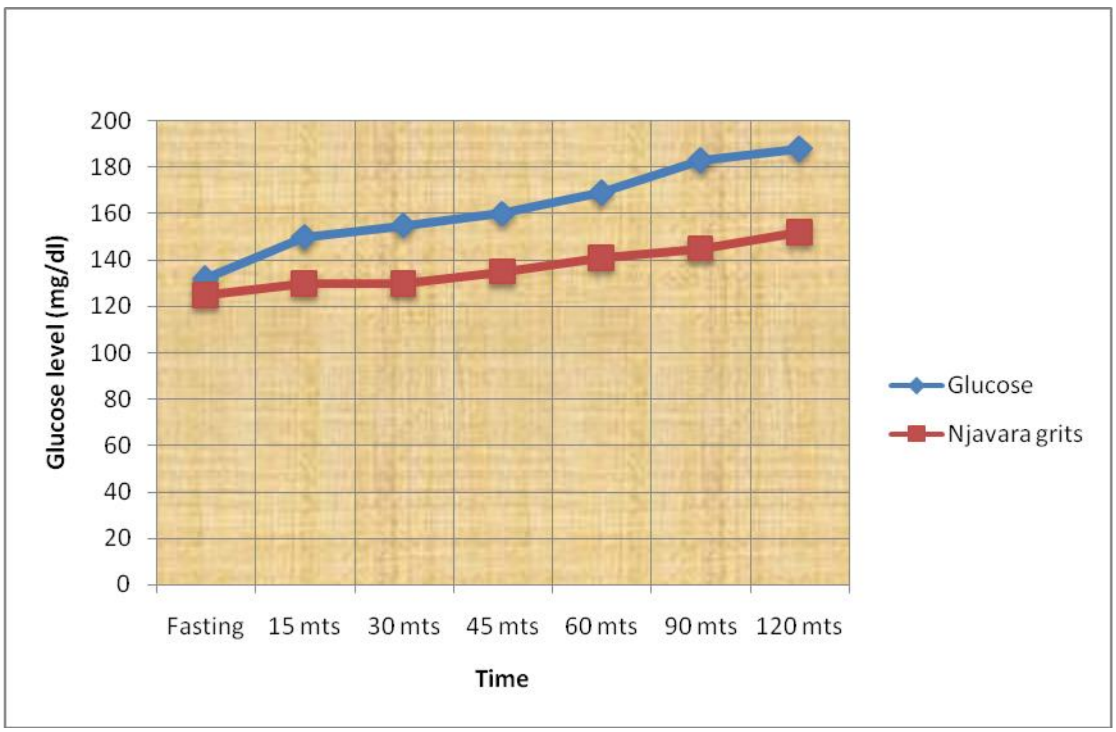


Fig. 21 GTT of subject B

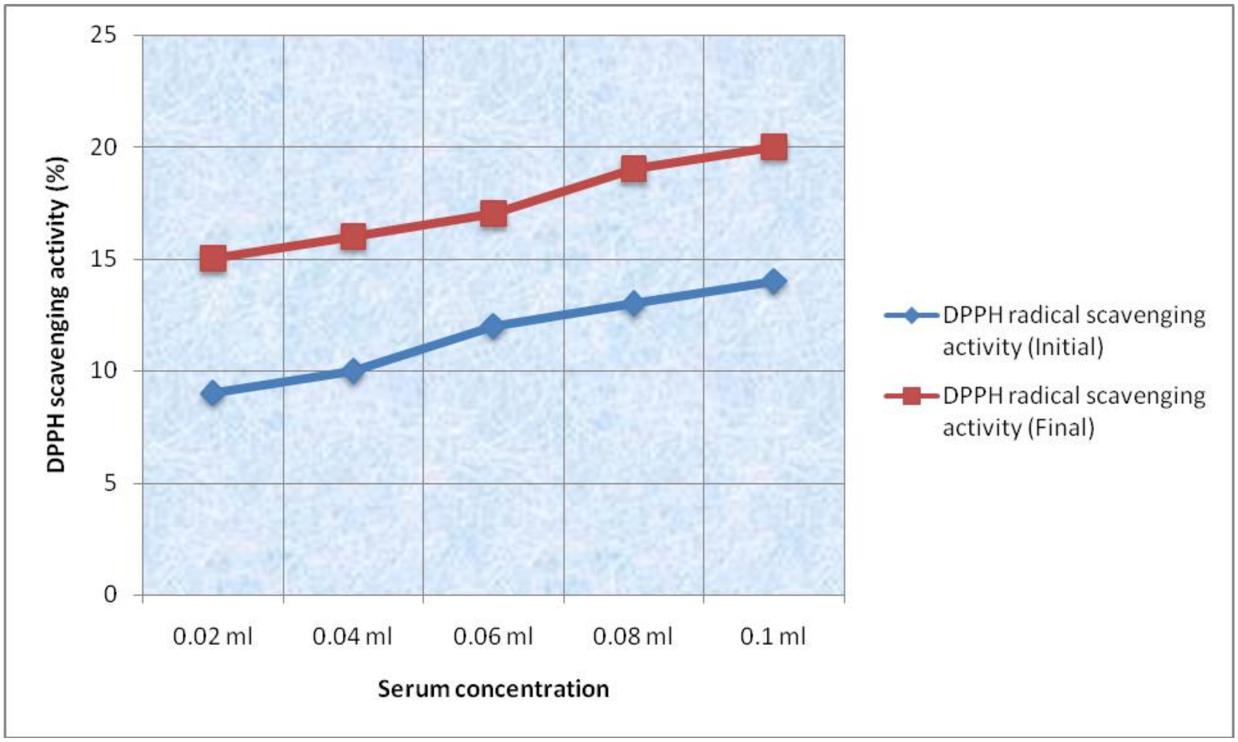


Fig. 22 DPPH radical scavenging activity of subject B (before and after supplementation)

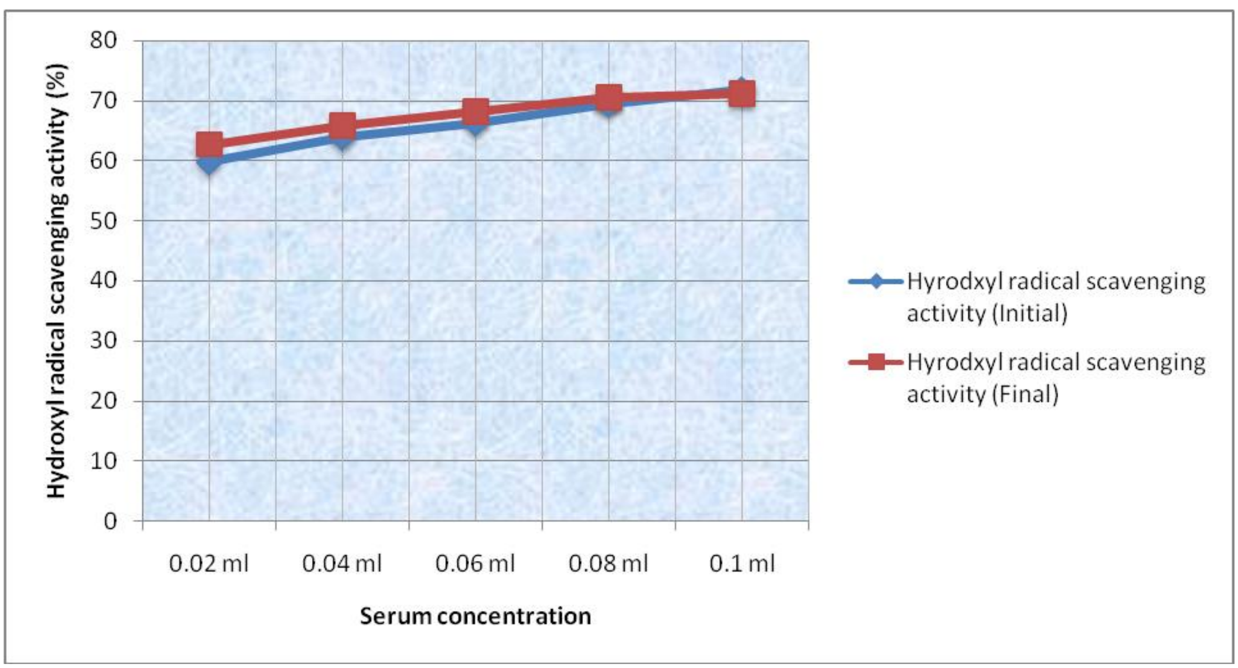


Fig.23 Hydroxyl radical scavenging activity of subject B (before and after supplementation)

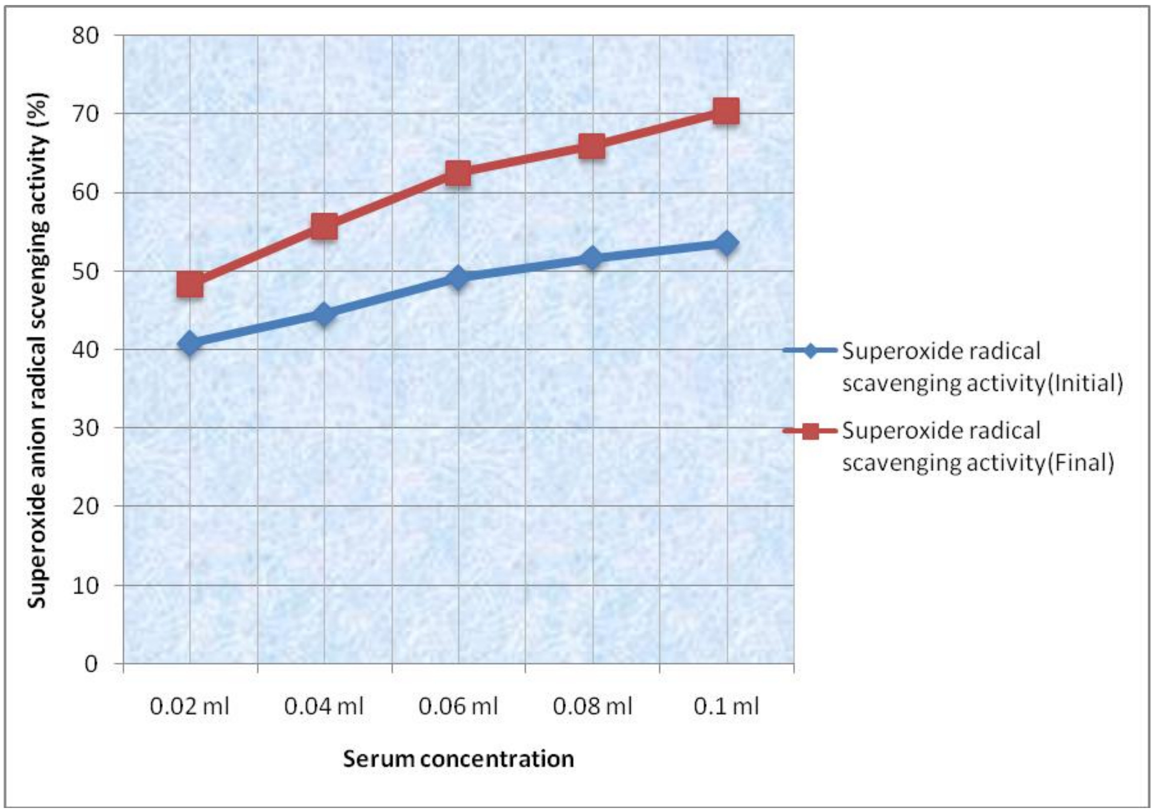


Fig. 24 Superoxide anion radical scavenging activity of subject B (before and after supplementation)

Subject C

1) **Socio economic profile**

Age	:	48 yrs.
Gender	:	Female
Religion	:	Hindu
Type of family	:	Nuclear
Family income (monthly) (Rs.)	:	> 2, 00,000
Educational status	:	PG and above
Occupation	:	Govt. employee

2) **Duration of diseases & other related problems**

The subject is having diabetes for the past 2 years. In her family, only her brother is having diabetes. There was no diabetes related diseases found in her. The subject doesn't have gestational diabetes and had menopause 4 years before the supplementation.

3) **Exercise pattern**

The subject was having a moderate activity level and used to go for regular walk for 45 minutes.

4) **Dietary pattern**

The subject is a vegetarian and includes cereals, pulses, vegetables, milk and milk products and tea in the daily diet. Her frequency of meals was three meals per day and uses coconut oil and sunflower oil for cooking. On an average, the subject consumed 1304 Kcal/day.

5) Anthropometric data

Height (cms)	:	157
Weight (Kg)	:	60
BMI	:	24.34
Category	:	Normal
Waist circumference (cms)	:	86
Hip circumference (cms)	:	96
W/H ratio	:	0.89

Interpretation

The subject comes under normal category of BMI, with a high waist hip ratio of 0.89. The subject was given 69.00 g of NB grits for 3 months.

6) Effect of *Njavara* grits on blood sugar level

Table 41 shows the fasting blood sugar levels of the subject before and after the supplementation.

Table 41. Changes in blood glucose levels of subject C

Monitoring intervals	Blood sugar levels (mg/dl)
Initial	140
Intermittent (after 45 days)	130
Final	110

The initial value of fasting blood sugar of subject C was 140 mg/dl (before the supplementation), after 45 days of supplementation it decreased to 130 mg/dl, and finally the blood sugar level decreased to 110 mg/dl after a feeding trial of 3 months (Fig.25).

7) Glucose Tolerance Test (GTT)

Fig. 26 shows the GTT of the subject, and the result revealed that for *Njavara* grits the values were lower when compared to reference food glucose.

8) Antioxidant properties

Fig. 27, 28 and 29 shows the antioxidant activities of subject C and the findings revealed that DPPH radical scavenging activity, hydroxyl radical scavenging activity and superoxide anion radical scavenging activity, were increased after three months supplementation.

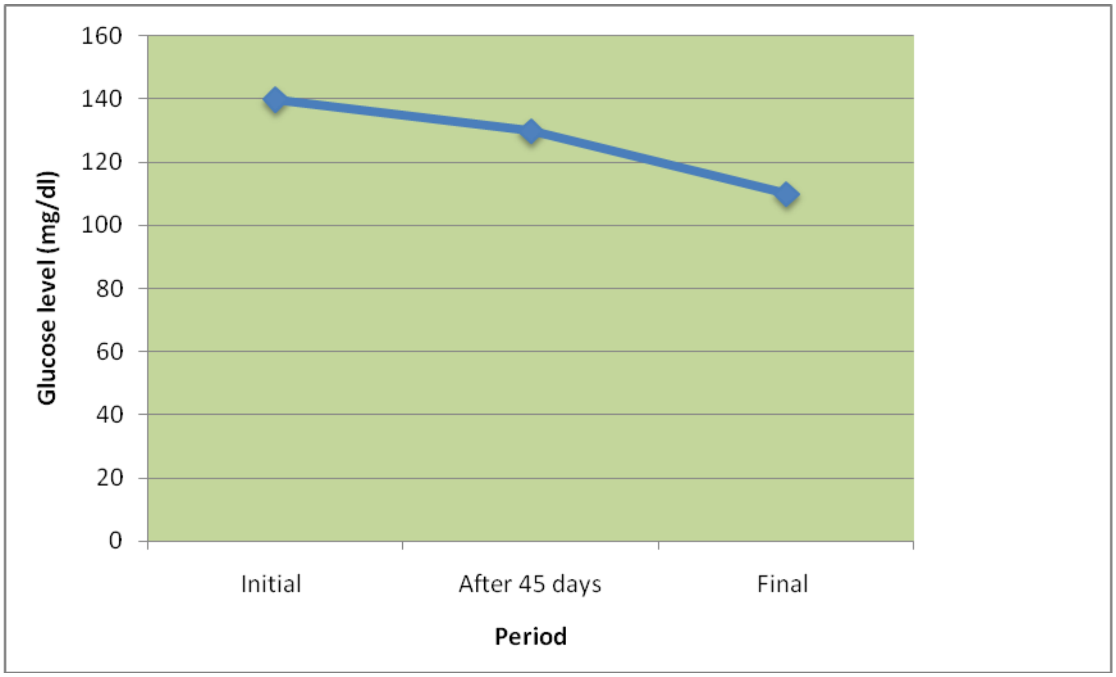


Fig. 25 Changes in blood sugar levels of subject C

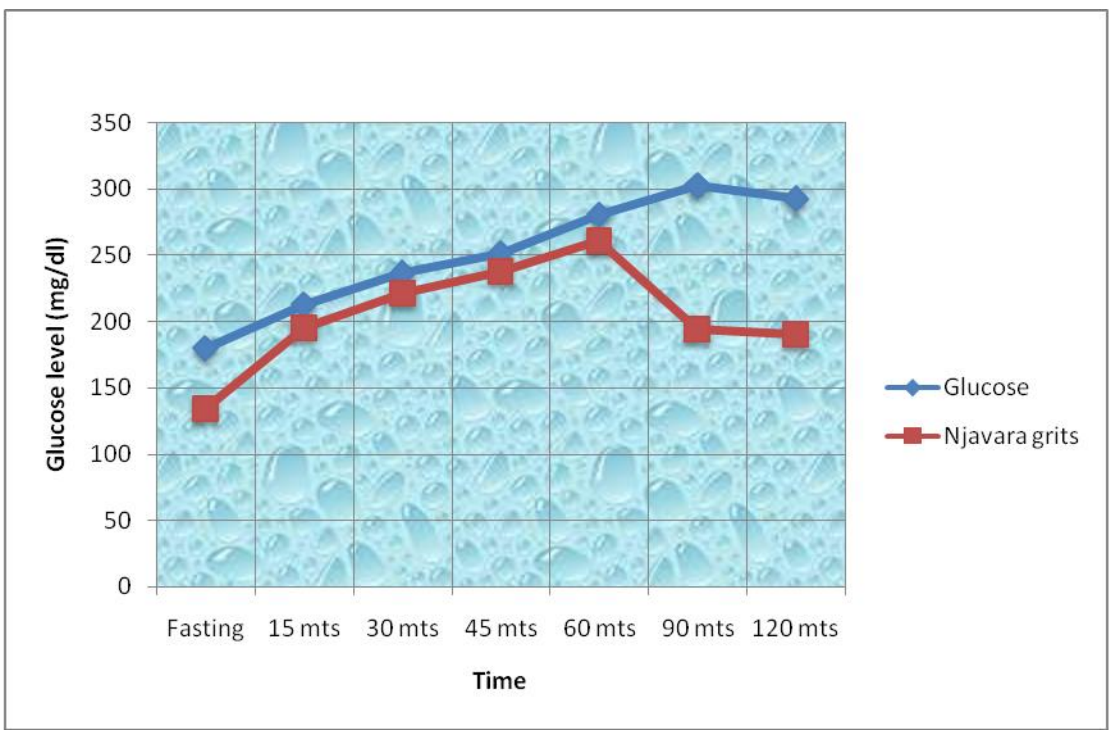


Fig. 26 GTT of subject C

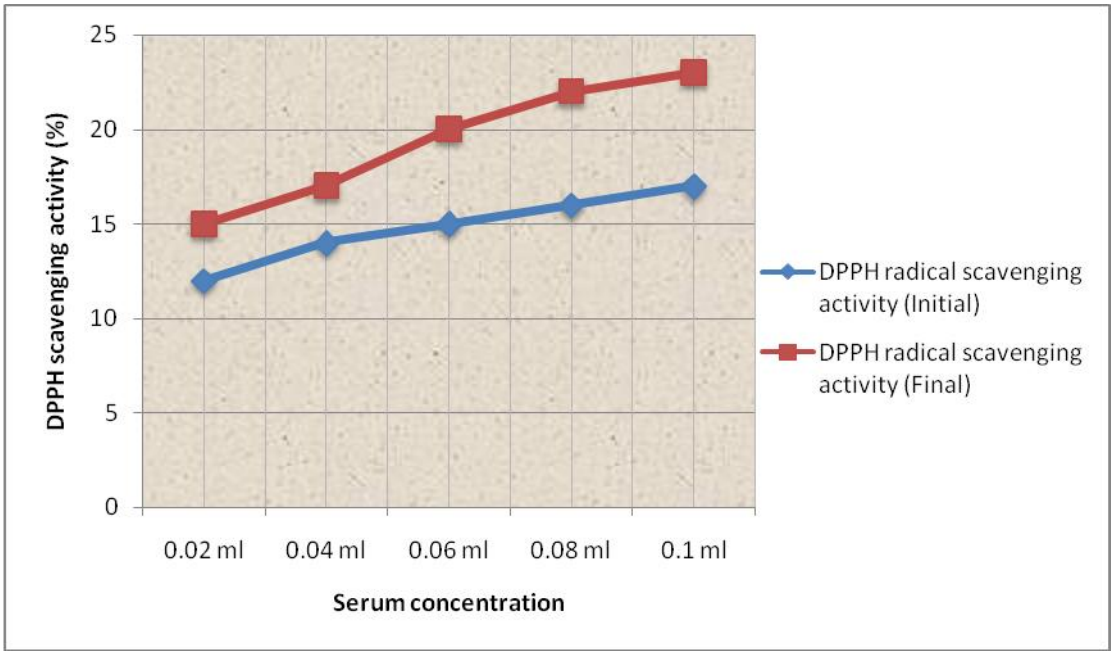


Fig. 27 DPPH radical scavenging activity of subject C (before and after supplementation)

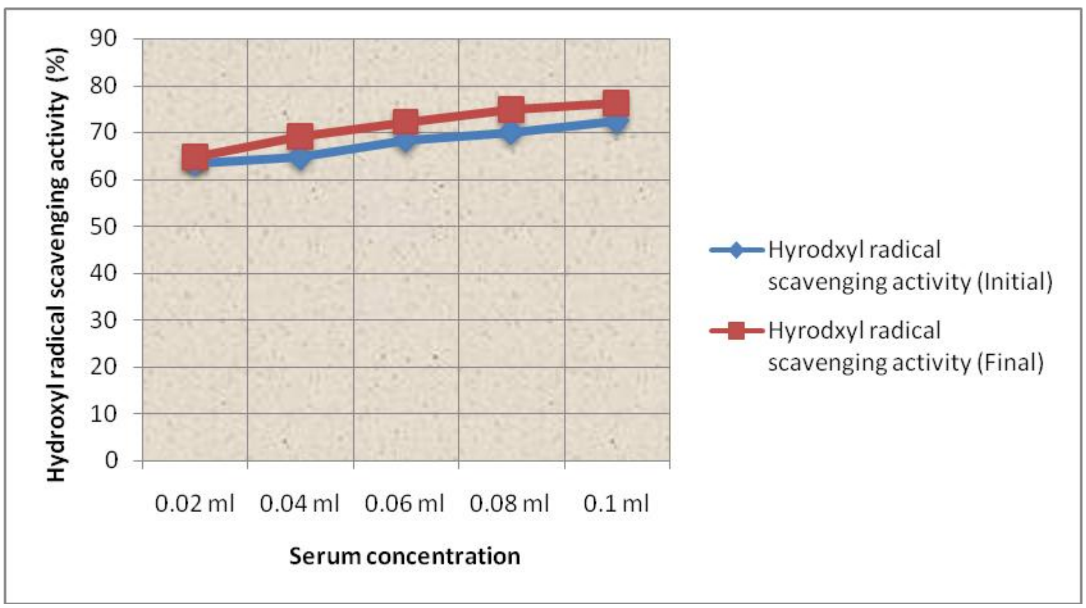


Fig. 28 Hydroxyl radical scavenging activity of subject C (before and after supplementation)

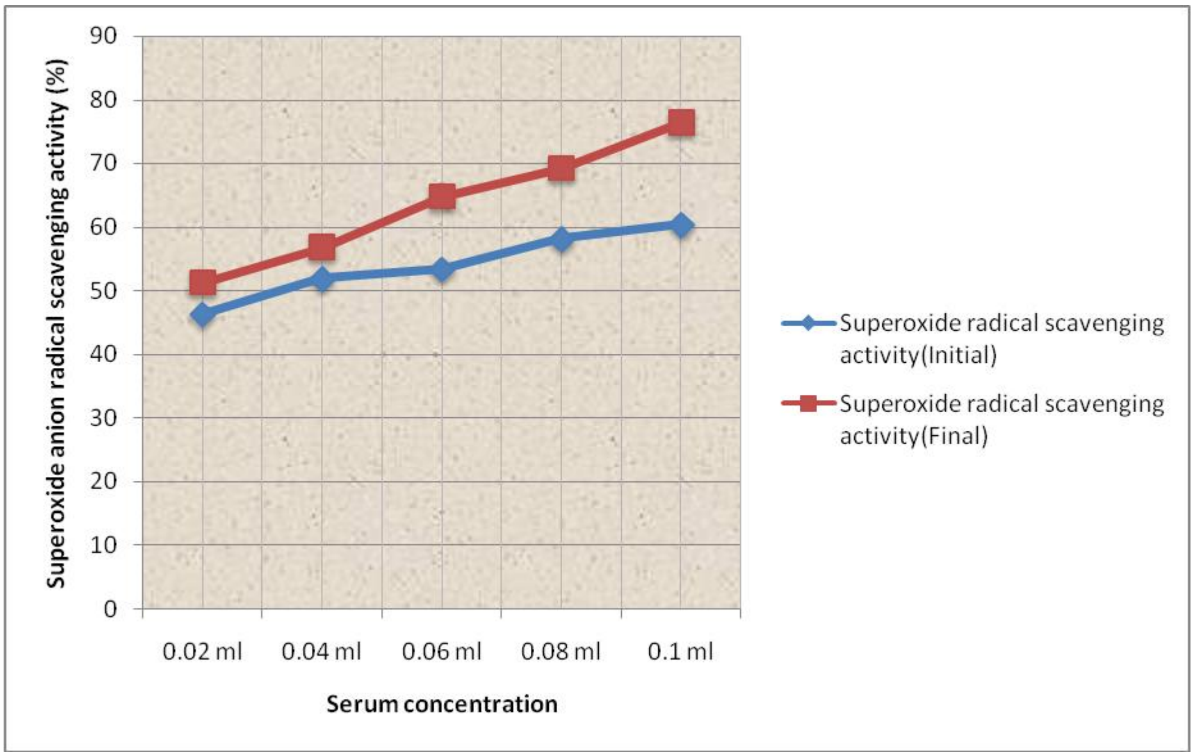


Fig. 29 Superoxide anion radical scavenging activity of subject C (before and after supplementation)

Subject D

1) **Socio economic profile**

Age	:	49 yrs.
Gender	:	Female
Religion	:	Hindu
Type of family	:	Nuclear
Family income (monthly) (Rs.)	:	50,000 – 60,000
Educational status	:	Plus two
Occupation	:	House wife

2) **Duration of diseases & other related problems**

The subject is having diabetes for the past 2 years. There was no family history of diabetes. The subject has cholesterol problem but it was under control. She doesn't have gestational diabetes and had menopause 5 years before the supplementation.

3) **Exercise pattern**

The subject is having a moderate activity level. She has a regular exercise pattern of walking for 30 minutes.

4) **Dietary pattern**

The subject is a vegetarian. She includes cereals, pulses, vegetables, milk and milk products, tea in her daily diet. Her frequency of meals was three meals per day and she uses coconut oil for cooking. Her average intake of calorie was 1642 Kcal/day.

5) Anthropometric data

Height (cms)	:	150
Weight (Kg)	:	53
BMI	:	23.56
Category	:	Normal
Waist circumference (cms)	:	99
Hip circumference (cms)	:	100
W/H ratio	:	0.94

Interpretation

This subject comes under normal category, with a BMI of 23.56 and a waist hip ratio of 0.94. The subject was given 63.00 g of NB grits for 3 months.

6) Effect of *Njavara* grits on blood sugar level

Table 42 shows the fasting blood sugar levels of the subject before and after the supplementation.

Table 42. Changes in blood glucose levels of subject D

Monitoring intervals	Blood sugar levels (mg/dl)
Initial	150
Intermittent (after 45 days)	130
Final	109

The initial value of fasting blood sugar of subject D was 150 mg/dl (before the supplementation). After 45 days of supplementation it decreased to 130 mg/dl, and finally the blood sugar level decreased to 109 mg/dl after a period of 3 months (Fig.30).

7) Glucose Tolerance Test (GTT)

Fig. 31 shows the GTT of the subject, and the result revealed that for *Njavara* grits the values were lower when compared to reference food glucose.

8) Antioxidant properties

Fig. 32, 33 and 34 shows the antioxidant activities of subject D and the findings revealed that DPPH radical scavenging activity, hydroxyl radical scavenging activity and superoxide anion radical scavenging activity were increased after three months supplementation.

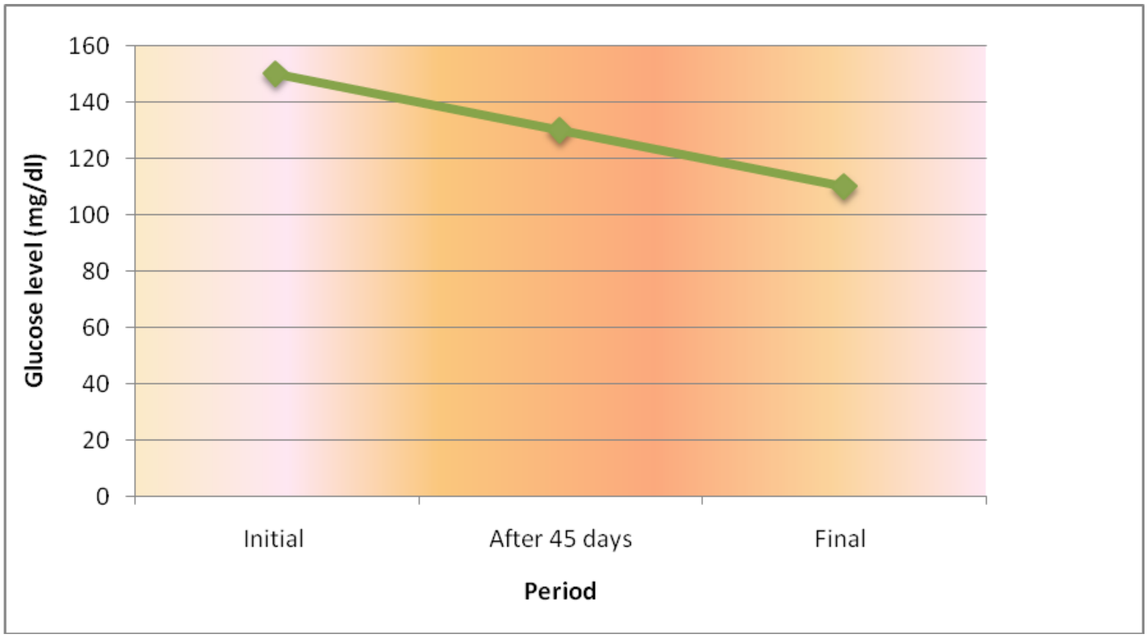


Fig. 30 Changes in blood sugar levels of subject D

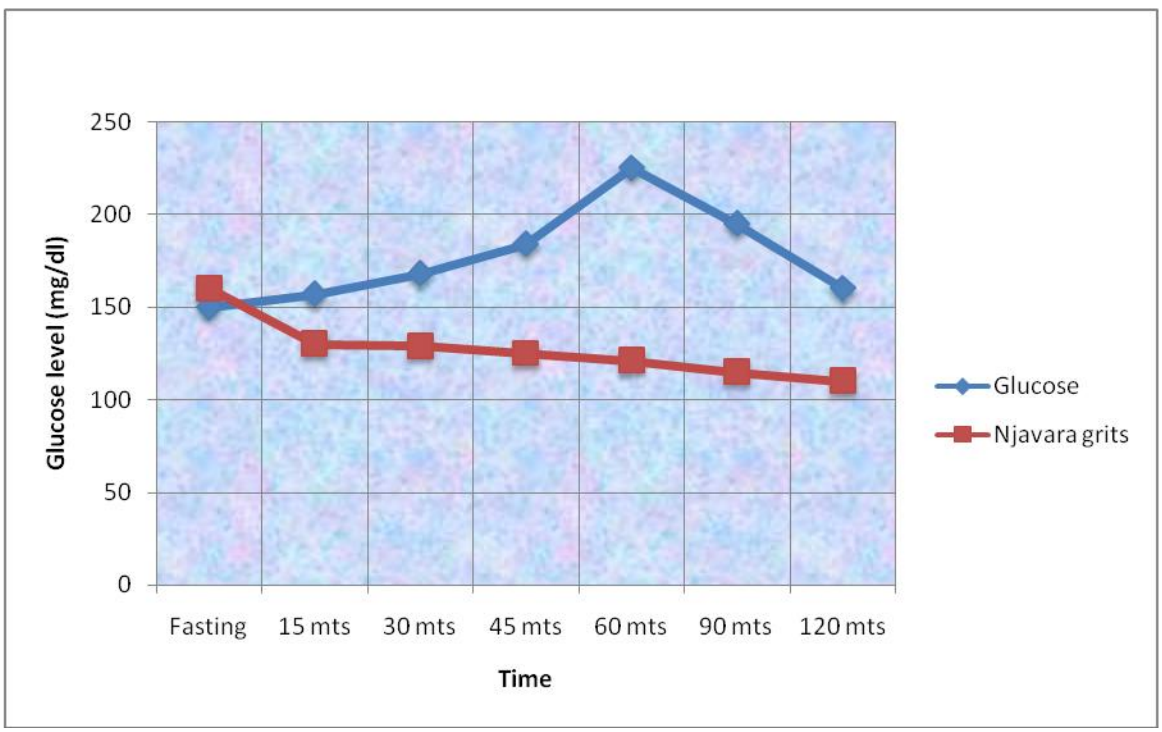


Fig. 31 GTT of subject D

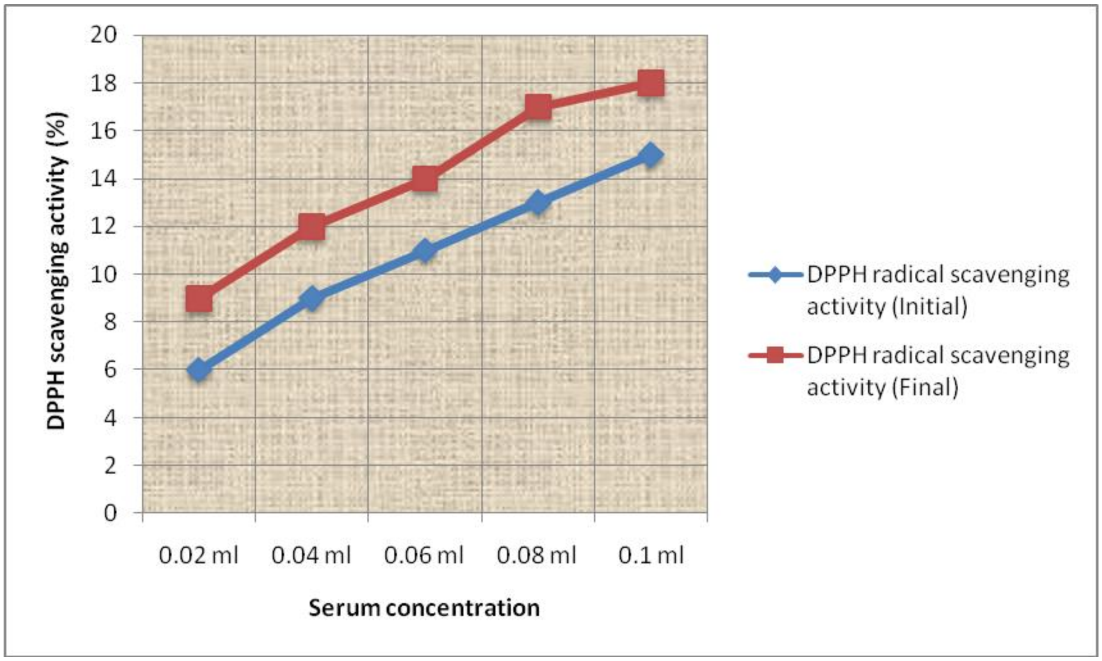


Fig. 32 DPPH radical scavenging activity of subject D (before and after supplementation)

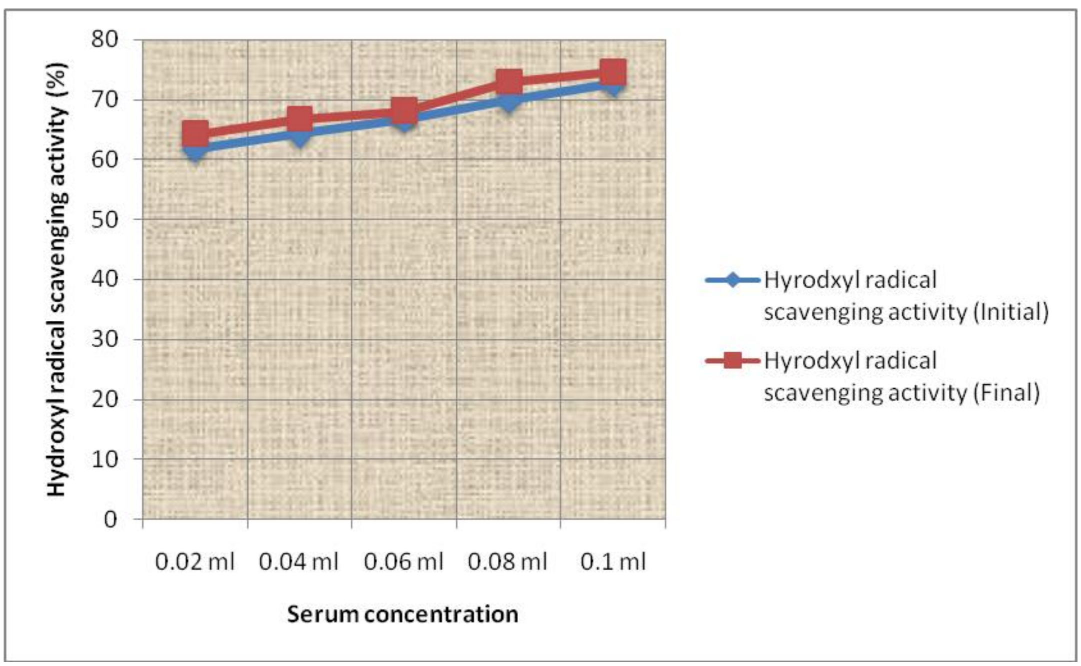


Fig. 33 Hydroxyl radical scavenging activity of subject D (before and after supplementation)

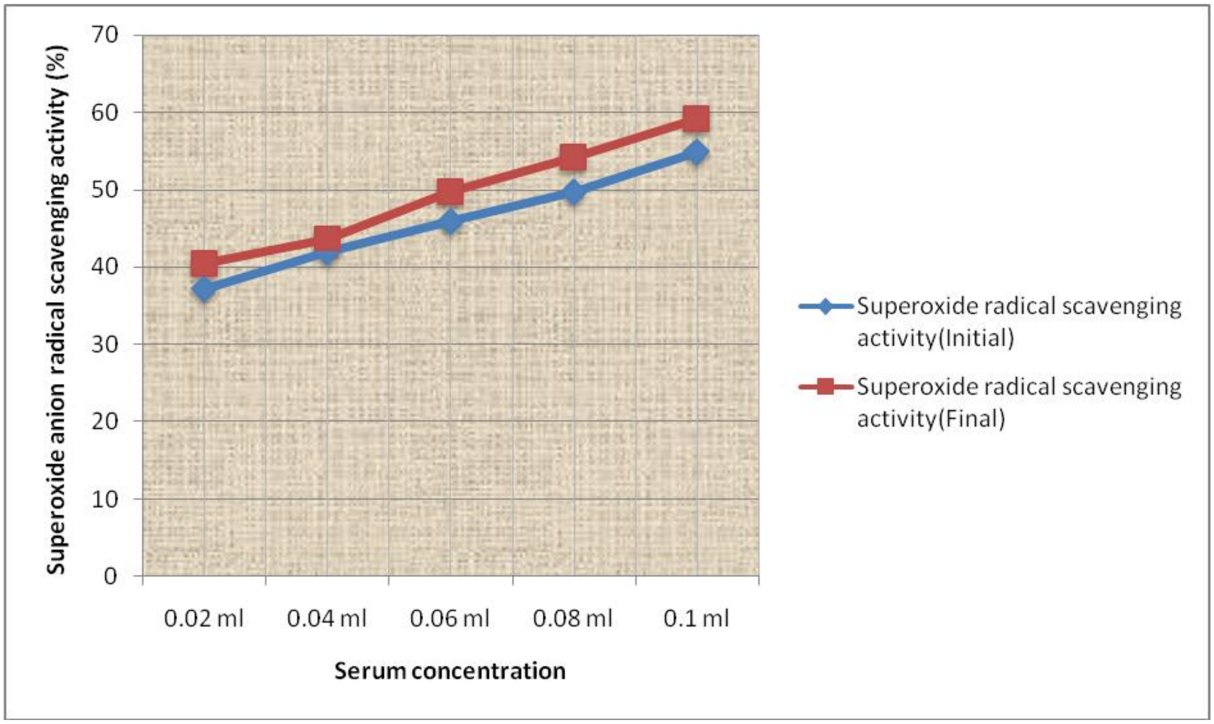


Fig. 34 Superoxide anion radical scavenging activity of subject D (before and after supplementation)

Subject E

1) Socio economic profile

Age	:	48 yrs.
Gender	:	Female
Religion	:	Hindu
Type of family	:	Nuclear
Family income (monthly) (Rs.)	:	> 1, 00,000
Educational status	:	Degree
Occupation	:	Bank employee

2) Duration of diseases & other related problems

The subject is having diabetes for the past 3years. There was no family history of diabetes. There was no diabetes related problems or gestational diabetes. The subject had menopause 4 months before the supplementation.

3) Exercise pattern

The subject was having a moderate activity level. She is not having a regular exercise pattern, but she walks for 30 minutes 2-3 times in a week.

4) Dietary pattern

The subject is a vegetarian and includes cereals, pulses, vegetables, milk and milk products and tea in her daily diet. Her frequency of meals was three meals per day and she used coconut oil for cooking. Subject E's average intake of calories per day was 1844 Kcal.

5) Anthropometric data

Height (cms)	:	162
Weight (Kg)	:	74
BMI	:	28.20
Category	:	Obese grade I
Waist circumference (cms)	:	101
Hip circumference (cms)	:	108
W/H ratio	:	0.99

Interpretation

This subject comes under obese grade I category, with a BMI of 28.20 and a high waist hip ratio of 0.99. The subject was given 56.00 g of NB grits for the supplementation.

6) Effect of *Njavara* grits on blood sugar level

Table 43 shows the changes in fasting blood sugar level of the subject before and after the supplementation.

Table. 43 Changes in blood glucose levels of subject E

Monitoring intervals	Blood sugar levels (mg/dl)
Initial	250
Intermittent (after 45 days)	220
Final	160

The initial value of fasting blood sugar of subject E was 250 mg/dl (before the supplementation). After 45 days of supplementation it decreased to 220 mg/dl, and finally the blood sugar level decreased to 160 mg/dl after the feeding trial (Fig.35).

7) Glucose Tolerance Test (GTT)

Fig. 36 shows the GTT of the subject, and the result revealed that for *Njavara* grits the values were lower when compared to reference food glucose.

8) Antioxidant properties

Fig. 37, 38 and 39 shows the antioxidant activities of subject E and the findings revealed that for DPPH radical scavenging activity, hydroxyl radical scavenging activity and superoxide anion radical scavenging activity were increased after three months supplementation.

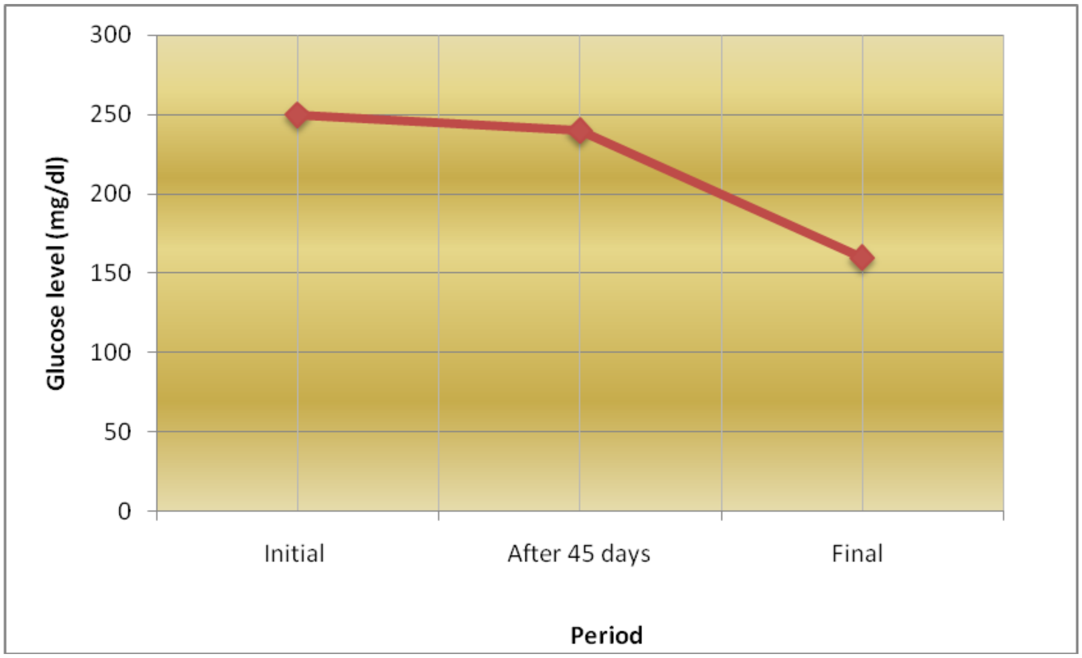


Fig. 35 Changes in blood sugar levels of subject E

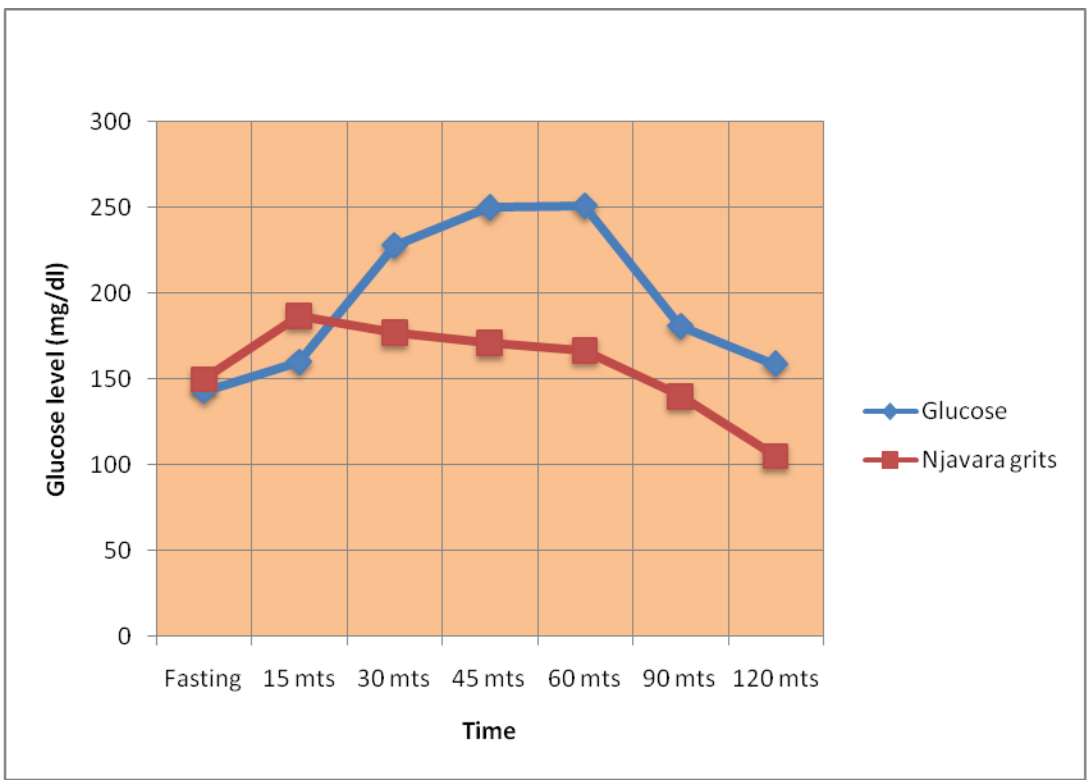


Fig. 36 GTT of subject E

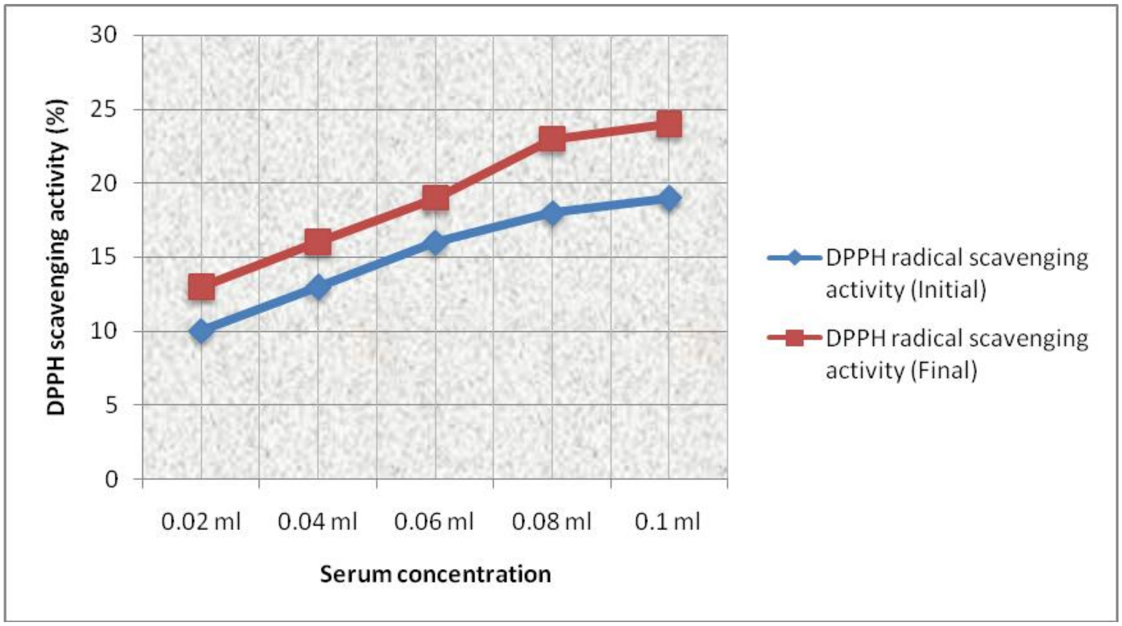


Fig. 37 DPPH radical scavenging activity of subject E (before and after supplementation)

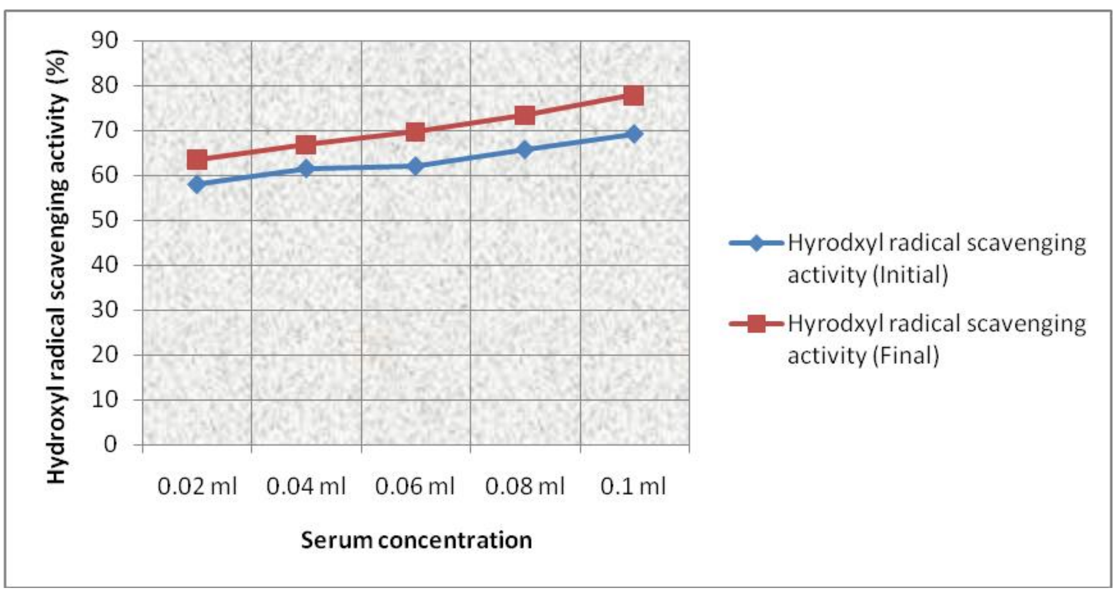


Fig. 38 Hydroxyl radical scavenging activity of subject E (before and after supplementation)

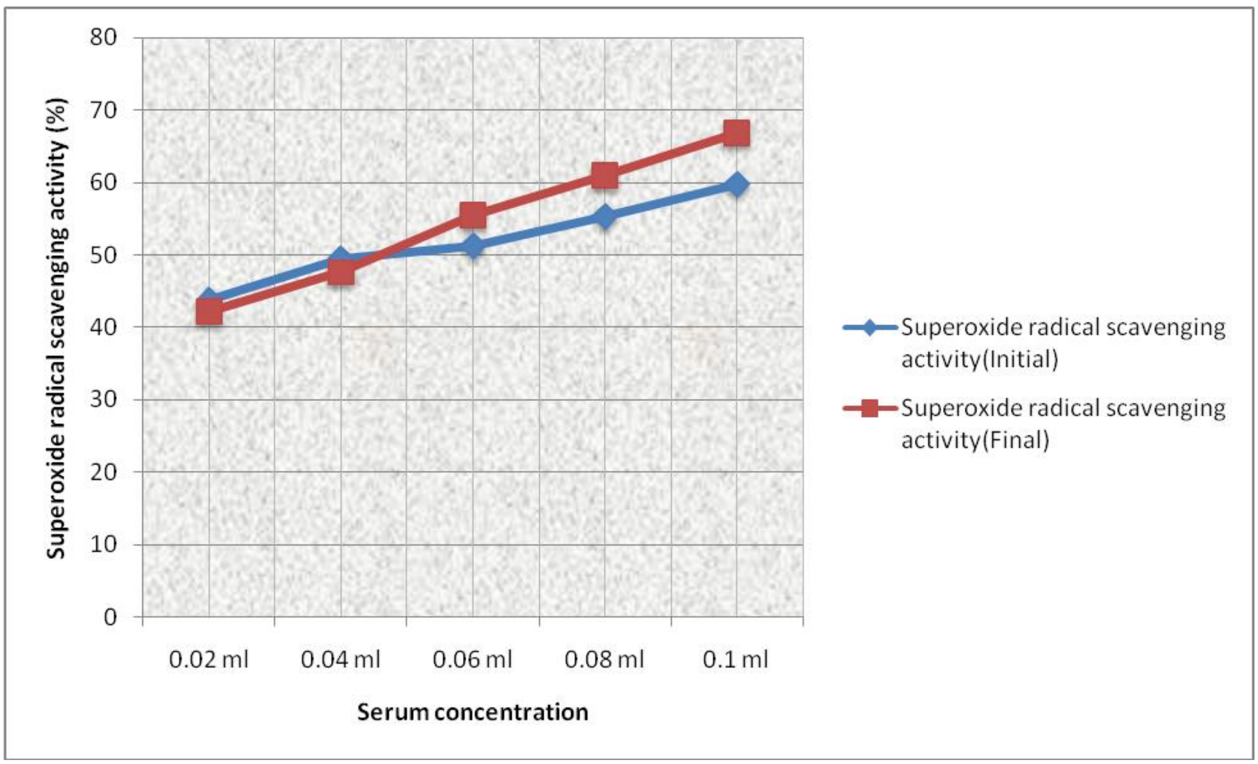


Fig. 39 Superoxide anion radical scavenging activity of Subject E (before and after supplementation)

Glycemic index

Table 44 shows the mean area under the plasma glucose response curve (AUC) for test food (*Njavara* grits) and reference food (Glucose). As indicated in table (43) glucose had an AUC of 360 mm^2 whereas test food had an AUC of 260 mm^2.

Table 44. Mean area under the curve of test food and reference food

Food	Area under the curve (mm^2)
Glucose (Reference food)	360.00
Njavara rice (Test food)	260.00

Based on AUC, the glycemic index of the test food (*Njavara* grits) was determined which is depicted in Table 45. As shown in table 45 (Figure 40) *Njavara* grits was having a GI of 72 which was around 28 per cent less than that of glucose.

Table 45. Glycemic Index of test food

Food	Glycemic response
Glucose	100
Njavara rice	72

Table 46 depicts the glycemic load of *Njavara* grits (test food).

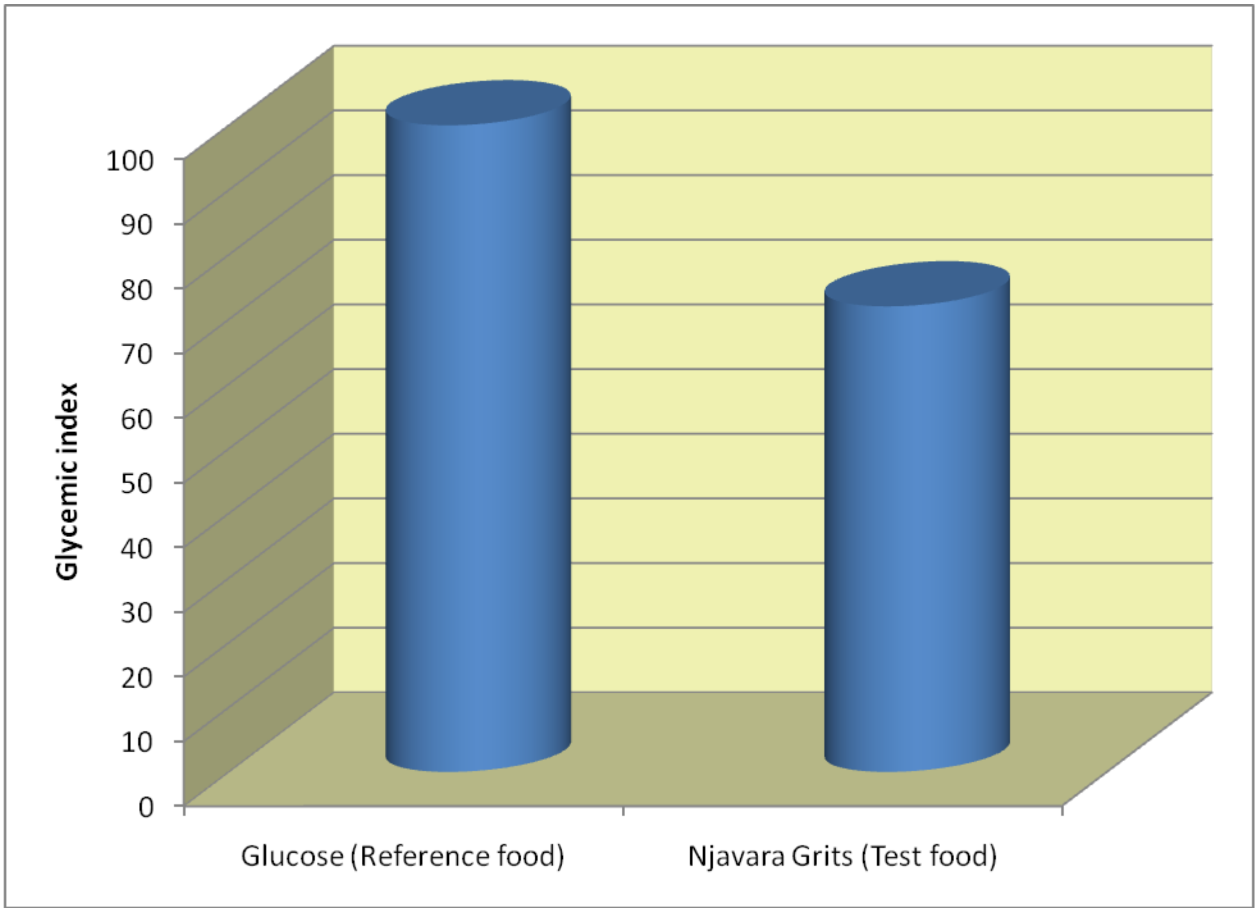


Fig.40 Comparison of Glycemic Index of the Test food and Reference food

Table 46. Glycemic load of test food

Food	**Glycemic load**
Njavara grits	36

The result of glycemic load revealed that *Njavara* grits had a glycemic load of 36.

DISCUSSION

5. DISCUSSION

The results of the present study entitled "Quality evaluation of medicinal rice (*Oryza sativa* L.) cv. *Njavara* for product development and therapeutic value" is presented under the following heads-

5.1. Ist Experiment – Quality evaluation of selected rice varieties

5.1.1. Physico-chemical characteristics

5.1.2. Nutritional composition

5.1.3. Antioxidant properties

5.2. IInd Experiment – Quality evaluation of processed *Njavara* grits

5.2.1. Physico-chemical characteristics

5.2.2. Nutritional composition

5.2.2. Antioxidant properties

5.2.3. Shelf-life quality

5.2.4. Selection of superior variety

5.3. IIIrd Experiment – Assessment of the therapeutic value of *Njavara* grits through case studies

5.3.1. Formulation, sensory evaluation and suitability of *Njavara* grits

5.3.2. Results of case study

5.1. I^st Phase – Quality evaluation of selected rice varieties

Quality evaluation of rice varieties has been investigated by a number of workers who had reported that no single parameter can be universally accepted as the sole criterion for judging the rice quality (Khan and Reddy, 2009). According to Malik and Choudhary (2001) rice quality depends on a combination of several physiochemical properties of the rice grains.

5.1.1. Physico-chemical characteristics

In the present study, both the *Njavara* varieties i.e. *Njavara* Black (NB) and *Njavara* Yellow (NY) and the control variety *Hraswa* were found to have red colour. According to Reddy (2000) *Njavara* has a wide range of colour.

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. In the present study, all the three varieties were found medium in shape. With respect to size, NB and *Hraswa* (control) variety were found to be medium, whereas NY was found to be short. In a study done by Nandini (1995), *Hraswa* was categorized under bold and *Njavara* under medium bold.

Physical dimensions of grain 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 important criteria of rice quality especially for developing new varieties for commercial production (Bandhyopadhyay and Roy, 1993; Dela and Khush, 2000). The kernel appearance, size, shape and nutritional value are important for judging the quality and preference of rice from one group of consumer to another (Dela and Khush, 2000; Sellappan et al., 2009 and Qin et al., 2008).

In the present study, the mean grain length of NB was 5.60 mm and that of NY was 5.30 mm and the mean length of the control variety *Hraswa* was 6.48 mm. The mean values for breadth of NB, NY and *Hraswa* were 2.12 mm, 1.88 mm and 2.42 mm respectively. Reddy (2000) found that the mean grain length and breadth of *Njavara* ranged between 7.6 mm to 9.3 mm and 2.65 mm to 3.33 mm respectively. In a study done by Korde et al. (2006) also, it was observed that mean length and breadth varies from 5.80 mm to 6.96 mm and 2.36 mm to 2.76 mm respectively in raw rice.

Length/width ratio for NB and NY were 2.65 and 2.84 respectively, while 2.68 was the L/B ratio obtained for *Hraswa*. Kernel shape and L/B ratio are important features for grain quality assessment (Rita and Sarawgi, 2008).

According to Oghbaei and Prakash (2010) length/breath ratio denoted the shape and size of a grain. The authors found a ratio of 2.70 for raw rice. Khatoon and Prakash (2007) reported a ratio of 2.84 and Khan and Reddy (2009) reported a ratio of 2.6 to 4.0 for different rice varieties. But Nandini (1995) reported an L/B of 2.31 for *Hraswa* and 2.16 for *Njavara* rice.

Moisture content of rice is a very important factor which markedly affects rice quality especially shelf life and milling quality (Sotelo et al., 2000). Grain should be dried to at least 14 per cent to avoid losses due to breakage in milling (Kunze, 2001). In the present study, NY recorded high moisture percentage. Nandini (1995) observed moisture content of 13.69 per cent in *Hraswa* and 12.75 per cent in *Njavara* rice in her study. According to Sotelo (2000) the moisture content of raw polished rice varies from 9.00 – 12.50 per cent. The moisture content reported by Khatoon and Prakash (2007) for four varieties of raw rice samples were found to be 9.50 – 10.50 per cent. These findings are in line with our results.

Thousand grain weight is a major determinant in adjudging the popularity of rice varieties. In the present study, thousand grain weight of the rice varieties varied significantly at 1 per cent level. The control variety *Hraswa* was found to have highest thousand grain weight (20.80 g), followed by NB (16.40 g) and NY (13.00 g). According to Elsy et al. (1992) *Njavara* rice varieties has a mean 1000 grain weight of 20.10 g but Reddy (2000) observed that it ranged between 18.50 g to 30.00 g. The results are in accordance with the findings of Sarkar et al (2004) who had reported that the 1000 grain weight of rice varieties to be 15.82 to 26.80 g. According to Luh (1999) values below 20 g indicate the presence of immature, damaged or unfilled grains thus *Njavara* has a very low 1000 grain weight.

Gelatinization temperature is the temperature at which rice starch begins to melt (gelatinize) and take up water. Rice has a gelatinization temperature of 65-85 °C (Aquerreta et al., 2007).

Kaddusmiah et al. (2002) reported that the gelatinization temperature is the range of temperature where in at least 90 per cent of the starch swell irreversibly in hot water with loss of crystalline and birefringence. The gelatinization temperature of rice varieties may be classified as low (55 to 69°C), intermediate (70 to 74°C) and high (>74°C) (Dela and Khush, 2000).

In the present study, the gelatinization temperature of all the three varieties including *Hraswa* was found to be high (>74°C). Similar results were reported by Simi and Abraham (2008). Singh et al. (2006) also observed that rice varieties with intermediate gelatinization temperature (70-74°C) are preferred all over the world, as at high gelatinization temperature rice remains mainly under cooked and hence not preferred.

The gelatinization of starch is influenced by many parameters such as amylose: amylopectin ratio, the degree of hydration and the size of starch granule

(Swamy et al., 2001). Gel consistency differs depending upon the source of starch and variety of grain. The results for gel consistency for 30 and 60 minutes in the rice varieties NB were 54.40 mm and 56.60 mm respectively, for NY 54.00 mm and 55.60 mm and that of control variety *Hraswa* it was 49.40 mm and 51.40 mm. The findings are further supported by Sarkar et al. (2004) who observed a gel consistency between 22 to 78 mm for scented and non-scented varieties. The gel consistency (GC) was measured in to soft, medium and hard. The GC of the rice samples ranged from 49.40 mm - 56.60 mm.

Bulk density of any material indicates the weight volume ratio and is an important parameter from storage point of view (Oghbaei and Prakash, 2010). Bulk density of rice samples in the present study was found to be between 0.96 – 1.00 g/ml, which was similar to the findings of Oghbaei and Prakash (2010). The bulk density is related to the kernel shape i.e. L/B ratio, the more round the kernel the greater the bulk density.

Chalkiness index is to evaluate a representative milled sample for the degree (extent) of chalkiness that will best describe the sample with respect to (a) white belly (b) white centre and (c) white back (www.knowledge.irri.org).

Results of the present study revealed that NY had the highest value of chalkiness index (5.02) while NB has the lowest chalkiness index (0.05) among the three rice varieties. But in contrast Cheng and co-workers (2006) who found that chalkiness of 15 rice varieties ranged from 13.80 to 64.36. The chalky grains reduce the palatability of cooked products and the presence of more than 20 per cent chalkiness in rice kernels is not acceptable in World markets.

Viscography represents the pasting property of rice. Pasting properties are reported to be influenced by granule size, amylose/amylopectin ratio, starch molecular characteristics and the condition of the thermal process employed to induce gelatinization (Zhou et al., 2003). Data on viscography of rice varieties of the present study shows that NB (16.80 pb) had the highest viscography value followed by NY (11.60 pb) and *Hraswa* (control variety) (10.00 pb).Similar results were reported by Deepa et al. (2011) and Simi and Abraham (2008).

Hardness of cooked rice is one of the most important criteria which determine the rice quality (Jiang et al., 2010). In the present study, among the three rice varieties control variety *Hraswa* has got the highest hardness (4.68 Kg), followed by NB (3.72 Kg) and NY (3.05 Kg). The results are supported by Webb et al. (2000). According to Pomernanz and Webb (2000) and Cameron and Wang (2005) rice hardness is important to many facets of the rice industry and has a positive correlation with stickiness.

Salient Findings

The results of the physical characteristics revealed that regarding the colour of the rice samples all the samples were red in colour. The size of the selected rice varieties revealed that NB and *Hraswa* was medium in size , whereas NY was found to be short in size. Shape of the rice varieties revealed that all the samples were medium in shape. The length and breadth of the rice was found to be highest in *Hraswa* (Control) variety, followed by NB and NY. The L/B ratio was recorded highest for NY among the three selected rice varieties.

Thousand grain weight was highest for variety *Hraswa* (control) and lowest for NY. Highest moisture content was found in NY, followed by *Hraswa* and then NB. *Hraswa* (control) variety had the highest value for gelatinization temperature and NY had the lowest value. Bulk density of the rice samples showed that NB had the highest value and *Hraswa* was having lowest value. NB had the highest value for gel consistency followed by NY and *Hraswa*.

Chalkiness index of the selected rice samples showed that NY had the highest value and NB had the lowest value. NB had the highest value for viscography and *Hraswa* (control) had the lowest value. Regarding the hardness of the rice samples, *Hraswa* (control) variety had the highest value and NY had the lowest.

5.1.2. Nutritional composition

Rice is a very widely used staple cereal and is a main source of energy for more than two-thirds of population in India (Mujoo and Ali, 2009), and is primarily a high energy or high calorie food (Singh et al., 2006).

It supplies almost 60 per cent of the dietary energy. In the present study, NY had the highest energy content of 357.00 Kcal/100g followed by *Hraswa* (337.00 Kcal) and NB (323.00 Kcal). The results are in line with the findings of Deepa et al. (2008) and Nandini (1995) who found energy content of 389 Kcal and 325 Kcal/100 g of rice for *Njavara* and *Hraswa* respectively.

Protein content of rice is important from nutritional point of view. Among cereal proteins, rice protein is biologically the richest by virtue of its high true digestibility (88 per cent) and relatively better net protein utilization (NPU) (Rai, 2009). Protein content of samples in the present study ranged between 8.77 to 12.15g. The findings are further supported by Reddy (2000) and Dhanvantari Ayurveda Academy (www.dhanvantari.in) who found that protein content of *Njavara* grains ranged from 9.00 to 19.00g. On the basis of nutritional value the *Njavara* varieties contained sufficient amount of protein.

Devi et al. (1997) stated that proteins present in food are a mixture of several fractions which differs in its solubility, amino acid composition and digestibility. Zelensky (2001) is of the opinion that the protein content of rice though relatively low in its nutrient value is much higher compared to other cereals. Rice protein is easily digested (up to 98 per cent) and contains all the essential amino acids (except lysine) than in other cereal crops.

In the present investigation, 18 amino acids including 8 essential amino acids were estimated in the rice samples to get more precise data on the quality of protein. When the values of essential amino acids of *Njavara* rice samples were compared to that of reference protein (Fig. 1) it was found that all the essential amino acids (except methionine and lysine) were almost equal to that of reference protein. When the comparison of values of essential amino acids of *Njavara* with that of *Hraswa* (control variety) was done, it was found that values of threonine, valine, methionine and isoleucine of *Hraswa* were lower than that of *Njavara* varieties.

In a study by Nair (2004) the total free amino acid content of *Njavara* grains are higher compared to other land races such as Ptb 10, Chitteni and Kunjukunju etc. The free amino acid contents of NB and NY (golden) were reported to be 0.316 mg/g and 0.089mg/g respectively (Menon, 1996a).

In the present study, the highest amount of non-essential amino acid present in the rice samples was glutamic acid followed by aspartic acid and proline (Table 10).

Amino acid score (AAS) of the rice samples revealed that NY had more number of amino acid score than NB. Data on Essential Amino Acid (EAA) Index revealed that NB had high EAA index compared to NY.

Menon and Potty (1999) reported that *Njavara* contains high sulphur containing amino acid, methionine, which is believed to be involved in the biosynthesis of thiamin. The authors also found that from the metabolism of the two amino acids, phenylalanine and tyrosine, an ubiquitous polyphenol is formed known as ferulic acid and this may be the possible reason of high total free amino acid content. A study by Shareesh (2007) done in KAU on *Njavara* rice indicated the presence of a gene fragment encoding protein, Christened Bowman-Birk Trypsin Inhibitor protein which is reported to have anti-carcinogenic, anti-inflammatory and anti-allergic properties.

Crude fibre is a mixture of substances which make up the frame work of plants and is composed of cellulose, hemicelluloses and lignin of the cell walls. Rice is reported to be a moderate source of fibre. Most of the crude fibres of rice are in the bran and outer layers, for this reason brown rice has higher crude fibre content than milled rice (Badawi and Ei-Hissewy, 2001).

The crude fibre content of the rice samples were significantly different (F=3.84*). NB had the highest crude fibre content among the three rice varieties (0.206 g) and *Hraswa* which is the control variety had the lowest crude fibre content (0.190 g). The results are in accordance with the studies carried out by Ramulu and Udayasekhararao (2007).

Soluble fibres are soluble in water, viscous in nature and nearly 100 per cent fermentable in large bowel (Anderson et al., 1994). In the present study, the soluble fibre content of the three rice samples were 0.114 g, 0.096 g and 0.056 g respectively for NB, NY and *Hraswa* (control). Dhanvantari Ayurveda Academy (www.dhanvantari.in) also reported similar findings.

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 (Hayakawa et al., 2000).

The starch content of the rice varieties ranged between 74.45 – 76.14 per cent and did not have much difference from the amount of starch in normal polished raw rice which was found to be 78.20 per cent.

A study done by Khatoon and Prakash (2007) revealed that raw rice had a starch content ranging from 79.90 to 83.00 per cent. Oghbaei and Prakash (2010) reported a starch content of about 80.42 per cent in uncooked raw rice and Sugeetha (2010) reported that varietal differences exist with respect to starch content of the rice varieties.

Amylose content of rice determines the hardness and stickiness of cooked rice. It is one of the most important determinants of rice quality. Rice contains both soluble and insoluble amylose. Insoluble amylose of rice directly affects kernel firmness and inversely affects stickiness and glossiness of cooked grain. Amylose higher than 25 per cent gives non-sticky soft or hard cooked rice. Rice having 20-25 per cent amylose gives soft, and relatively sticky cooked rice (Anonymous, 1997).

In the present study, it was noticed that all the rice varieties viz. NB, NY and control variety *Hraswa* were identified to be medium amylose rice. Preference for intermediate amylose types is the case for the majority of the South and Southeast Asian countries as well.

Amylopectin is the major starch constituent and is the only starch fraction of waxy (glutinous) rice. In the present study, the amylopectin content varied significantly among the rice varieties. The amylopectin content of the three rice varieties ranged between 50.08 – 54.50 per cent.

Sugeetha (2010) found the amylopectin content of raw rice as 59.90 per cent, which is similar to the results of the present investigation.

The ratio of amylose to amylopectin in starch is characteristic of the plant species and is under genetic control. In the present investigation, it was noticed that amylose to amylopectin ratio of rice varieties varied significantly with an F value of 22.38 ** (0.39-0.49). Similar findings were also reported by Sugeetha (2010). According to Leloup et al. (1991) when the ratio of amylose and amylopectin exceeds 2.5 it can have a strong influence on the swelling index and based on which it can be concluded that the ratio doesn't affect the swelling index of *Njavara.*

Minerals are essential for normal metabolic functions and are required components in a balanced diet.

The ash content of a foodstuff is the organic residue remaining after the organic matter has been burnt away (Kirk and Sawyer, 1991). It was also noted that there was a significant difference (F=12.07 **) among the varieties with respect to ash content. Similar results were reported by Deepa et al. (2008) for *Njavara* rice.

Calcium is an important component of a healthy diet and a mineral necessary for life. The National Osteoporosis Foundation says, "Calcium plays an important role in building stronger, denser bones early in life and keeping bones strong and healthy later in life." (Grant et al., 2005). In the present study calcium content of rice samples ranged from 10.80 - 12.80 mg/100g of rice. Deepa et al. (2008) reported that *Njavara* rice had 11.6 mg/100g of Ca, while Nandini (1995) reported a Ca content of 10.24 mg/100g in *Hraswa*, which is in agreement with the present study. Moldenhauer et al. (2008) observed that raw milled rice contains 13 mg of Ca/100g of rice, while Oghbaei and Prakash (2010) reported Ca content in the range of 13.07 to 19.36 mg/100g of rice.

The phosphorus content of rice is generally high, about 4 per cent of which is present as phytic acid.

In the present study, it was noticed that the phosphorus (P) content of *Njavara* varieties were exceptionally high when compared to the control variety *Hraswa*. The average value of phosphorus in NB was found to be 352.40 mg/100g while that of NY was 351.40 mg/100g of rice. This is in agreement with the findings of Deepa et al. (2008). The high calcium and phosphorous content in Njavara could be helpful in improving muscle activity in patients suffering from muscle wasting. The phosphorus content in *Hraswa* was found to be only 135.40 mg/100 g of rice. Similar findings were observed by Nandini (1995).

Rice is a principal source of B-vitamins especially thiamine and riboflavin (Rai, 2009). In the present study, the thiamine content was found to be highest in NB (0.98mg/100g) and lowest in control variety *Hraswa* (0.40 mg/100g).

The values are in agreement with the findings of Deepa et al. (2008) who found thiamine content of 0.52 mg in *Njavara* rice, which is about 27-32 per cent more when compared to non-medicinal varieties Jyothi and IR 64. The high thiamine content in *Njavara* rice could be useful in treating muscle weakness, neuritis and other symptoms related to deficiency of vitamin B_1 (Menon, 2004).

Chang and Luh (2001) reported the composition of white rice to be 0.07 mg for thiamine. In another study, the thiamine contents of raw milled rice varied from 0.025 -0.045 mg/100g (Sotelo et al.2000).

The riboflavin content of rice samples in the present investigation varied between 0.047 – 0.061 mg/100g. According to Deepa et al. (2008) *Njavara* grains had about 4 to 25 per cent more riboflavin content when compared to Jyoti and IR 64. Chang and Luh (2001) reported a riboflavin content of 0.03 mg/100g in raw rice.

The variation in the vitamin content of rice reported by various authors may be due to the application of fertilizer, variation in maturity period and the degree of processing (Grewal and Sangha, 2006).

Polyphenols are major plant compounds with potential antioxidant activity. This activity is believed to be mainly due to their redox properties, which play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides (Petti and Scully, 2009; Han et al., 2004).

Present study revealed that among the three rice varieties NB had the highest total phenol content (0.41 mg/100g of rice). Rao and co-workers (2010) in *Njavara* rice bran reported that total phenolic content of *Njavara* is significantly high when compared to Jyoti, Yamini and Vasumathi.

Trace element is a dietary mineral that is needed in very minute quantities for the proper growth, development and physiology of the organism (Bowen, 2006).

In the present study, trace elements like Fe, Mn, Zn, Cu and Se were determined.

When NB and NY were compared, it can be seen that Fe content was equal in both the varieties, whereas Mn content was found to be higher in NY when compared to NB. Regarding Cu content NB had higher value compared to NY. Oghbaei and Prakash (2010) reported that the Fe content in rice was in the range of 1.11 to 1.57 mg/100g. Moldenhauer et al. (2008) reported that milled rice contains 0.2 mg Fe. Similar results were reported by Hossam et al. (2010).

Selenium is a component of the unusual amino acids selenocysteine and selenomethionine. In humans, Se is a trace element nutrient that functions as co-factor for reaction of antioxidant enzymes, such as glutathione peroxidases and certain forms of thioredoxin reductase found in animals and in some plants (ipi.oregonstate.edu). The present study revealed that control variety *Hraswa* had the highest Se content (7.41µg/ml). According to Morris and Levander (1990) polished rice had a Se content of 0.334 µg/gm of rice.

Salient Findings

The salient findings of the nutritional/chemical composition are presented below.

The assessment of the calorific value/energy of the three rice varieties revealed that NY had the highest values for calories when compared to the other two varieties. The protein content was found to be higher in NB and lowest in *Hraswa* (control) variety. The evaluation of protein quality revealed that values of essential amino acids like threonine, valine, methionine and isoleucine of *Hraswa* (control variety) were lower than the values of raw *Njavara* rice samples. NY had more number of amino acid score than NB. Essential Amino Acid (EAA) Index showed that, NB had high value than NY.

NB had the highest crude fibre and soluble fibre content and *Hraswa* (control) variety had the lowest crude and soluble fibre content.

Significant variation was found in the values of total starch among the rice varieties. *Hraswa* (control) was having the highest value, whereas NY was having the lowest value. In the case of Amylose content, all the three varieties were found to be having intermediate amylose content. *Hraswa* (control) variety was having highest value for amylopectin and amylose-amylopectin ratio. Among the three rice varieties selected, NB had the highest ash content.

Regarding the mineral content, NB had highest calcium and phosphorus content. Results of the B-vitamins revealed that NB had the highest value for thiamine and riboflavin. NB had the highest value for total phenol content.

Trace element content of the rice samples revealed that for Fe, NB had the highest value, for Mn, Zn, Cu and Se *Hraswa* (control) variety had the highest value.

5.1.3. Antioxidant properties

Plants and their products have been used for many years for human health. There are still many plants which have various medicinal values but still not explored and used. Plants contain many novel compounds with medicinal values which need scientific exploration. The free radicals are produced in aerobic cells due to consumption of oxygen in cell growth (Barros et al., 2007; Singh et al., 2007). Free radicals cause decrease in membrane fluidity, loss of enzyme receptor activity and damage to membrane protein leading to death (Li et al., 2007). These free radicals are involved in different disorders like ageing, cancer, cardiovascular disease, diabetes, rheumatoid arthritis, epilepsy and degradation of essential fatty acids (Barros et al., 2007; Singh et al., 2007). Antioxidant helps in treatment of above disorders. Antioxidants may be defined as substances whose presence in relatively low concentrations significantly inhibits the rate of oxidation of the target within the biological systems (Lai et al., 2009). According to Geethakutty et al. (2004) *Njavara* is a good antioxidant.

5.1.3. a) DPPH radical scavenging activity

DPPH is a free radical compound which has been extensively used to determine the free radical scavenging activity is considered as a good invitro model widely used to assess antioxidant effica within a very short time (Marxen et al., 2007; Lee et al., 2007).

Results of the present study indicated that *Njavara* yellow variety had shown highest DPPH scavenging activity compared to the other two rice varieties, with an IC_{50} value of 31.52 µg/ml for NY, 33.73 µg/ml for NB and 52.39 µg/ml for *Hraswa* control variety. This is in tune with the results of Smitha et al. (2012) who found that Njavara black variety (bran) was having higher scavenging activity with 2, 2-diphenyl-1-picryl hydrazyl radical with an IC_{50} value of 84.66 µg/ml

A study on *Njavara* rice bran by Rao et al. (2010) revealed that *Njavara* had the highest DPPH scavenging activity among the four tested rice varieties viz. *Njavara* (IC_{50} value of 30.85 µg/ml), Vasumathi (IC_{50} value of 87.72 µg/ml), Yamini (IC_{50} value of 70.58 µg/ml) and Jyothi (IC_{50} value of 48.88 µg/ml).

5.1.3. b) Hydroxyl radical scavenging activity

The hydroxyl radical is a very reactive oxygen species (ROS) with a short half-life, and is considered to be responsible for much of the biological damage inherent to free radical pathology (Hochestein and Atallah, 2008). This radical has the ability to cause strand breakage in DNA, 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, 2003).

In the present investigation, NY showed the highest hydroxyl radical scavenging activity with an IC_{50} value of 46.00 µg/ml) followed by NB (IC_{50} value of 55.68 µg/ml) and *Hraswa* (control variety) had IC_{50} value of 60.78 µg/ml. Similar findings was reported by Smitha (2012).

5.1.3. c) Super oxide anion-radical scavenging activity

Over-production of superoxide anion radical has long been known as the starting point of ROS/RNS accumulation in cells, contributing to redox imbalance and other associated deleterious physiological consequences (Pervaiz and Clement, 2007).

Results of the present study revealed that among the three rice varieties NB showed the highest value for superoxide activity. The IC_{50} value of NB was 48.78 µg/ml, whereas NY has an IC_{50} value of 55.43 µg/ml and *Hraswa* control variety had an IC_{50} value of 60 µg/ml. Similar findings were reported by Smitha et al. (2012).

Salient Findings

The results of the DPPH and Hydroxyl radical scavenging activity revealed that among the three rice varieties selected NY had the highest DPPH and hydroxyl radical scavenging activity with an IC_{50} value of 31.62 µg/ml and 46 µg/ml respectively.

Regarding the superoxide anion radical activity, NB had the highest activity with an IC_{50} value of 48.78 µg/ml.

5.2. IInd Phase – Quality evaluation of processed *Njavara* rice (Rava)

5.2.1. Physico-chemical characteristics

Results of moisture content revealed that grits had a higher moisture percentage compared to raw form. The moisture percentage increased from 11.60 to 15.36 in NB (G), whereas in NY (G) it has increased from 13.00 to 15.12 per cent. Statistical analysis revealed that there is a significant increase in the moisture percentage at 1 per cent level after it was converted into grits form. Similar studies were reported by Oghbaei and Prakash (2010).

Gelatinization temperature strongly influences the cooking quality of rice. In the present, *Njavara* had a significantly higher value for gelatinization temperature compared to raw *Njavara* rice. The gelatinization temperature was found to increase with parboiling process. Increased gelatinization temperature as a result of parboiling was also reported by Shilpa and Krishnan (2010).

Data regarding the bulk density revealed that the values had decreased but the decrease was not significant statistically. Similar results were reported by Oghbaei and Prakash (2010), where the authors found a decrease in the bulk density of parboiled rice which was not significant.

Gel consistency values revealed that there was a significant increase in gel consistency both at 30 minutes and 60 minutes. Khatoon and Prakash (2007) also reported an increase in gel consistency as a result of parboiling. In the present study also parboiling was done before converting the rice into grits so parboiling may be the reason for the increase in the gel consistency values.

Data on viscography shows that NB had higher viscography values in both raw and grits form. This may be due to the high moisture percentage noted as a result of parboiling. Similar findings were reported by Fitzgerald et al. (2003), who found that moisture percentage is directly proportional to viscography i.e. as the moisture percentage of rice increases viscography of the rice also increases.

Salient Findings

The results of the physical characteristics depicted that regarding moisture content of the grits, NB had highest value compared to NY (G). Gelatinization temperature increased in both the varieties with NB having the highest value. Bulk density values had decreased in both the varieties. Gel consistency and viscography values also had increased in both the varieties.

5.2.2. Chemical/Nutritional composition

According to Bhattacharjee et al. (2002) rice is a rich source of energy and it contributes over 20 per cent of the total calorie intake of the human population.

An increase in calorific value was noticed after converting into grits which may be due to parboiling. NY (G) had a value of 364.00 Kcal where as that of NB (G) had 342.00 Kcal. During parboiling, the brown outer layers (scutellum and grain) adhere to the grain and most of the nutrients in it are driven into the interior of the grain. Similar findings were also reported by Heinemann et al. (2005) and Sugeetha (2010).

The increase in energy content after processing is due to imbibitions of rice bran oil into the endosperm at the time of parboiling which enables a higher calorific value (Bhavani, 2002).

Protein is the second most abundant constituent in rice. Though the protein content of rice is relatively low, its nutrient value is much higher compared to other cereals.

In the present study, the protein was found to be decreased as a result of parboiling. A significant difference between the rice varieties was observed with respect to protein content (F=83.13**).

The study conducted by Sadhna et al. (1998) and Oghbaei and Prakash (2010) reported that parboiled rice was found to have lower protein when compared to raw rice.

For the quality of protein, 18 amino acids (including essential amino acids) were estimated.

When the values of the essential amino acid of *Njavara* grits were compared with that of raw *Njavara* rice, it was noted that both the *Njavara* grits were having lower essential amino acid values than raw rice. Sadhna et al. (1998) reported similar findings that parboiled rice was found to have lowest free amino acids compared to raw rice.

Values of non-essential amino acids of *Njavara* grits (Table 25) revealed that NB (G) had high values except arginine in comparison with NY (G). The amino acid score of *Njavara* grits depicted that NB (G) had higher amino acid content such as phenylalanine, methionine, threonine and leucine when compared to NY (G) (Table 26). Menon (1996a) reported that *Njavara* black glumed variety contains amino acids DL-2-amino-n-butyric acid and DL-iso-leucine while, golden yellow glumed *Njavara* contains L-Histidine monochloride, L-ornithine monochloride and DL-iso-leucine. According to Menon and Potty (2004) the free amino acid content of black glumed *Njavara* type ranged from 0.316 to 0.814 mg/g with an average of 0.534 mg/g and yellow glumed type ranged from 0.089 to 0.424 g/g averaging 0.212 mg/g.

Mirko et al. (2001) reported that cereals form quantitatively most important sources of dietary fibre.

Crude fibre content of *Njavara* after the treatment of parboiling increased significantly (F= 45.65**). This may be due to the fact that the nutrients from fibrous outer layers are forced into the centre of the grain during parboiling. Similar findings were reported by Heinemann et al. (2005). The mean value of crude fibre of NB(G) was 0.236g/100g and that of NY(G) was 0.254g/100g of rice, whereas the raw values were 0.206g for NB and 0.198 g for NY. According to Devi and Geervani (2000) varietal difference has no implication on increase in dietary fibre content as a result of parboiling.

Soluble fibre content of *Njavara* in the present study after converting it into grits was found to be 0.056g for (NB (G) [NB (raw) = 0.114g] and 0.096 for NY (G) [NY(raw)=0.0.096]. When these values were statistically analysed, significant interaction was obtained among the varieties, treatments and varieties and treatments (F=7.47*, 53.22** and 52.49** respectively).

A study by Hossam et al. (2010) reported that the soluble fibre of white rice was found to be 0.33 per cent and that of brown rice as 1.70 per cent but Lovis (2003) noticed that total fibre in white and brown was 2.4 and 4.6 per cent respectively. The soluble dietary fibre of rice as reported by Devi and Geervani (2000) from three varieties of rice lies in the range of 3.2 to 3.6g/100g

According to Srilakshmi (2004) the major carbohydrate of rice is starch which is 72-75 per cent. In the present study, it was noticed that the starch content of rice varieties decreased as a result of parboiling. This may be due to the fact that during parboiling, starch granules gelatinize and squeeze together, making the endosperm hard and compact (Gill et al., 2003). In the present study, starch content of NB (G) and NY (G) was 57.98 per cent and 56.84 per cent respectively.

Cooking quality of rice depends mainly on amylose content and gelatinization temperature and gel consistency. According to Hettiarachchy et al. (1997) differences in amylose content are attributes in part of the differences in the environmental conditions in which the crop is grown, particularly temperature.

In the present study, the treatment i.e. parboiling had a significant effect on the *Njavara* rice samples. A significant decrease in amylose content was observed after parboiling. This may be due to the gruel loss of gluten into the gruel. Similar effects were reported by Roy et al. (2009) and Korde et al. (2006).

In the present study, the amylopectin content of the *Njavara* grits are 39.24 per cent for NB (G) and 40.08 per cent for NY (G). When the data was statistically analysed it was noticed that there was a significant decrease in varieties, treatment and between varieties and treatments (F=36.32**, 204.95** and 204.32** respectively).

In the present investigation, the amylose-amylopectin ratio varied significantly among the treatments, varieties and varieties and treatments. The mean ratio of NB (G) was 0.48 and NY (G) was 0.42. A slight variation was found among the values of raw *Njavara* and grits and this may be due to variation in total starch and total amylose content. Similar findings were reported by Kadan et al. (2007).

Total ash content of *Njavara* grits revealed that there was an increase in the total ash content. The values of NB was increased from 1.52 per cent to 1.64 per cent and that of NY from 1.26 per cent to 1.52 per cent. Sadhna et al. (1998) and Heinemann et al. (2005) also reported similar findings.

According to Gujral and Singh (2001) raw rice had lower mineral contents than parboiled rice and this indicated that there was diffusion of minerals into the rice kernel during the steeping and steaming process. This is in tune with the findings of the present study.

In the present study, the Ca content was decreased as a result of parboiling. In the raw form the Ca content of NB was 12.80 mg/100g and after parboiling it become 8.60 mg and the raw value of NY was 12.20 mg whereas in grits form it decreased to 9.00 mg/100g of rice.

Similar phenomenon was also observed for phosphorus content. The values of phosphorus decreased from 352.40 mg to 325.40 mg in *Njavara* black variety where as in the case of *Njavara* yellow variety it decreased from 351.40 mg to 326.00 mg/100g of rice. This may be due to the decrease in total ash content. Heinemann et al. (2005) also reported a similar trend in parboiled rice.

Rice is a fair source of water soluble B-complex vitamins. In the present investigation it was noted that both thiamine and riboflavin contents have increased as a result of parboiling.

Thiamine content of the NB (G) was 1.018 mg/100g (raw = 0.98mg/100g) and that of NY(R) was 0.143mg/100g (raw=0.58 mg/100g)

Riboflavin content of the *Njavara* varieties has also increased from 0.051 mg/100g to 0,097 mg/100g in NB and from 0.048 mg/100g to 0.067 mg/100g in NY.

Thiamine and riboflavin content of parboiled rice as reported by Oghbaei and Prakash (2010) was 0.18 and 0.22 mg/100g respectively. Parboiled rice had significantly higher contents of both thiamine and riboflavin which redistributes the water soluble vitamins in the whole kernel thus reducing the surface losses occurring due to the polishing. In a study by Sotelo et al. (2000) the thiamine content of raw milled rice varied from 0.025 -.0.045 mg/100g and after parboiling, there was slight increase from 0.03 to 0.051mg/100g. This is in tune with the findings of the present study. The variation in the vitamin content of rice reported by various authors may be due to the application of fertilizer, variation in maturity period and the degree of processing (Grewal and Sangha, 2006).

Polyphenols are the principal plant compounds evidencing antioxidant activity (Zheng and Wang, 2001). In the present investigation, the total phenolic content of the rice samples were found to be increased. The total phenolic content of NB (G) was 0.42 mg (raw value 0.41 mg) and that of NY (G) was 0.33 mg (raw rice value 0.29 mg).

According to Tian et al. (2004) phenolic compound are mostly located in the bran layer of rice grain and this may be the reason for its increase in content after processing. During parboiling, the phenolic compound in the barn of the rice grain may diffuse into the rice kernel as in the case of B-vitamins, which is in line with the findings of the present investigation.

Trace element contents of the *Njavara* grits in the present study revealed that all the trace elements viz., Fe, Mn, Zn and Cu had an increase in their content. The increase in Fe content in both *Njavara* varieties was very high when compared to raw value and all the other trace element values were had only slight increase after it was converted into grits.

Selenium is an essential micronutrient for human beings (www.wafoods.org).

In the present study, it was noticed that there was an increase in the Se content, in grits form. The Se content increased to 9.79 µg/ml from 4.73µg/ml in NB and 9.37 µg/ml from 2.22 µg/ml in NY.

Se content in brown rice is about 14 per cent (www.wafoods.com). Morris and Levander (1990) reported that brown rice contains about 0.383 µg/gm. In a study by Yoshida and Yosumoto (2004) found that mean Se content of 69 rice varieties was 43±27 µg/g. The authors reported that Se levels may vary by the site at which the rice was grown but not by cultivar.

Salient Findings

The results revealed that the energy content of the NY grits was higher when compared to NB grits. It was found that processing influences the calorific values of rice positively. The protein content was found to be highest in NY grits and the results shows a decrease in protein content due to processing when compared to raw values. As a result of processing the essential and non-essential content of both the *Njavara* varieties were found to decrease and the decrease was found more in NY grits. The EAA index of the grits showed that NB grits had higher value.

NY grits had the highest crude fibre content among the two samples. NY grits had higher soluble content. The results revealed that soluble fibre content had decreased as result of processing. Values of total starch depicted that NY grits had higher value than NB grits. Processing had decreased the total starch content. NB grits was having the highest amylose content. Amylose content was also tend to lower after processing. NY grits was having highest value for amylopectin, NB grits was having highest value for amylose-amylopectin ratio.

The ash content of the two grit types revealed that NB grits had higher ash content and it also revealed that after processing the values had increased. Regarding the mineral content, NY grits had highest calcium and phosphorus content.

Trace element contents of the *Njavara* rice samples in the present study revealed that all the trace elements viz., Fe, Mn, Zn Cu and Se had an increase in their content after processing with NB grits having the highest value for all the trace elements.

Values of B-vitamins increased as result of processing. NB grits had the highest value for thiamine and riboflavin.

NB grits had the highest value for total phenol content and the results revealed that after processing the total phenol content has increased.

5.2.2. Antioxidant properties

Antioxidants are defense against free radical and oxidative attacks. They act as free radical but also the accompanying damaging effects in the body. Antioxidants can be water soluble or lipid soluble, because they are found in both lipid and water portion of cells, protecting vital molecules such as proteins, and DNA from being oxidized by the free radicals. Antioxidants are found in numerous foods and even produced naturally by the human body (Vinson et al., 2001).

5.2.1. a) DPPH radical scavenging activity

The results of the present study indicated that processing had decreased the DPPH activity in both the *Njavara* varieties.

The results revealed that NY (G) has an IC_{50} value of 35.89µg/ml (raw IC_{50} value = 31.62µg/ml) and that of NB (G) was 47.80 µg/ml (raw IC_{50} value = 33.73µg/ml). This indicates that NY (G) had more DPPH activity compared to NB (G). Statistically there was a significant decrease in the antioxidant activity of both the *Njavara* ecotypes (F= 6638.00**).

In a study by Sawaddiwong et al. (2008) had shown that when brown rice was soaked at different temperature viz. 25°C, 30°C, 35°C and 40°C and when its DPPH activity was studied, it was noticed that the DPPH activity decreased with increase in temperature. It was also observed that other antioxidant assay like ABTS radical scavenging activity, ferric reducing antioxidant power were also found to be decreased.

5.2.2. b) Hydroxyl radical scavenging activity

According to Aurand et al. (2000) hydroxyl radicals are the major active oxygen species causing lipid oxidation and enormous biological damage.

The results of the present study revealed that parboiling had decreased the hydroxyl radical scavenging activity in both *Njavara* black and yellow varieties with an IC_{50} value of 53.78 µg/ml in NY (G) and 60.40 µg/ml in NB (G). When the data was statistically analysed it was found that the antioxidant activity had decreased in NB and NY (F=.4008.82**) (Table 33).

5.2.3. c) Super oxide anion-radical scavenging activity

Superoxide anions indirectly initiated lipid peroxidation as a result of superoxide and hydrogen peroxide serving as precursors of singlet O_2 and hydroxyl radicals (Okuda et al., 2003).

In the present investigation it was noticed that processing had decreased superoxide anion-radical scavenging activity in both the rice varieties. With an IC_{50} value of NB (G) = 50.93 µg/ml (raw NB= 48.78 µg/ml) and NY (G) = 60.00 µg/ml (raw NY= 55.43 µg/ml). Compared to the 1st experiment the activity of superoxide anion radical activity also decreased with an F of 1447.37** for varieties.

A study done by Sarithakumari (2007) showed that boiled *Njavara* treatment has increased the activity of scavenging enzymes thereby decreasing the lipid peroxidation products. The findings are in accordance with the results of the Robak and Grylewski (2001).

Salient Findings

The results of the DPPH activity among the *Njavara* grits revealed that NY grits has more DPPH scavenging activity compared to NB grits (IC_{50} Value of NY grits = 35.89 µg/ml). The results also revealed that the DPPH activity of both the rice varieties have fallen down compared to the raw form in the 1st experiment.

Hydroxyl radical scavenging activity of the rice samples revealed that NY grits (IC_{50} value = 46.00 µg/ml) had the highest hydroxyl radical scavenging activity. NB grits had the highest superoxide anion radical activity (IC_{50} Value of NB grits = 50.93 µg/ml).

5.2.3. Shelf-life quality

Assessment of shelf life quality is important since it determines the suitability of a particular ingredient for product development (Livingstone et al., 1993). The mechanism and kinetics of the food deterioration can be controlled by storage techniques applied (Varsany, 1993).

According to Shankar (1993) factors like raw material quality, storage temperature, storage containers, process employed and the environment in which it is processed affects the shelf life quality.

In the present investigation, the grit was stored in polypropylene covers at ambient conditions for a period of six months, in order to assess the shelf life quality of the grit. The moisture content and peroxide value of the grits were analysed at monthly interval.

- Moisture content

In the case of moisture which is an important parameter in the shelf-life quality assessment, it was noticed that there was not much increase in the moisture content before and after the storage period in both the samples. It was found that there was no significant difference between the rice varieties. Solanki (2000) reported similar findings in ready to eat mixes.

An increase in the moisture content of the flaked rice-based wafers has been reported by Kulkarni et al (1999). Pandey (2002) reported that most of the stored products are considered to be safe when stored at particular moisture content.

- Peroxide value

The extent of peroxidation in the stored grits was estimated and found that rate of peroxidation has not affected the quality of the stored grits. The estimated peroxide value was very low and may be due to the low fat content in the grits.

In a study by Rao et al. (2006) reported that when parboiled and raw milled rice (both husked and unhusked) was stored for 1 year, there was an increase in peroxide value but it was highest for husked rice. In a product (sevian) prepared from rice and other ingredients it was found that the peroxide value changed from 0 to 1.07 meq when stored for one month (Banerjee and Tripathi, 2000).

- Insect infestation and microbial analysis of stored *Njavara* grits

The incidence of insect attack was not observed in *Njavara* grits during the storage period of six months. Pingale et al. (2006) and Singh (2009) also observed the absence of insect attack in rice when stored for one year.

Leela et al. (2001) opined that processed foods and other food materials provide ample scope for contamination with spoilage and pathogenic micro-organisms, thus necessitating microbiological quality assessment as an integral part of processing. Hence, microbial assessment of the developed *Njavara* grits was carried out.

In the present investigation, the microbial load was found to be more in NB than in NY. For the first three months and there was no fungal attack in both the samples (grits).

Highest bacterial load was found in NB with a mean value of $3.25x10^3$ cfu/g and that of fungal growth was $1.22x10^3$ cfu/g. In NY, the mean value of bacterial load was 2.162×10^3 cfu/g and that of fungus was 0.748×10^3cfu/g. These values are within the ISI specification, which stated in SPI 8 part XI 1981 (ISI, 1981) prescribed bacterial count per gram should not exceed 50,000 and the count for yeast and mould should not exceed 10/g (Nimmy, 1996).

Joarder et al. (1999) is of the opinion that the moisture content has been found to influence favouarbly the higher loads of fungi. But storage period has little influence on the fungal content when the moisture content of stored samples remains below 16 per cent. This is in line with the findings of the present investigation because in the present study the moisture content was below 16 percent.

Salient Findings

The moisture percentage of the *Njavara* grits showed an increase (not significant) in their content, with NB grits having higher value. Peroxide value was found to be low in both the samples. The *Njavara* grits was completely free of insect infestation. The microbial assessment revealed that the highest microbial load was found in NB grits and these levels were in the ISI prescribed specification.

5.2.4. Selection of superior variety

The selection of superior variety was done based on the following parameters viz., energy, protein, crude fibre, soluble fibre, total starch, amylose, amylopectin, amylose-amlyopectin ratio, total ash, calcium, phosphorus, thiamine, riboflavin, total phenols and trace elements using the discriminant function analysis. The results revealed that varieties *Njavara* black (NB) and *Njavara* yellow (NY) were not having much difference in their attributes. Leenakumary (2004) is of the same opinion that both the types of *Njavara* doesn't have any variation.

5.3. IIIrd Phase – Assessment of the therapeutic value of *Njavara* grits through case studies

5.3.1. Formulation, sensory evaluation and suitability of *Njavara* grits

To formulate the *Njavara* grits, the rice was first parboiled, sundried and milled. This milled rice was converted to grits using a pulveriser.

The yield ratio of grits was 1.43 and processing loss was found to be 0.3 per cent. The cost of the grits was found to be Rs.96/Kg.

Various breakfast recipes like uppuma, porridge, kozhukatta and oratti were tried in the laboratory incorporating *Njavara* grits, in order to ensure the prompt inclusion of the supplement by the subjects.

These formulated recipes were assessed by a group of selected judges for sensory evaluation.

According to Lii and Chang (1999), the eating quality of rice is usually judged by the sensory evaluation and it varies according to personal preferences.

Sensory evaluation is defined as "A scientific discipline and to evoke, measure, analyse and interpret those responses to products that are perceived by the senses of sight, smell, touch, taste and hearing (Stone and Sidel, 2003).

Quality attributes selected in this study were colour, appearance, flavour, taste and doneness. The attributes such as appearance, doneness and flavor are final criteria of cooking quality and determine palatability or eating characteristics of cooked rice.

Colour, one of the important visual attributes has been used to judge the overall quality of food for a very long time. If the colour is unattractive, a potential consumer may not be impressed by other attributes.

The highest score obtained for colour was for porridge and lowest for kozukatta.

Appearance is the criterion for the desirability of any food product. According to Srilakshmi (2004) the appearance of the food product is contributed by surface characteristics, viz, size, shape, colour, transparency, opaqueness, turbidity and dullness.

In the present study, uppuma and kozhukatta got the highest score for appearance (4.00).

Flavour is one of the most important sensory attributes that affects acceptability of foods (Prinyawatkul et al., 2003). According to Hayat et al. (2005) flavour is the blend of taste and smell perceptions noted when the food is in the mouth.

In the present study, the recipe uppuma got the highest score for flavour and taste. Next to uppuma, oratti (4.00) got the highest score for flavour and kozhukatta (3.80) got the highest score for taste.

For the attribute doneness also, uppuma got the highest score, followed by kozhukatta, porridge and oratti.

According to Savithri et al. (1990) the overall acceptability depends on the concentration of amount of particular components, the nutritional and other hidden attributes of the food and its palatability or sensory quality.

In the present investigation, uppuma was the recipe which got the highest score for overall acceptability (22.80) and oratti (15.40) got the lowest score for overall acceptability.

5.3.2. Results of case study

Five subjects having diabetes in the age group of 40-50 years and not on medication were selected for the case study (Subject A-E). Among the five subjects, two were male (A and B) and three were females (Subject C to E). All the subjects were Hindu and were from nuclear family.

The monthly income of the subjects ranged from 30,000 to 2, 00,000. Their educational qualification ranged from plus two to PG and above, with occupation business (subject A), Govt. employee (B and C), subject D was a housewife whereas subject E was a bank employee.

Duration of the diseases revealed that subject A, C and D were having diabetes for the past 2 years, whereas subject B too was having diabetes for the last nine months and Subject E for the past 3 years. Except subject B and C, all others were not having any diabetic history.

The two male subjects were found to have no exercise habit and both of them were non-vegetarians, whereas female subjects were found to have the habit of doing exercise and all of them were vegetarians.

Their anthropometric data revealed that subject A and E were coming under Obese grade I category, with a waist-hip ratio of 1.05 and 0.99 respectively. Whereas the other three subjects i.e. subject B, C and D were of normal category, with a waist hip ratio of 0.96, 0.89 and 0.94 respectively.

Abdominal obesity is defined as a waist-hip ratio of above 0.90 for males and above 0.85 for females. (http://whqlibdoc.who.int.publications /2011/9789241501491_ eng.pdf). Price et al. (2006) is of the opinion that waist hip ratio (WHR) has been found to be a more efficient predictor of mortality than BMI.).

In the present investigation, it can be seen that the two subjects i.e. subject A and B were having waist-hip ratio more than 0.90 and the three females also were having high waist –hip ratio of above 0.85.

The National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) stated that women with waist–hip ratios of more than 0.8, and men with more than 1.0, are at increased health risk because of their fat distribution (Marlowe et al., 2005). The author also stated that a WHR of 0.7 for women and 0.9 for men has been shown to correlate strongly with general health. Women within the 0.7 range have optimal levels of estrogen and are less susceptible to major diseases such as diabetes, cardiovascular disorders and ovarian cancers. Men with WHRs below 0.9, similarly, have been shown to be healthier. In the present study all the subjects were shown to have high waist-hip ratio and this may be one of the reasons for them to be diabetic.

Frequency of consumption of various food groups in the diet revealed that all subjects were included cereals, pulses, vegetables, milk, milk products and tea daily. The average intake of energy as calculated from their diet was 2025 Kcal for subject A, 1617 Kcal for subject B, 1304 Kcal fro subject C, 1642 Kcal for subject D and 1844 Kcal for subject E.

5.3.2.1. Efficacy of *Njavara* on the blood sugar levels

. The subjects were given *Njavara* grits for a period of three months based on their requirement.

The results indicated that all the subjects have shown a decrease in the blood sugar level. The initial blood sugar levels of subject A, B, C, D and E were 193 mg/dl, 140mg/dl, 140 mg/dl, 150mg/dl and 250 mg/dl and their final blood sugar level was 173 mg/dl, 90 mg/dl, 110mg/dl, 109 mg/dl and 160 mg/dl respectively.

The present investigation revealed that *Njavara* has glucose lowering effect. The exact factor for this effect is not known but there can be a number of factors like amino acids, soluble fibre, antioxidants, phenolic compound, minerals and B-complex vitamins.

Several studies have reported that amino acids improves insulin resistance (Arwert et al., 2003; Pasini et al., 2008; Solerte et al., 2008, 2004; Pellegrino et al., 2008). Study by Gannon and Nuttal (2000) suggested that the process of gluconeogenesis from protein occurs during a 24-hr. period, and the slowly and evenly produced glucose can be disposed of over a long period of time. A study by Anuradha (2009) suggested that individual amino acids, especially the ones bestowed with antioxidant property like N-acetyl cysteine and taurine seem to have beneficial effects by their ability to reduce intracellular oxidative stress generation and glycooxidation. Other amino acids like glycine and lysine may be good for the prevention of glycation. The author also suggested that nutritional intervention with taurine, phenylalanine or branched chain amino acids can improve insulin sensitivity and post-prandial glucose disposal.

A number of studies have been published regarding the relationship between soluble fibre and management of diabetes. Wursch and Sunyer (2007) are of the opinion that diabetic individuals can be benefit from diets that are high in β-glucan, which is a component of soluble fibre. Other studies which supports this are Misra and Jha (2011) and Chandalia et al.(2000).

Antioxidants are substances which can prevent the generation of reactive species (free radicals) and also scavenging of these free radicals (Johansen et al., 2007). Diabetes is such a disease in which free radicals are produced and antioxidants plays an important role in preventing this (Reena et al., 2010; Maritim et al., 2003).

Polyphenol also acts as antioxidant. In a study by Sabu et al. (2002) found that an aqueous solution of green tea polyphenols was found to inhibit lipid peroxidation and they also reduced serum glucose level in alloxan diabetic rats.

Selenium which is a mineral also acts as antioxidant (Mukerjee et al., 2008). The authors suggested that selenium plays a role in reducing the oxidative stress associated with diabetes.

Other minerals which may be helpful in the management of diabetes are manganese (Mn), Magnesium (Mg), Zinc (Zn) and Copper (Cu) (Zargar et al., 1998).

B-complex vitamins also have ability to lower blood levels of homocysteine (as sulphur containing amino acid). Hence, the B-vitamins are also essential for breakdown of carbohydrates into glucose, and also help in normal functioning of the nervous system.

5.3.2.2. Assessment of Glucose Tolerance Test (GTT)

GTT is the determinant of diabetic characteristics of the subjects. Results of the GTT conducted among the selected five subjects under the study indicated that *Njavara* grits have lower GTT values as compared to Glucose (reference food). According to Mohan (1997), in a severe diabetic, the plasma glucose is likely to be high at any time of the day and therefore random plasma glucose is often enough for diagnosis. However, this is not very much helpful in identifying the mild diabetes and hence GTT is a must. The GTT helps to diagnose diabetes early and also helps in assessing the severity of the case.

5.3.2.3. Glycemic index and Glycemic load of Njavara grits

According to Wolever et al. (1991) the area under the glycemic index curve for each food taken by the same subjects and the resulting values were arranged to obtain the glycemic index value of the food. The Area Under the Curve (AUC) of test food (*Njavara* grits) and reference food (Glucose) were plotted in a graph paper. The AUC of the reference food was found to be 360.00 mm^2 and that of test food was 260.00 mm^2.

The results are in accordance with the findings of Wolever et al. (2004) who found that foods having long glycemic index may be beneficial in the treatment of Type 2 diabetes.

GI of the food is defined as the incremental area under the two hour blood glucose response curve (AUC) following a 12 hr. fasting and ingestion of a food with a certain quantity of available carbohydrate (usually 50 g) (Jenkins, 2008). Glucose has a glycemic index of 100, by definition, and other foods have a lower glycemic index. Glycemic index is defined for each type of food, independent of the amount of food consumed. Glycemic load accounts for the amount of food consumed and are calculated in terms of glycemic index.

Glycemic Load (G.L) of food is a number that estimates how much the food will raise a person's blood glucose level after eating it (Das, 2007).

Glycemic index of the test food calculated based on the area under curve indicated that *Njavara* rice has a glycemic index of 72 and glycemic load was found to be 36. Study by Deepa et al. (2010) reported that glycemic load (GL) and glycemic index (G.I) of *Njavara* were similar to Jyothi and IR 64 (non-medicinal variety).

Miller et al. (2002) determined the GI values for 12 rice products, using eight healthy subjects and found that GI of the rices ranged from 64 +/- 9 to 93 +/- 11, where glucose = 100. The high amylose rice gave a lower GI. Only high-amylose varieties are potentially useful in low-GI diets. Similar results were reported by Zhang et al. (2011).

Antioxidant properties

During normal physiological processes, the human body generates small amounts of free radicals of oxygen, such as the hydroxyl (OH−), superoxide (O2−), nitric oxide (NO−), and lipid peroxyl (LOO−) radicals. The presence of too many free radicals in the human body will result in their attacking protein, lipid, DNA and other biomolecules, thereby causing damage to the cell structure, and interfering with the body's normal metabolism, all of which result in disease and acceleration of the aging process (Devasagayam et al., 2004).

The effects of free radicals on human beings are closely related to toxicity, disease and aging (Maxwell, 1995). Most living species have an efficient defense system to protect themselves against the oxidative stress induced by Reactive Oxygen Species (ROS) (Sato et al., 1996). Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases including cancer, atherosclerosis and diabetes (Stajner et al., 1998; Sanchez-Moreno et al., 1999; Malencic et al., 2000). The antioxidants can interfere with the oxidation process by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers.

a) DPPH radical scavenging activity

Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid peroxidation (Blokhina et al., 2003; Rice-Evans et al., 1997). The DPPH radical scavenging activity has been extensively used for screening antioxidants from fruits and vegetable juices or extract (Robards et al., 1999; Sánchez-Moreno, 2002).

The results of the DPPH scavenging activity in the blood samples of the subjects revealed that after supplementation of *Njavara* rava, their DPPH radical activity has increased on a dose dependent manner.

In a study done by Chrzczanowicz et al. (2008) mean values of DPPH radical scavenging activity obtained in human serum (healthy subjects) were 11.2+/-3.3 per cent.

b) Hydroxyl radical scavenging activity

Hydroxyl and superoxide radicals are the two most representative free radicals. In cellular oxidation reactions, superoxide radical is normally formed first, and its effects can be magnified because it produces other kinds of cell damaging free radicals and oxidizing agents. However, the damaging action of the hydroxyl radical is the strongest among free radicals. Synthetic compounds are found to be strong radical scavengers but usually they have side effects (Zhou et al., 1991). Neutralization of this radical activity by naturally occurring substances mainly by supplementation of food having antioxidant property is becoming one of the most acceptable modes of modern therapy (Pal et al., 2010).

In the present investigation, the results revealed that after the supplementation of *Njavara* grits for three months the hydroxyl scavenging activity of the serum has increased (Fig. 30) when compared to the activity level before supplementation (Fig. 29).

c) Superoxide anion-radical scavenging activity

Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive species (Krishnendu et al., 2011). One risk of the superoxide generation is related to its interaction with nitric oxide to form peroxinitrite (Yamagishi et al., 2001) which is a potent oxidant that causes nitrosamine stress in the organ systems

The superoxide scavenging activity of serum of subjects under study of the present investigation has shown an increase after the feeding trial, which is also on a dose dependent manner.

Salient Findings

In the third experiment the following was the results:

The yield ratio, processing loss and cost of the grits was found to be 1.43, 0.3 per cent and Rs.96/Kg. For assessing the therapeutic value, different breakfast recipes like uppuma, porridge, kozhukatta and oratti were tried in the laboratory incorporating *Njavara* grits. The formulated recipes were then subjected to sensory evaluation. *Njavara* uppuma was having the highest score.

The *Njavara* grits was given to five subjects in age group of 40-50 years who were diabetic but not on medication. Out of three subjects two were males and three were females. All of them belonged to Hindu community and also were from nuclear family. Socio economic profile revealed that all subjects were having a good family income between Rs. 30,000/- to Rs. 2, 00,000/-. Dietary pattern of the subjects showed that, three subjects were vegetarians, and the other two were non-vegetarians and follows three meals per day pattern. BMI of subjects revealed that three subjects were having normal BMI, whereas the other two subjects were falling under obese grade I category.

For assessing the efficacy of *Njavara* on the blood sugar levels, a feeding trial for 3 months was conducted. Blood sugar levels were monitored during 0, 45 and 90th day of supplementation. The results revealed that for all subjects blood sugar levels decreased after supplementation study.

Glucose Tolerance Tests were conducted in the subjects to assess their glycemic index and glycemic load of *Njavara* grits. The results revealed that GTT of Njavara grits was lower than the reference food (Glucose). Mean glycemic response of the *Njavara* grits was found to be 72. Glycemic load of *Njavara* grits was found to be 36.

The findings revealed that after the supplementation of *Njavara* the DPPH scavenging activity, hydroxyl radical activity and superoxide anion-radical scavenging activity of the blood samples of all the five subjects under study have increased.

Conclusion

Medicinal plants are a source of antioxidants and bioactive compounds present in them are responsible for the prevention of oxidative stress that leads to many degenerative diseases and conditions (Smitha et al., 2012). The present study was done to ascertain the nutritive and therapeutic value of *Njavara* rice.

The result revealed that Njavara rice has good nutritional value especially with a good protein (raw Njavara values of amino acids was comparable to that of reference protein i.e. egg), phosphorus, selenium, Fe, Cu, Zn and Mn content. It also has antioxidant property like DPPH scavenging activity, hydroxyl radical activity and superoxide radical scavenging activity.

The therapeutic value of the *Njavara* revealed that it has a positive effect in reducing the sugar levels of subjects under study but it doesn’t give a conclusive result because of the small number of subjects taken.

Recommendations

For conclusive results, the study can further be expanded to large subjects with more focus on antioxidant properties especially serum antioxidants and also in various disease conditions like heart diseases, cancer and neurological problems.

SUMMARY

6. SUMMARY

A study entitled "Quality evaluation of medicinal rice (*Oryza sativa* L.) cv. *Njavara* for product development and therapeutic value" was conducted to assess the major quality parameters such as physico-chemical characteristics, nutritional composition and antioxidant activity. Two ecotypes of *Njavara* rice and one KAU short duration variety *Hraswa* (control), were collected from Rice Research Station of KAU located at Moncompu, Agricultural Research Station, located at Mannuthy, Thrissur to conduct the study and the results obtained are summarized below.

The study was conducted in three experiments. The first experiment encompasses the quality evaluation of the selected rice varieties. In the second experiment, a product was developed (grits) using both the ecotypes of *Njavara* and its physico-chemical/nutritional composition and antioxidant activity, sensory and shelf life quality were assessed. In the third experiment, the grits was tested for its therapeutic value viz. glycemic index and antioxidant properties.

In the first experiment, the parameters selected under physical characteristics were moisture, size, shape, length, width, L/B ratio, colour, thousand grain weight, gel consistency, bulk density, gelatinization temperature, chalkiness index, viscography and hardness.

The results of physico-chemical characteristics are summarized below:

- Regarding the colour of the rice samples all the samples were red in colour.
- The size of the selected rice varieties revealed that NB and *Hraswa* was medium in size, whereas NY was found to be short in size.

- Shape of the rice varieties revealed that all the samples were medium in shape.
- The length and breadth of the rice was found to be highest in *Hraswa* (Control) variety, followed by NB and NY.
- The L/B ratio was recorded highest for NY among the three selected rice varieties.
- Thousand grain weight was highest for variety *Hraswa* (control) and lowest for NY.
- Highest moisture content was found in NY, followed by *Hraswa* and NB.
- *Hraswa* (control) variety had the highest value for gelatinization temperature and NY had the lowest value.
- Bulk density of the rice samples showed that NB had the highest value and *Hraswa* had the lowest.
- NB had the highest value for gel consistency followed by NY and *Hraswa*.
- Chalkiness index of the selected rice samples showed that NY had the highest value and NB had the lowest value.
- Among the three rice samples selected NB had the highest value for viscography and *Hraswa* (control) had the lowest value.
- Regarding the hardness of the rice samples, *Hraswa* (control) variety had the highest value and NY had the lowest.

The nutritional composition of the rice varieties were ascertained by estimating energy, protein, crude fibre, soluble fibre, total starch, amylose, amylopectin, amylose-amlyopectin ratio, total ash, calcium, phosphorus, thiamine, riboflavin, total phenols and trace elements.

- The assessment of the calorific value/energy of the three rice varieties revealed that NY had the highest values for calories when compared to the other two varieties. The protein content was found to be highest in NB and lowest in *Hraswa* (control) variety. The evaluation of protein quality revealed that values of essential amino acids like threonine, valine, methionine and isoleucine of *Hraswa* (control variety) were lower than the values of raw *Njavara* rice samples, whereas leucine, phenylalanine, lysine and tryptophan values of *Hraswa* were higher than *Njavara* black and yellow varieties. Amino acid score (AAS) of the rice samples revealed that for NB, the highest amino acid score was obtained for phenylalanine, valine and threonine, where as for NY the amino acid score was highest for phenylalanine, tryptophan and leucine. On a comparative basis, NY had more number of amino acid score than NB. Essential Amino Acid (EAA) Index showed that, control variety *Hraswa* had high value.
- NB had the highest crude fibre content and *Hraswa* (control) variety was having the lowest crude fibre content among the selected rice samples.
- Soluble content was more for NB and lowest for *Hraswa* (control) variety.
- Significant variation was found in the values of total starch in rice varieties. *Hraswa* (control) was having the highest value, whereas NY was having the lowest value.
- In the case of Amylose content, *Hraswa* (control) variety was having highest value, followed by NY and NB.
- *Hraswa* (control) variety was having highest value for amylopectin and amylose-amylopectin ratio.
- Among the three rice varieties selected, NB had the highest ash content.

- Regarding the mineral content, NB had highest calcium and phosphorus content and *Hraswa* (control) variety was having the lowest value for calcium and phosphorus content.
- Results of the B-vitamins revealed that NB had the highest value for thiamine and riboflavin, whereas *Hraswa* (control) variety was having the lowest value.
- NB had the highest value for total phenol content and NY had the lowest value.
- Trace element content of the rice samples revealed that for Fe, NB had the highest value, for Mn, Zn, Cu and Se *Hraswa* (control) variety had the highest value. Lowest value for Mn and Zn was found in NB and for Cu and Se the lowest value was found in NY.

Antioxidant activities were determined using DPPH scavenging activity, hydroxyl radical scavenging activity and super oxide anion-radical scavenging activity.

- The results of the DPPH scavenging activity revealed that among the three rice varieties selected NY had the highest DPPH activity with an with an IC_{50} value of 31.62 µg/ml.
- Hydroxyl radical scavenging activity of the rice samples revealed that NY (IC_{50} Value = 46 µg/ml) had the highest activity.
- NB (IC_{50} value of 48.78 µg/ml) showed the highest superoxide anion radical activity, followed by NY and *Hraswa* (control) variety.

In the second experiment, *Njavara* rice varieties (using both the *Njavara* ecotypes) were first parboiled and then converted to grits form and its physico-chemical characteristics, nutritional composition, sensory and shelf life qualities were assessed.

The results of the physical characteristics revealed that

- The moisture content of the grits was higher when compared to raw form, with NB (G) having the highest value.
- Gelatinization temperature increased in both the varieties with NB having the highest value.
- Bulk density values have decreased from 1.00 to 0.99 in NB and from 0.99 g/ml to 0.98 g/ml in NY.
- Data on gel consistency and viscography shows that in both varieties the values have increased, with the highest value noted in NB.

- The results of the nutritional composition revealed that the energy content of the NY grits was higher when compared to NB grits. It was found that processing influences the calorific values of rice positively.
- The protein content was found to be highest in NY grits and the results shows a decrease in protein content due to processing when compared to raw values. As a result of processing the essential and non-essential content of both the *Njavara* varieties were found to decrease and the decrease was found more in NY grits when compared to NB grits. The EAA index of the rice samples showed that NB grits had higher value.
- NY grits had the highest crude fibre content among the two grit samples.
- NY grits had higher soluble content; the results revealed that soluble content has decreased as result of processing.

- Values of total starch shows that NY grits had higher value than NB grits. Processing has decreased the total starch content.
- NB grits was having the highest amylose content. Amylose content was also tend to lower after processing.
- NY grits was having highest value for amylopectin; NB grits was having highest value for amylose-amylopectin ratio.
- The ash content of the two grit types revealed that NB grits had higher ash content and it also shows that after processing the values had increased.
- Regarding the mineral content, NY grits had highest calcium and phosphorus content.
- Values of B-vitamins shows an increase in contents due to processing. NB grits had the highest value for thiamine and riboflavin.
- NB grits had the highest value for total phenol content and the results revealed that after processing the total phenol content has increased.
- Trace element contents of the *Njavara* rice samples in the present study revealed that all the trace elements viz., Fe, Mn, Zn Cu and Se had an increase in their content after processing with NB grits having the highest value for all the trace elements.
- Antioxidant activities of *Njavara* grits of both types revealed that NY grits has more DPPH scavenging activity compared to NB grits (IC_{50} Value of NY grits = 35.89 µg/ml). The results also revealed that the DPPH activity of both the rice varieties have fallen down compared to the raw form in the 1st experiment.
- Hydroxyl radical scavenging activity of the rice samples revealed that NY grits (- IC_{50} value = 46.00 µg/ml) had the highest hydroxyl radical scavenging activity.

- NB grits had the highest superoxide anion radical activity (IC_{50} Value of NB grits = 50.93 µg/ml).
- Shelf life quality of the stored *Njavara* grits revealed an increase in moisture percentage as a result of storage.
- Peroxide value was found to be low in both the samples.
- The *Njavara* grits was completely free of insect infestation.
- The microbial assessment revealed that the highest microbial load was found in NB grits in comparison with NY grits but these levels were in the prescribed specifications.

Superior variety was selected based on the following parameters viz., energy, protein, crude fibre, soluble fibre, total starch, amylose, amylopectin, amylose-amylopectin ratio, total ash, calcium, phosphorus, thiamine, riboflavin, total phenols and trace elements.

- The results showed that NB grits was found to be superior in quality but statistically the values of NY grits and NB grits were not having much difference.

In the third experiment the *Njavara* grits was tested for its therapeutic value viz. glycemic index and antioxidant properties.

- The yield ratio, processing loss and cost of the grits were found to be 1.43, 0.3 per cent and Rs.96/Kg respectively.
- For assessing the therapeutic value, different breakfast recipes like uppuma, porridge, kozhukatta and oratti were tried in the laboratory incorporating *Njavara* grits.

The formulated recipes were then subjected to sensory evaluation. *Njavara* grits uppuma was having the highest score.

- The *Njavara* grits was given to five subjects in age group of 40-50 years who were diabetic but not on medication. Out of three subjects two were males and three were females. All of them belonged to Hindu community and also were from nuclear family. Socio economic profile revealed that all subjects were having a good family income between Rs. 30,000/- to Rs. 2, 00,000/-. One subject was doing business, three were having government job and one subject was a house wife. Dietary pattern of the subjects showed that, three subjects were vegetarians, and the other two were non-vegetarians and followed three meals per day pattern. BMI of subjects revealed that three subjects were having normal BMI, whereas the other two subjects were falling under obese grade I category. All the subjects were having high waist-hip ratio.

- For assessing the efficacy of *Njavara* on the blood sugar levels, a feeding trial for 3 months was conducted. Blood sugar levels were monitored initially (before the supplementation), intermittently and finally (after the supplementation). The results revealed that for all subjects, blood sugar levels decreased after supplementation study.

- Glucose Tolerance Test was conducted in the subjects to assess their glycemic index and glycemic load of *Njavara* grits. The results revealed that GTT of Njavara grits was lower than the reference food (Glucose).

- Mean glycemic response of the *Njavara* grits was found to be 72.

- Glycemic load of *Njavara* grits was found to be 36.

- Antioxidant properties in the blood samples of the subjects were studied and the results revealed that after the supplementation of *Njavara* the DPPH scavenging activity, hydroxyl radical activity and superoxide anion-radical scavenging activity of the blood samples of all the five subjects under study had increased considerably.

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QUALITY EVALUATION OF MEDICINAL RICE (*Oryza sativa* L.) cv. *Njavara* FOR PRODUCT DEVELOPMENT AND THERAPEUTIC VALUE

RESHMI. R

Abstract of the thesis submitted in partial fulfillment of the requirement for the degree of

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8. ABSTRACT

A study entitled "Quality evaluation of medicinal rice (*Oryza sativa* L.) cv. *Njavara* for product development and therapeutic value" was conducted to assess the major quality parameters such as physico-chemical characteristics, nutritional composition and antioxidant activity.

Two eco types of *Njavara* rice Njavara black (NB) and Njavara yellow (NY) were procured from RRS, Moncompu and one eco type of *Hraswa* was obtained from ARS, Mannuthy. *Hraswa* was treated as the control.

The study was conducted in three phases. The first phase experiment envisaged the quality evaluation of the selected rice varieties. In the second phase of experimentation, a product (grits) was developed using both the ecotypes of *Njavara;* Its physical, chemical, nutritional composition, sensory and shelf life qualities was assessed and finally in the third phase of experiments the product was tested for its therapeutic value viz. glycemic index and antioxidant properties.

In the first experiment, the parameters selected to study under physico-chemical characteristics were moisture, size, shape, length, width, L/B ratio, colour, thousand grain weight, gel consistency, bulk density, gelatinization temperature, chalkiness index, viscography and hardness.

Regarding the colour of the rice samples all the samples were red in colour. The L/B ratio and moisture content was recorded highest for NY among the three selected rice varieties. Thousand grain weight was highest for the variety *Hraswa* (control) and lowest for NY.

The assessment of the calorific value/energy of the three rice varieties revealed that NY had the highest value for calories when compared to the other two varieties. The protein content was found to be higher in NB and lowest in *Hraswa* (control) variety. The evaluation of protein quality showed that values of essential amino acids like threonine, valine, methionine and isoleucine of *Hraswa* (control variety) was lower than the values of raw *Njavara* rice samples. Whereas leucine, phenylalanine, lysine and tryptophan values of *Hraswa* were higher than *Njavara* black and yellow varieties. Amino acid score (AAS) of the rice samples revealed that NY had more amino acid score than NB.

Soluble fibre content was more for NB and lower for *Hraswa* (control) variety. NB had the highest value for total phenol content. Trace element content of the rice samples revealed that NB had the highest value for Fe. As for Mn, Zn, Cu and Se, *Hraswa* (control) variety had the highest values.

The results of the antioxidant properties revealed that for DPPH scavenging activity and hydroxyl radical activity NY had highest antioxidant activity, with an IC_{50} value of 31.62 µg/ml for DPPH and 46.00 µg/ml for hydroxyl radical scavenging activity. For superoxide radical scavenging activity, NB had the highest activity with an IC_{50} value of 48.78 µg/ml.

In the second phase of the experiment, both the *Njavara* ecotypes were first parboiled and then converted to grits form and its nutritional composition, sensory and shelf life qualities were assessed.

The results revealed that the energy content of the NY (G) was higher when compared to NB (G). It was found that processing influences the calorific values of rice positively. The protein content was found to be highest in NY (G) and the results showed a decrease in protein content due to processing when compared to raw values. As a result of processing the essential and non-essential content of both the *Njavara* varieties were found to decrease and the decrease was found more in NY (G) when compared to NBG). The EAA index of the rice samples showed that NB (G) had higher value.

NY (G) had higher soluble fibre content; the results revealed that soluble fibre content has decreased as result of processing. NB (G) had the highest value for total phenol content and the results revealed that after processing the total phenol content had increased. Trace element analysis of the *Njavara* rice samples in the present study revealed that all the trace elements viz., Fe, Mn, Zn Cu and Se increased in content after processing with NB(G) having the highest value for all the trace elements.

Antioxidant properties showed that for DPPH scavenging activity NY grits had highest antioxidant activity with an IC_{50} value of 35.89 µg/ml. For hydroxyl radical scavenging activity also NY grits was having highest activity. ($IC_{50} =$ 53.78 µg/ml). Superoxide radical scavenging activity showed that NB grits had highest activity (IC_{50} value = 48.78 µg/ml).

To ascertain the shelf life quality of the developed product, moisture percentage, peroxide value, insect count and microbial growth was determined.

The assessment of microbial growth revealed that the highest microbial load was found in NB(G) but these levels were within the prescribed specification.

Results of the selection of superior variety showed that NB (G) was found to be superior in quality, but statistically the values of NY (G) and NB(G) were not having much difference.

In the third phase of experiments, *Njavara* grits were tested for their therapeutic value viz. glycemic index and antioxidant properties.

For assessing the therapeutic value, different breakfast recipes like uppuma, porridge, kozhukatta and oratti were tried in the laboratory incorporating *Njavara* rice grits. The formulated recipes were then subjected to sensory evaluation. *Njavara* grits uppuma obtained the highest score for overall acceptability.

For assessing the efficacy of *Njavara* on the blood sugar levels, a feeding trial for 3 months was conducted among five diabetic subjects in the age group of 40-50 years. Blood sugar levels was monitored initially (before the supplementation) and finally (after the supplementation). The results revealed that, for all subjects, blood sugar levels decreased after supplementation study.

Mean glycemic index and Glycemic load of the *Njavara* rice was found to be 72 and 36 respectively.

Antioxidant properties in the blood samples of the subjects revealed that after the supplementation with *Njavara* the DPPH scavenging activity, hydroxyl radical activity and superoxide anion-radical scavenging activity of all the five subjects under study had appreciably increased.

APPENDICES

APPENDIX I

SPECIMEN EVALUATION CARD FOR TRIANGLE TEST

Name: Date:

Product: Time:

Two of the three samples are identical

Determine the odd samples

Pair No.	Code no. of samples	Code no. of odd samples
1.		
2.		
3.		
4.		

APPENDIX II

SCORE CARD FOR THE ASSESSMENT OF SENSORY EVALUATION OF THE DEVELOPED PRODUCT

Criteria	**Points**
1. Appearance	
Excellent	5
Very Good	4
Good	3
Fair	2
Poor	1
2. Colour	
Excellent	5
Very Good	4
Good	3
Fair	2
Poor	1
3. Flavour	
Excellent	5
Very Good	4
Good	3
Fair	2
Poor	1
4. Texture	
Excellent	5
Very Good	4
Good	3
Fair	2
Poor	1
5. Taste	
Excellent	5
Very Good	4
Good	3
Fair	2
Poor	1

APPENDIX III

QUESTIONNAIRE FOR ELICITING THE PERSONAL CHARACTERISTICS OF THE SUBJECTS

1. Name:
2. Age:
3. Educational status
4. Occupation:
5. Type of family
6. Family size
7. Total family income
8. No. of family members having diabetes
9. Duration since the onset of disease
10. Associated disease condition

    a. No disease   b. Hypertension   c. Dyslipidemia

    d. Hypothyroid   e. Others (specify)

11. Habit of smoking
12. History of gestational diabetes
13. Menopause stage
14. Activity level

    a. Sedentary   b. Moderate   c. Heavy

15. Exercise frequency

    a. No exercise   b. Regular   c. Irregular

    d. Once in a week   e. 2-3 times /week

16. Type of exercise

a. No exercise b. Walking c. Aerobics

d. Using equipments at home e. Attending health club/gym

17. Duration of exercise

a. Nil b. <15 minutes c. 15 – 30 minutes

d. > 30 minutes

18. Anthropometric data

a. Height (cms) b. Weight (Kg) c. BMI

d. Waist circumference (cms) e. Hip circumference (cms) f. W/H ratio

19. Blood sugar level

20. Dietary habits

a. Vegetarian b. Non-vegetarian

21. Frequency of use of food items

a. Cereals b. Pulses c. Vegetables d. Fish/Meat

e. Milk & Milk products f. Miscellaneous

22. Average intake of energy

23. Frequency of meals

a. 3 meals/day b. 4 meals/day c. 5 meals/day d.>5 meals/day

24. Oil used

a. Coconut oil b. Sunflower oil c. Palm oil

d. Olive oil

e. Gingelly oil f. Combination of oils

APPENDIX IV

CALORIE CALCULATION

Men- 48 Kg – 1^{st} 5 feet (150 cm = 48 Kg + 2.76Kg for extra inch).

Women – 45.5 Kg - 1^{st} 5 feet (150 cm = 45.5 Kg + 2.3Kg for extra inch).

Sedentary worker

- Obese = 20-25Kcal/Kg ideal body wt./day
- Normal = 30 Kcal/Kg ideal body wt./day
- Underweight = 35 Kcal/Kg ideal body wt./day

Moderate worker

- Obese = 30 Kcal/Kg ideal body wt./day
- Normal = 35 Kcal/Kg ideal body wt./day
- Underweight = 40 Kcal/Kg ideal body wt./day

Heavy worker

- Obese = 35Kcal/Kg ideal body wt./day
- Normal = 40 Kcal/Kg ideal body wt./day
- Underweight = 45 – 50 Kcal/Kg ideal body wt./day

For. e.g.

Ht. 150 cm, Wt. 45 Kg, Gender = Male, Activity level = Sedentary

His ideal wt. should be 48Kg

Sedentary underweight = 35 Kcal/Kg ideal body wt./day

= 48X35 Kcal/Kg ideal body wt./day

=1680 Kcal/day

= 1008 Kcal (60% of 1680 Kcal/day)

= 252 Kcal for one time (1680/4)

= 69 g (252x100/364) (364 Kcal = energy value for *Njavara*)

APPENDIX V

ORAL GLUCOSE TOLERANCE TEST (GTT)

GTT is carried out after 12 hrs. of overnight fasting. Glucose 75g in adults is orally administered. Before the glucose load and 15 minutes to 120 minutes after the administration of glucose blood samples were collected and glucose levels were estimated.

The diagnostic criteria for diabetes and impaired glucose tolerance, the fasting blood sugar levels vary between 80-110 mg/100ml. The blood sugar levels increase after the glucose load and come down to base level within 2 hrs.

WHO criteria with glucose load 75g for adult or 1.75g/Kg/body wt. (To a maximum of 75g).

	Glucose concentration		
	Venous whole blood	**Capillary whole blood (mg/dl)**	**Venous plasma (mg/dl)**
	Normal	**Diabetes Mellitus**	**Confirmed**
Fasting	120	120	140
2 hours after glucose load	180	200	200
	Impaired glucose tolerance		
Fasting	120	120	140
2 hours after glucose load	120-180	140-200	140-200

(WHO, 1980)