MEDICINAL PROPERTIES AND PROCESS OPTIMISATION FOR GABA ENRICHMENT IN RICE

By

SIMLA THOMAS

(2018-24-002)

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF COMMUNITY SCIENCE

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2023

MEDICINAL PROPERTIES AND PROCESS OPTIMISATION FOR GABA ENRICHMENT IN RICE

By

SIMLA THOMAS

(2018-24-002)

THESIS

Submitted in partial fulfilment of the

requirement of the degree of

Doctor of Philosophy in Community Science

(FOOD SCIENCE AND NUTRITION)

Faculty of Agriculture

KERALA AGRICULTURAL UNIVERSITY

DEPARTMENT OF COMMUNITY SCIENCE

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR – 680656

KERALA, INDIA

2023

DECLARATION

I hereby declare that the thesis entitled "**Medicinal properties and process optimisation for GABA enrichment in rice**" is a bonafide record of research work done by me during the course of research and the thesis has not been previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

15/12/23

Vellanikkara

Date : 15/12/2023

SIMLA THOMAS

(2018-24-002)

CERTIFICATE

Certified that the thesis entitled "**Medicinal properties and process optimisation for GABA enrichment in rice**" is a bonafide record of research work done independently by **Ms. SIMLA THOMAS** under my guidance and supervision that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellanikkara
Date: 15/12/2023

Dr. ANEENA E. R.
Major Advisor
Associate Professor
Krishi Vigyan Kendra, Thrissur

CERTIFICATE

We, the undersigned members of the advisory committee of Ms. Simla Thomas (2018-24-002), a candidate for the degree of **Doctor of Philosophy in Community Science** with the major field in **Food Science and Nutrition**, agree that the thesis entitled, "**Medicinal properties and process optimisation for GABA enrichment in rice"** may be submitted by **Ms. Simla Thomas** in partial fulfilment of the requirement for the degree.

15/12/23

Dr. Aneena E. R.
Major Advisor
Associate Professor
Krishi Vigyan Kendra
Thrissur

Seeja

Dr. Seeja Thomachan Panjikkaran
Associate Professor and Head
Dept. of Community Science
College of Agriculture
Vellanikkara

15/12/23

Dr. Sharon C. L.
Assistant Professor
Dept. of Community Science
College of Agriculture
Vellanikkara

15/12/23

Dr. P. Prameela
Professor and Head
Dept. of Agronomy
College of Agriculture
Vellanikkara

15/12/23

Dr. Berin Pathrose
Associate Professor
Dept. of Agricultural Entomology
College of Agriculture
Vellanikkara

EXTERNAL EXAMINER

15/12/2023

ACKNOWLEDGEMENT

I express my heartfelt thankfulness to God Almighty for the blessings showered upon me during my course of study. The strength and courage which was shown to me aided me in completing this strenuous work.

I consider myself exceedingly fortunate, delightful and privileged to have ***Dr. Aneena E.R.****, Associate Professor, Krishi Vigyan Kendra, Thrissur to lead my advisory committee. Words cannot explain my deep sense of gratitude towards her. I am incredibly delighted to place on record my profound gratitude for her expert guidance, critical and valuable suggestions, consistent encouragement, inexhaustible patience, friendly manner, and mental support during the course of the investigation. Her wide knowledge, constructive ideas, undeserved assistance, expertise, and, most importantly, understanding and passion during the research time all contributed significantly to the successful completion of the thesis. My sincere and heartfelt gratitude will always be with her.*

I consider it an honour to express my heartfelt gratitude to ***Dr. Seeja Thomachan Panjikkaran.****, Associate Professor and Head, Department of Community Science, College of Agriculture and* ***Dr. Sharon C. L.****, Assistant Professor, Department of Community Science, College of Agriculture, Kerala Agricultural University, Vellanikkara, for their invaluable assistance, unending support, suggestions, and constant encouragement throughout the duration of the work.*

Words cannot convey how grateful I am to ***Dr. Berin Pathrose****, Associate Professor, Department of Agricultural Entomology, College of Agriculture, Kerala Agricultural University, Vellanikkara, and member of my advisory committee, for his critical comments, motivation, and constant support. I appreciate all of his assistance and cooperation for the successful completion of my research work. Also, I would like to convey my sincere gratitude to all the staff and students in the pesticide lab, KAU, for their immense support.*

Dr. P. Prameela, *Professor and Head of the Department of Agronomy, College of Agriculture, Kerala Agricultural University, Vellanikkara, and a member of my advisory group, has provided critical input, motivation, and encouragement. I am grateful for all of her help and cooperation.*

*I'd like to take this moment to thank **Dr. Suman K. T.** and **Dr. Lakshmi P. S.** for their kind assistance and helpful recommendations.*

*I am deeply indebted to **Dr. S. Krishnan**, Rtd. Professor, Dept. Agricultural Statistics, and **Dr. Pratheesh P. Gopinath**, Assistant professor and Head, Department of Agricultural Statistics for their expert advice, constant motivation, and help he rendered during the statistical interpretations and **Mrs. Norma Xavier C**, Assistant Professor and former Head of the Department, Department of Community Science, for her help advice and motivation.*

*I express my sincere thanks to the Amala Cancer Research Centre, Dept. of Animal Nutrition (KVASU), Department of Agronomy and Radio Tracer Laboratory for all the facilities provided, for their cooperation and support during the conduct of the research. I sincerely thank **Dr. A. T. Francis** and **Sreekumaran S**, Librarian for their support and guidance in organising research materials.*

*Let me express my sincere thanks to all my friends. A special thanks to my batchmates **Ajisha** and **Athira** for the moral support they rendered throughout. I am genuinely indebted to my seniors **Shahanas** Chechi, **Remya** chechi and I would like to extend my sincere gratitude to **Vidya** chechi, **Sruthy** as well as my juniors **Rammya molu, Nivya, Rajeesha, Somitha, Amrutha, Reshma, Gayathri, Neha and Visakh** for their unfailing support and encouragement. They helped me, supported me, encouraged me and gave me enough mental strength to go through all the difficulties of my life and study. For their help, my seniors and juniors have my sincere gratitude. For their genuine assistance and unwavering cooperation, I would like to thank **Kumari Chechi** and **Rose Chechi** of the Department of Community Science.*

I am genuinely indebted to all my friends and well-wishers who supported me throughout my journey. I also remember all the staff and students of KAU for their support and assistance, received during my academic years.

*For providing the essential assistance, a special thank you to **Mr. Aravind** and **Mrs. Rajitha**, Student's Computer Club, College of Agriculture, Kerala Agricultural University, Vellanikkara. We gratefully acknowledge the **KAU Junior Research Fellowship** funding.*

I also bow my head to thank my ***Pappa, Mummy, Appachan, Ammachi, Valyettan, Valyechi, Kunjettan, Kunjechi, Leo*** *chetan****, Leena*** *chechi****, Teddy*** *chettan****, Reny*** *chechi****, Ethan, Cathy, George, Antony, Emmu, Rosa*** *and* ***Joseph*** *for their love and constant support. I am also grateful for the boundless affection, eternal love and deep concern and prayers of my beloved grandparents Mr.* ***C.T Poulose*** *and Mrs.* ***Mary Poulose,*** *Mr.* ***C.K Thomas*** *and Mrs.* ***Rosy Thomas****. Last but not least, words have no power to express my love towards my husband* ***Noel*** *and my little king* ***Daveed*** *for being the pillars of unfailing encouragement.*

I am genuinely indebted to all my friends, relatives and well-wishers, who supported me throughout my journey. Their everlasting faith, love and more presence in every aspect of my life, has meant everything to me and will always be cherished.

Simla Thomas

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Sl. No	Appendix
1.	Score card for assessing the organoleptic qualities of cooked rice *(Jyothi /Njavara/Chitteni*)
2.	Score card for the selection of the best suitable condition for GABA enrichment
3.	Procedure for the preparation of processed rice products from GABA enriched rice
4.	Score card for assessing the organoleptic qualities of processed rice products of GABA enriched rice
5.	HPLC chromatogram of GABA derivatized with 2 hydroxynaphthaldehyde for GABA enriched rice varieties of *Jyothi, Njavara* and *Chitteni*
6.	Organoleptic qualities of germinated and cooked *Jyothi /Njavara/Chitteni* rice
7.	Organoleptic qualities of cooked GABA enriched rice of *Jyothi, Njavara* and *Chitteni*
8.	Organoleptic qualities of cooked GABA enriched rice of *Jyothi, Njavara* and *Chitteni* during storage
9.	Organoleptic qualities of processed products from GABA enriched rice of *Jyothi, Njavara* and *Chitteni*

INTRODUCTION

1. INTRODUCTION

"Rice is the best, the most nutritive, and unquestionably the most widespread staple in the world"

(Auguste Escoffier)

Rice, the grain of life, vitality, and vigour, is a staple for greater than 50 per cent of the global population and constitutes a major pillar of food security. It is an ancient grain that has been used as food by humans for over 10,000 years and has fed more people than any other crop. Rice (*Oryza sativa L.*) has become the "Grain of life" for 2.4 billion Asians, with Asia accounting for 92 per cent of the world's rice production and 90 per cent of the world's rice consumption. Rice is grown in an area of 157 million ha worldwide, with Asia accounting for 90 per cent. It is the most significant cereal grain, accounting for global human per capita energy (21 %) and protein (15 %) (IRRI, 2002). Therefore, there is an increasing concern regarding rice cultivation and processing to deal with the growing global population (Charoenthaikij *et al*., 2009; Horie, 2019). India has the most rice acreage, 43.4 million hectares, with 118 million tonnes of production (Singh and Pun, 2020).

Kerala is well known for its rich genetic diversity of traditional rice varieties, including many medicinal ones. Black, brown, red, dark red-purple dark purple, black-purple and dark blue grains are the various pigmented rice varieties. Several rice varieties are indigenous to Kerala, (*Jyothi* PTB 39), a widely cultivated, popular and high yielding variety in Kerala and Karnataka. The traditional unique medicinal rice variety *Njavara* (yellow glumed), is known for its therapeutic potential used in ayurvedic treatments like panchakarma and to treat rheumatism, neurological problems, rejuvenation of muscles, *etc*. and *Chitteni*, is a traditional rice variety of Kerala which is released as PTB 20. The whole brown rice grain retains bran and embryo, which contain many nutritive and bioactive compounds (Zhang *et al*., 2022). However, due to its lower acceptability when cooked, brown rice is consumed at a lower rate than white rice. The increasing demand for nutritious food among health conscious people has prompted both the scientific community and the food industry to develop novel products with improved nutritional and functional attributes and

possess improved nutritional and functional attributes, as well as discernible sensory characteristics (Pino *et al*., 2022). Germination is a low cost and effective method for enhancing textural and organoleptic attributes, flavour and taste components, phytonutrient bioavailability, and functional and medicinal properties of rice grains. Brown rice grains that have been soaked in water and allowed to sprout before being cooked or processed are referred to as germinated brown rice (GBR). The germination process involves activating the dormant enzymes and nutrients in the grain and enhancing its digestibility. It is reported that the content of phytic acid, gamma oryzanol, inositol, ferulic acid, tocotrienols, zinc, potassium, magnesium, gamma amino butyric acid, prolyl endopeptidase inhibitor, vitamin B1, lysine, vitamin E, niacin, vitamin B2 and dietary fibre increased dramatically in germinated brown rice. Traditional and coloured rice varieties are found to be an effective source of antioxidants and possess, antiproliferative activities too. Germination activates various phytochemicals like tocols, amino acids, sterols, flavones, phenolics, tannin, essential oils and γ -oryzanols (Chaiyasut *et al*., 2016), which aids in promoting human health by lowering the level of free radicals exists in the human body. GBR administration reduces blood sugar levels, strengthens the immune system, decreases blood pressure, attenuates atherosclerosis, prevents the growth of cancer cells, and assists in treating Alzheimer's and anxiety-related disorders. The antioxidant and radical scavenging properties of pigmented rice can help promote human health by lowering the concentration of reactive oxygen species in the human body. Thus, traditional rice cultivars could be a high quality food source for a well balanced diet.

Germination improves nutritional and bioactive component bioavailability, including GABA. Because of its ubiquitous nature, this amino acid has received much more attention in recent decades. GABA is a four-carbon nonprotein amino acid that already exists in brown rice, and enhances its quantity through germination. The synthesis of gamma-aminobutyric acid (GABA) occurs through the process of decarboxylation of L-glutamic acid, which is facilitated by the enzyme glutamate decarboxylase (Singh and Sharma, 2017; Sahab *et al*., 2020). It functions as an inhibitory neurotransmitter in the human brain, which promotes relaxation and sleep, lowers blood pressure, tension, anxiety, stress and depression, boosts immunity and

also has diuretic properties (Hepsomali *et al*., 2020). In addition, GABA prevents diabetes, regulates blood cholesterol levels, and inhibits the cell growth and migration of leukaemia and colon cancer cells (Ngo and Vo, 2019). GABA is recognised as a functional component of a balanced diet due to its health benefits and biological activities. There is a dearth of information regarding the GABA content of Indian brown rice varieties and their utilisation.

GABA enriched food production methods, notably rice, are gaining popularity due to increased awareness of the health benefits of GABA. The GABA concentration of germinated brown rice is two-fold higher than brown rice and ten-fold higher than milled white rice (Patil and Khan, 2011). The amount of GABA in germinated brown rice (GBR) is influenced by various factors including variety (Karladee and Suriyong, 2012), seed germination (Kaosa-ard and Songsermpong, 2012), and length of soaking (Singh *et al*., 2017). There has not yet been a thorough investigation into the potential for GABA development and utilisation, the impact of different hydrothermal methods on the GABA content in germinated traditional rice cultivars and improved varieties.

Hence, the present study entitled 'Medicinal properties and process optimisation for GABA enrichment in rice' has been undertaken with the following objectives.

1. To assess the medicinal properties and to optimise conditions for Gamma Amino Butyric Acid (GABA) enrichment in rice varieties
2. To assess the effect of processing on GABA content in rice

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Literature pertaining to the study entitled 'Medicinal properties and process optimisation for GABA enrichment in rice' are reviewed under the following headings.

2.1. Rice -The global staple

2.1.1. Nutritional qualities of rice cultivars

2.1.2. Germinated brown rice (GBR) – A key to improving nutrition

2.2. Gamma aminobutyric acid – A natural calming neurotransmitter

2.3. Health benefits of GABA

2.3.1. Neuroprotective effect

2.3.2. Anti hypertensive effect

2.3.3. Anti diabetic effect

2.3.4. Anti cancer effect

2.3.5. Antioxidant effect

2.3.6. Anti-inflammatory effect

2.3.7. Antimicrobial effect

2.3.8. Anti allergic effect

2.3.9. Hepatoprotective effect

2.3.10. Renoprotective effect

2.3.11. Intestinal protective effect

2.3.12. Other pharmaceutical properties

2.4. Factors affecting the production of GABA in rice

2.4.1. Effect of cultivar and harvest on GABA content

2.4.2. Effect of pH and temperature on GABA content

2.4.3. Effect of moisture on GABA content

2.4.4. Effect of soaking and germination on GABA content

2.4.5. Effect of drying on GABA content

2.4.6. Effect of cooking on GABA content

2.4.7. Effect of environmental stress on GABA content

2.4.8. Effect of microbes on GABA content

2.5. GABA enriched functional foods

2.5.1. Cereal based products

2.5.2. Pulse based products

2.5.3. Dairy based products

2.5.4. Vegetable based products

2.5.5. Beverages

2.1. Rice -The global staple

Rice is a sacred grain that is responsible for shaping the traditions, cultures, and food habits of billions of people worldwide. Every third person on Earth consumes rice on a daily basis in one or another form. Because it is closely knitted with the daily lives of more than half of humankind worldwide, rice has been referred to as the "grain of life." The majority of people around the world eat it as a staple food (Noonari *et al*., 2015). It is a vital part of social life, connected to rituals, festivals, and religious offerings and it also serves as a sign of affluence.

Rice plays a vital role in the world's food security, poverty eradication, and socioeconomic growth. Food security in Asia has traditionally focused production, marketing and consumption of rice. Rice is cultivated in 157 million ha in the world, of which 90 per cent of the area is in Asia. According to FAO (2020), seven countries account for 80 per cent of global rice production. China (32.7%), India (26%), Indonesia (10.2%), Bangladesh (7.5%), Vietnam (6.8%), Thailand (5.3%), Myanmar (4.8%), the Philippines (2.8%), Brazil (2.0%), and Japan (1.9%) are the top ten rice producing countries in the world.

India has surpassed China to become the world's second largest rice producer, with the largest rice harvesting area. In India, rice is a major staple food and a vital component of the food security and economy of the rural population. Small farmers with holdings of less than one hectare primarily cultivate rice. In India, 40 per cent of all food production and nearly close to 65 per cent of the population are dependent on rice (Bishwajith *et al*., 2013) and have the largest acreage of 45.07 million hectares under rice with a production of 122.27 million tonnes (Murthy *et al*., 2023). West

Bengal, Assam, Andhra Pradesh, Uttar Pradesh, Madhya Pradesh, Orissa, Kerala, Bihar, Tamil Nadu, Maharashtra, Punjab, and Karnataka are the primary rice growing states, accounting for 92 per cent of the nation's acreage and production, respectively (Mukesh *et al*., 2018).

One of the country's main rice biodiversity reservoirs is Kerala. Despite this, there are no published scientific studies on the precise number of traditional cultivars found in the state. However, it is known that Kerala cultivated mostly close to 2000 traditional cultivars (Leenakumari, 2012). These included indigenous, aromatic, and medicinal rice varieties that were well suited to varying edaphic conditions. Among these, *Jyothi* (PTB 39) is a short duration, popular high yielding variety in Kerala and Karnataka with long and bold grains and is consumed as table rice.

Njavara (yellow glumed), is a traditional, unique rice cultivar endemic to Kerala, known for its therapeutic potential used in the ayurvedic treatments of panchakarma and to treat rheumatism, neurological problems, rejuvenation of muscles *etc*. *Njavara* morphologically resembles typical rice with the husk colour changing from golden yellow to brownish black (Menon, 2004). Two types of *Njavara,* yellow and black glumed, are used in Ayurvedic treatments (Mohanlal, 2011). Due to highly valued rice with distinctive therapeutic potentials and unique flavour, *Njavara* is known as “Gold with Fragrance”. *Chitteni*, is a bright red indigenous rice variety released as PTB-20 from Kerala, grown during the Mundakan season (July to December).

2.1.1. Nutritional qualities of rice cultivars

The nutritional quality of rice is affected by the chemical composition like starch, moisture, carbohydrate, protein, fat and mineral content. The moisture level of rice is an essential component that has a substantial impact on the overall quality of rice grains. Starch expands as a result of moisture adsorption, which produces compressive stress that can result in fissured grain, which typically breaks during milling (Kunze, 2001). Additionally, Deepa *et al*. (2008) and Reshmi (2012) reported that, *Jyothi* and *Njavara* had a moisture content of around 13 per cent. As

per the findings of Pillai *et al.* (2020), the moisture level of the rice varieties ranged from 9.23 per cent in *Njavara*, 11.13 per cent in *Chitteni* to 11.23 per cent in *Jyothi*. To preserve the quality and extended shelf life, moisture content in a range from 12 to 14 per cent is recommended in rice.

The calorific content of seventeen rice cultivars of Kerala was assessed by Nandini (1995), who identified that the indigenous cultivars gave higher caloric values than the commonly used rice cultivars. Deepa *et al*. (2008) pointed that, the energy value of *Jyothi* and *Njavara* was 1570 kJ/100g and 1630 kJ/100g consecutively. The energy value of *Njavara* was reported as 357 Kcal/100g (Reshmi and Nandini, 2012) and *Jyothi* was reported as 335.81 Kcal by Chandhini (2015). As per the findings of Pillai *et al*. (2020) the *Jyothi*, *Njavara*, and *Chitteni* rice cultivars have gross energy values of 1427.25 kJ/100g, 1393.56 kJ/100g, and 1367.57 kJ/100g, respectively.

As per Yousaf (2000), freshly harvested rice contains almost 80 per cent of starch derived carbohydrates, glucose, sucrose and dextrin. The carbohydrate content in the *Njavara* and *Jyothi* cultivars was 73.5 g/ 100g and 72.8 g/100g respectively (Deepa *et al.*, 2008). As per Shijagurumayum *et al*. (2018), the carbohydrate content in pigmented red rice cultivars ranged from 64g/100g to 80g/100g respectively, and among the rice cultivars high carbohydrate content was observed in *Jyothi* (68.43 %), *Chitteni* (67.39 %) and *Njavara* (65.20 %) respectively (Pillai *et al*., 2020).

The starch of raw and parboiled rice of *Jyothi* was reported as 79.61 per cent and 75.13 per cent respectively and Reshmi and Nandini (2012) pointed out that, the starch content of *Njavara* was 74.45 per cent. According to Nadh (2018), the starch content of red rice cultivars was discovered to be between 65.83 g/100g to 70.50 g/100g.

The quantity, quality and type of protein are essential components in nutrition and Juliano (1994) indicated that, rice protein is made up of two major proteins (globulin (12 %), glutelin (80 %) and two minor proteins albumin (5 %), prolamin (3 %). Rice protein has a superior nutritional quality compared to other cereal proteins

because it is dense in lysine, the important amino acid, as per the findings of Bean and Nishita (2000). Protein is one of the abundant components in rice, next to starch and its nutritional quality depends on amino acid composition and digestibility (Xie *et al*., 2008). Protein content ranged from 6.99-10.17 per cent and glutamic acid concentration ranged from 10.1–15.2 mg/g, respectively in GBR. During the germination of brown rice, glutamate decarboxylase catalyses the decarboxylation of L-glutamic acid to produce GABA, a four-carbon non-protein amino acid (Choe *et al*., 2021).

As per the findings of Deepa *et al.* (2008), dehusked *Njavara* consists of 9.5 per cent of protein and *Jyothi* consists of 8.11 per cent protein (Sathyan, 2012). Similarly, Reshmi and Nandini (2012) indicated that, *Njavara* (yellow) contains a protein content of around 11.80 g. Likewise, Pillai *et al*. (2020) observed the crude protein content of 10.20 per cent, 11.27 per cent and 7.77 per cent in the *Jyothi, Njavara* and *Chitteni* cultivars.

Brown rice contains a fat content ranging from 2.1 to 3.2 g/100g as per the findings of Sugimoto *et al*. (1998) and Yadav and Jindal (2007) noticed a fat content in Indian rice cultivars in a range of 0.54 to 0.82 per cent. According to the findings of Deepa *et al*. (2008), a crude lipid content of 2.48 g/100g and 2.60 g/100g consisted of *Njavara* and *Jyothi* cultivars. Rice fat is made up of unsaturated fatty acids and the fat content decreases with milling because, it is more abundant in the rice bran region (Saleh *et al*., 2013). Instead, Reddy *et al*. (2017) stated the fat content of pigmented rice varieties ranged from 3.05-3.73 per cent and as per the findings of Pillai *et al*. (2020), *Jyothi, Njavara* and *Chitteni* had a crude lipid content of 3.26 per cent, 3.24 per cent and 3.10 per cent.

Rice contains insoluble fibre. According to Nandini (1995), among the seventeen selected rice varieties of Kerala, indigenous cultivars had higher total fibre content than hybrid derivatives. Furthermore, Srilakshmi (2003) found that the fibre content in rice is mainly made up of hemicelluloses that consist of pentoses, arabinose and xylose. The total fibre content in *Jyothi* was 5.82 per cent and *Njavara* was 8.08 per cent as per the study conducted by Deepa *et al*. (2008). Meanwhile, Reshmi and

Nadhini (2012) observed that, *Njavara* (yellow) had a crude fibre content of 0.198 g. According to Sathyan (2012) and Nadh (2018), the *Jyothi* rice variety had a crude fibre content of 1.07 percent and 0.18 g, respectively. According to the study, Pillai *et al.* (2020) reported, a total dietary fibre of 5.10 per cent, 5.94 per cent and 5.22 per cent consisting in *Jyothi, Njavara* and *Chitteni* cultivars.

Pigmented rice cultivars are a good source of minerals and as per the findings of Deepa *et al.* (2008), *Njavara* and *Jyothi* rice cultivars reported a calcium content of 11.6 mg/100g and 9.70 mg/100g respectively. Lakshmi (2011) and Sathyan (2012) indicated, that parboiled and raw rice of *Jyothi* contains a calcium content of 6.50 mg/ 100g and 5.94 mg/ 100g and *Njavara* (yellow) contained a calcium content of 12.20 mg/100g (Reshmi and Nandini, 2012). Simultaneously, Chandhini (2015) revealed that, the calcium content in the red rice variety *Jyothi* was 6.6 mg/100g respectively. A calcium content of 18.02 per cent, 15.81 per cent and 13.20 per cent was reported in *Jyothi, Njavara* and *Chitteni* cultivars (Pillai *et al*., 2020).

Rice contains a very low amount of iron (Gregorio *et al*., 2000) and Deepa *et al.* (2008) pointed, 1.93 mg/100g and 3.95 mg/100g of iron in both *Njavara* and *Jyothi* rice cultivars. The iron content of parboiled *Jyothi* rice is 1.97 mg/100g (Lakshmi, 2011) and Sathyan (2012) noticed, an iron content of 1.94 mg/100g in the *Jyothi* rice variety. As per the findings of Pillai *et al*. (2020), *Jyothi, Njavara* and *Chitteni* had an iron content of 3.04 per cent, 2.76 per cent and 2.38 per cent respectively.

A study put forth by Deepa *et al*. (2008) reported a higher phosphorus content of 354 mg/ 100g in the *Njavara* variety followed by *Jyothi* (324 mg/100g). A phosphorus content of 161.83 mg/ 100g was noticed by Lakshmi (2011) in the parboiled *Jyothi* variety. Similarly, Reshmi and Nandini (2012) noticed phosphorous content of 351.40 mg/100g in *Njavara* (yellow) and according to Chandhini (2015), the phosphorus content of *Jyothi* obtained as 133.2 mg/100g respectively. Nadh (2018) observed a phosphorus content of 130.10 mg/100g in the *Jyothi* variety.

Furthermore, Sotelo *et al*. (2000) indicated that, polished rice grains have a low amount of zinc whereas, the rice bran contains the highest zinc content. According

to Brar *et al.* (2011), rice cultivars were divided into three groups according to the zinc concentration as high (>2.5 mg/kg), medium (1.41-2.4 mg/100g) and low (0.0 – 1.40 mg/100g). The zinc content of 3.31 per cent, 3.07 per cent and 3.31 per cent is found in *Njavara, Jyothi* and *Chitteni* rice cultivars (Pillai *et al*., 2020).

As per the results of Deepa *et al.* (2008), *Njavara* and *Jyothi* had a magnesium content of 216 mg/100g and 150 mg/100g.

Rice is considered as a good source of vitamin B group, especially riboflavin and thiamine (Rai, 2009). Gopalan *et al*. (2012) noted that, the thiamine content was found to be between 0.35 to 0.44 mg/ 100g in brown rice. Meanwhile, Deepa *et al*. (2008), indicated, thiamine, riboflavin and niacin content of 0.52 mg, 0.071 mg and 7.32 mg in *Njavara* and 0.35 mg, 0.053 mg and 7.15 mg in *Jyothi* variety. According to a study, *Njavara* (yellow) contained 0.058 mg/100g of thiamine and 0.048 mg/100g of riboflavin (Reshmi and Nandini, 2012). Meanwhile, Nadh (2018) reported that the thiamine content in *Jyothi* variety was 0.060 mg/100g.

2.1.2. Germinated brown rice (GBR) – A key to improving nutrition

Brown rice grains that have been soaked in water and allowed to sprout before being cooked or processed are referred to as "germinated brown rice (GBR)." The process of germination involves activating the dormant enzymes and nutrients in the rice, increasing its nutrient content and enhancing its digestibility (Taylor and Njintang, 2012). Germinated brown rice (GBR) is also called as sprouted brown rice and it is considered whole food after germination, because rice is just removed from its hull, causing a least damaging effect on its nutritional content. GBR is distinct from regular brown rice, because GBR has gone through the germination process in which the sprouting of rice embryo occurs in favourable atmospheric conditions. Brown rice can be sprouted by soaking it in water between 35°C and 40°C for 10 to 12 hours, draining it, and keeping it moist for 20 to 24 hours. The brown rice grain emerges sprouting with a length of 0.5–1 mm (Patil and Khan, 2011). The accumulation of nutrients was found to be highest in this stage.

Suda *et al.* (1986) pointed out, increased activity of the enzyme amylase, which converts starch into simple carbohydrates. Carbohydrates were used as a source of energy for growth and development (German and Dillard, 2006). The breakdown of starch within the cotyledon into smaller molecules such as glucose and fructose serves as a source of energy for cellular division, facilitating the maturation and growth processes of seeds (Nonogaki *et al*., 2010). In addition, the alterations in carbohydrate after germination can be explained by Singh and Sharma, 2017).

The amount of amylose, tends to decrease as brown rice germinates. Compared to ungerminated rice, brown rice frequently has a lower amylose level. According to Imam *et al.* (2014), the total carbohydrates in GBR are 15 per cent less than in white rice. Similarly, the content of starch in GBR was lowered after pre-germination compared to brown rice (Noomhorm *et al*., 2014). Germination can cause modifications in rice starch granules. Germinated brown rice starch granules have been seen to change in granule size distributions and become more porous (Zhang *et al*., 2020). The gelatinisation characteristics of rice and cooking quality can be affected by the reduction in amylose content.

Sprouting increases the amino acid concentration and oligosaccharides in cereal grains, in rice (Manna *et al*., 1995). As per the results of Bau *et al.* (1997), the protein increase was caused by the production of enzyme proteins or a compositional change as a result of the decomposition of additional components. Similarly, Ohtsubo *et al.* (2004) pointed that, the protein was found to be increased after the germination of brown rice. After 72 hours of germination, GBR contains noticeably more alanine, glycine, and aspartic acid than brown rice, according to research by Ohtsubo *et al.* (2005). However, Urbano *et al.* (2005), Khatoon and Prakash (2006) and Ghavidel and Prakash (2007) revealed that total protein was found to be increased after germination. Meanwhile, Komatsuzaki *et al.* (2007) reported that, pre germination for 24 h reduced the contents of glutamine. Simultaneously, Vellupillai *et al.* (2009) identified that increased protease activity causes a decrease in total protein content while increasing amino acid content. Simultaneously, Nonogaki *et al*. (2010) reported that protein synthesis happens during imbibition, and hormonal shifts

are required for effective germination completion. Instead, Maksup *et al.* (2018) observed that, germination improved the proteins that produce phenolic acids in response to oxidative stress.

Fat is used as the principal source of carbon for seed development (Bau *et al*., 1997), and studies conducted by Dhaliwal and Aggarwal (1999), El-Adawy *et al*. (2004), Ghavidel and Prakash (2007), and Hahm *et al*. (2008) found that the fat content decreased with an increase in the time of germination. Additionally, Hahm *et al.* (2008) reported that, for energy formation, fatty acids are getting oxidised to water and carbon dioxide. Likewise, Choi *et al.* (2009) investigated that, oleic acid abundance was found to be trending downward in GBR during germination, but palmitic acid and linoleic acid concentrations were trending upward.

According to a study conducted by Azizah and Zainon (1997), soaking rice increased the total dietary fibre. This implies that the amount of dietary fibre during the soaking period prior to the sprouting period is affected by the germination process. The amount of resistant starch, which functions like dietary fibre and resists digestion in the small intestine, has been reported to increase with the germination of brown rice. The improvement in the nutritional quality of germinated brown rice is thought to have a result of this increase in resistant starch. The soluble fibre in brown rice was raised by pre germination by 70 per cent. Furthermore, Rao and Muralikrishna (2004) stated that, pre-germination causes brown rice's xylose, mannose, and glucose contents to fall while its rhamnose and arabinose contents increased. The occurrence of the fibrous bran layer of rice, in germinated rice contributed to the presence of the total amount of dietary fibre (Ohtsubo *et al*., 2005).

GABA, vitamin B1, lysine, dietary fibre, vitamin E, magnesium, niacin, and vitamin B6 are some of the nutrients whose concentrations have increased dramatically (Kayahara *et al*. 2001). Instead, Bahadur (2003) found out that, eating unpolished (brown) rice is preferable since it has more iron, fibre, minerals and vitamins than polished white rice, which is eliminated during milling. Additionally, germination is essential for boosting nutrients essential for optimal health. Meanwhile, El-Adawy *et al*. (2004) reported that, germination was also linked to an

increase in vitamin content and the bioavailability of nutrients like trace elements and minerals. Instead, Trachoo *et al*. (2006) stated that, sprouting enhanced the amount of various nutrients like, reducing sugar, total protein and B vitamins in GBR, which was greater than that of white rice and brown rice. Likewise, Usuki *et al.* (2007) studied GBR, γ-tocotrienols were more prevalent than other members of the vitamin E family. According to Kaushik *et al*. (2010), germination increases the concentration of minerals like copper, manganese, calcium, zinc, niacin, riboflavin and ascorbic acid. Germinated brown rice digests and absorbs well and includes more nutrients like GABA than regular brown rice. Vitamin B1 is more abundant in GBR than in brown rice (Watchararparpaiboon *et al*., 2010). When compared to brown rice, it appears that pre germination significantly changes a number of macro and micronutrients in GBR.

According to Kayahara and Tsukahara (2001), ferulic acid, inositols, phytic acid, potassium, tocotrienols, gamma oryzanol, zinc and prolyl endopeptidase inhibitors were increased in GBR. According to Tian *et al.* (2004), free ferulic, coumaric, and sinapic acid concentrations in GBR were substantially greater than those in brown rice. Another study by Ohtsubo *et al.* (2005) identified that, after 72 hours of germination, the amount of ferulic acid increased by 26 per cent. The concentration of phytic acid in brown rice was dramatically decreased prior to germination, while the rate of zinc absorption remained unchanged, suggesting that the zinc in GBR may also bind to other substances in the body (Liang *et al*., 2008). Meanwhile, Imam *et al.* (2012) stated that, due to its enhanced phenolic compound level, GBR has better antioxidant capacity. After germination, anaerobic treatment enhanced the number of phenolic acids in GBR, and antioxidant capacity was more noticeable in black or red rice than brown rice (Shen *et al.*, 2015). Simultaneously, Zhao *et al.* (2018) stated that, the GBR had higher concentrations of vanillin, vanillic acid, ferulic acid, syringic acid and coumaric acid.

Due to the texture softening and augmentation of flavour in grains, the breakdown of high-molecular-weight polymer yields bio functional compounds and enhances sensory properties (Beal and Mottram, 1993). Due to the neutralisation of phytic acid, an increased bioavailability is observed for nutrients and minerals during

the development of the sprout, GBR has a soft and smooth texture than regular brown rice, according to Kayahara and Tsukahara (2001). According to the sensory analysis, cooked germinated rice is more tastier, smoother, swollen, and coherent than cooked conventional brown rice. In addition to softening the texture, the germination of brown rice was employed to enrich the flavour and nutritional qualities (Ohtsubo *et al*., 2005). This suggests that it can be cooked more easily and is simpler to digest. According to Jiamyangyuen and Ooraikul (2008), longer soaking and germination times led to improved cooking and textural qualities of rice. They claimed that germinated rice expands in size and needs less time to cook due to the water absorption by the grains through germination.

As per Kayahara *et al*. (2001), germination causes an inner change those results in the release of more nutrients in addition to an increase in the existing nutrients in GBR. Likewise, Rasolt (2008) discovered that a growth factor, acylated sterylgulcoside (ASG) is found after germination in brown rice. Acylated sterylgulcoside reportedly normalises dysfunctional enzymes and aids in diabetic blood sugar control by raising levels of favourable enzymes that reduce diabetes. For people with diabetes mellitus, a lower carbohydrate intake is advantageous. Additionally, those who have hypercholesterolemia and cardiovascular disease can benefit from increasing their overall dietary fibre intake and reducing their fat intake.

The nutritional qualities of rice are enhanced by germination, but usually shortens grain's shelf life. The activated enzymes during the sprouting, changing the composition of the rice grain and increasing its susceptibility to deterioration. GBR is sold in dry form to increase the shelf life (Patil and Khan, 2011). Meanwhile, Chen *et al.* (2017) studied the germination effect on the shelf life of rice, and reported that, compared with the ungerminated rice, the germinated counterparts had a significantly shorter shelf life. In samples of germinated rice, they noticed changes like an increase in moisture content, accelerated lipid oxidation, and higher microbial growth. These alterations result in a shorter shelf life and can cause rancidity, off flavours, and spoilage.

2.2. Gamma aminobutyric acid (GABA) – A natural calming neurotransmitter

GABA is a four carbon non protein amino acid (Figure 1) present in plants and is also essential for the smooth functioning of the central nervous system (CNS) in humans. Plant research gained momentum when it was discovered that GABA concentration was first identified in potato tuber and later identified in the human brain (Roberts and Frankel, 1950). In plants, GABA regulates pH, bypasses the Tricarboxylic Acid (TCA) cycle, balances carbon and nitrogen, and stores nitrogen in plants. It is also useful for signaling and communication, plant defense, and as a suitable osmolyte (Rashmi *et al*., 2018). Other than GABA, neurotransmitters like serotonin, dopamine, acetylcholine, melatonin, noradrenaline, catecholamines and biogenic amines aid plant protection (Ramakrishna and Roshchina, 2018).

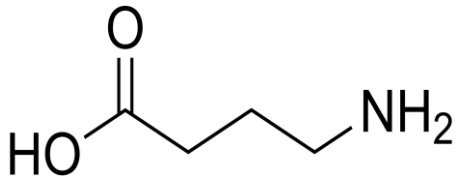


Figure 1. Chemical structure of GABA

Gamma amino butyric acid (GABA), the key coordinator of brain activity is an inhibitory neurotransmitter and non protein amino acid seen in the central nervous system (CNS) of the mammalian brain. Glutamate is converted to form GABA through decarboxylation, with the action of the enzyme glutamate decarboxylase (GAD) (Shelp *et al*., 1999). This reaction takes place in both neurons and astrocytes and needs the co-factor pyridoxal phosphate (PLP). GABA plays a crucial role in the regulation of transsynaptic signalling in terms of both temporal and spatial aspects. Additionally, it is engaged in modulating the temporal patterns of neuronal excitability, sustaining activity within certain brain regions, and maintaining the delicate equilibrium between neuronal excitation and inhibition. GABA's inhibitory role in the brain is crucial and known as the substance that naturally calms the brain (Klausberger and Somogyi, 2008).

According to Shelp *et al*. (2012), diamine oxidase (DAO) is the most significant rate limiting enzyme in the indirect formation of GABA from polyamines. GABA or gamma aminobutyric acid metabolism involves the GABA conversion into succinic semialdehyde (SSA) and then to succinate (Chua *et al*., 2019). This process is known as GABA shunt. GABA converts glutamate into succinate in the GABA shunt. According to Hepsomali *et al.* (2020), 60-75 per cent of synapses are GABAergic in nature.

The mechanistic action of GABA, which is produced in the human brain occurs by inhibiting the nerve signals. Pre and postsynaptic neurons and development and localisation of receptors are the important factors that are essential for the proper functioning of synapses. GABA acts as a tropical signal and facilitates synapse formation, enhances the neuronal growth, and promotes the production of particular proteins. The GABA exerts its inhibitory effect, through three major groups of GABA receptors, namely alpha (A), beta (B) and gamma (C) subunits which further determines the pharmacological efficiency. Fast inhibitory synaptic transmissions, rapid mood changes, and regulation of neuronal excitability are mediated by inotropic $GABA_A$ receptors (Borden *et al*., 1994). Likewise, Meldrum and Chapman (1999) found out that, $GABA_B$ receptors help to slow inhibitory transmissions, which is essential for pain, mood and memory. When the $GABA_A$ receptor is activated, chloride conductance increases across the cell membrane immediately and significantly, inhibiting the neuron and preventing it from producing an action potential (Mody and Pearce, 2004). The selective activation of the third class of GABA receptors in the mammalian central nervous system (CNS) is achieved by the GABA analogue cis-4-aminocrotonic acid. The pharmacological characteristics of $GABA_C$ receptors are distinct in that they do not exhibit inhibition by bicuculline and remain unaffected by barbiturates, baclofen, neuroactive steroids, or benzodiazepines (Yogeeswari *et al*., 2006).

GABA is impermeable to the blood brain barrier (BBB) and all the actions after the administration or injection of GABA reflect within the peripheral tissues, automatic nervous system, or blood vessels. Additionally, Bowery (2002) revealed

that, the metabotropic and ionotropic receptors are types of ligand-gated chloride channels that exhibit rapid activation and are responsible for facilitating swift inhibitory processes. In contrast, $GABA_B$ receptors are indirectly linked to calcium or potassium channels through G-proteins, resulting in delayed and sustained inhibitory responses (Nuss, 2015).

There are three types of GABA transporters, namely GAT-1 (also known as GAT-A), GAT-2 (a transporter sensitive to β-alanine), and GAT-3 (also known as GAT-B, which is sensitive to both nipecotic acid and alanine) (Kakee *et al*., 2001). Among these, the main GABA transporters GAT-3 and GAT-1, are situated in the spinal cord and brain, expressed by some astrocytes and neurons. Another GABA transporters are GAT-2 and BGT-1 which are present in lower levels in the brain and as higher amount in the meninges, liver and kidney (Zhou and Danbolt, 2013). By reabsorbing the transmitter and clearing the synapses, the GABA concentration in the extracellular space is regulated by ABA transporters. After binding with GABA, the ABA transporters are translocated into the cytoplasm. Instead of being broken down, the GABA transmitters are cleared by GABA transporters through reabsorption from the neural cleft. To regulate the GABA receptors' activity, an extracellular GABA concentration is maintained by the plasma membrane GABA transporters close to the synapse. The GABA transporter aids in the creation of a GABA equilibrium and performs the reverse action if necessary to maintain the basic level of GABA in the system (Scimemi, 2014).

2.3. Health benefits of GABA

The pharmacological and therapeutic properties of gamma-aminobutyric acid (GABA) have been shown in relation to several organs and non-neuronal peripheral tissues. These properties include anti cancer, antioxidant, anti diabetic, anti hypertensive, anti microbial, anti allergic, anti-inflammatory effects, as well as intestinal protection, renal protection, and hepatoprotection. As a result, GABA is considered as a potent alternative therapeutic agent for the diagnosis, therapy, and prevention of different diseases.

According to Ngo and Vo (2019), GABA is known as the primary inhibitory neurotransmitter in the central nervous system (CNS). The physiological functions of GABA include synaptic transmission regulation, neuron development promotion and provide relaxation and lower the rate of depression and insomnia. The decreased activity of GABA lead to diseases like schizophrenia, autism, Tourette's syndrome, Huntington's disease, epilepsy, Parkingson's disease, Dystonia *etc*.

2.3.1. Neuroprotective effect

ROS or Reactive oxygen species, cytokines and nitric oxide are considered inflammatory facilitators that of released when nervous tissue is damaged by an injury. These mediators can lead to multiple neuronal degenerations in the central nervous system (CNS) such as Alzheimer's, multiple sclerosis and Parkinson's disease (Kaminsky *et al*, 2016; Bagli *et al*., 2016). GABA supplementation has also demonstrated a protective effect against neurological conditions like insomnia, somnipathy, anxiety, dementia and depression. According to Hepsomali *et al.* (2020), the consumption of GABA improved immunity, sleep quality, memory, cognitive function and relaxation.

According to Rungtip *et al.* (2012), germinated brown rice (GBR) and brown rice (BR) extract can preserve the cells by significantly increasing the cell survival up to 29.3 per cent ± 0.01 per cent and 13.4 per cent ± 0.07 per cent and can decrease ROS formation, prevent SK-N-SH cells generation and cell death. Additionally, Ismail *et al*. (2012) investigated the protective effects of hydrogen peroxide (H_2O_2) against cellular apoptosis in human SH-SY5Y cells by germinated brown rice consumption. It was also found that, GBR prevented phosphatidylserine (PS) translocation and decreased mitochondrial membrane potential (MMP) in SH-SY5Y cells, which are essential components of apoptosis and subsequently led to cell death. Likewise, oral supplementation of GABA containing fermented rice flour (GFRF) increased the activities of antioxidant enzymes in the cortex and cerebellum with a significant increase in GABA levels, decreased oxidative stress, conferring neuroprotection (Divyashri *et al*., 2017). The protective effects of germinated brown rice (GBR) against glutamate toxicity in HT22 hippocampus neurons were studied by

Oo *et al.* (2020). According to Promtang *et al.* (2023), the results of the study indicated that GBR had a significant anti-apoptotic activity, effectively inhibiting cell death in differentiated HT22 cells. This effect was achieved by disrupting the re-entry of cells into the cell cycle and inhibiting apoptosis through its interaction with the $GABA_A$ receptor.

Neurologic disorders are linked to brain or nervous system dysfunction, which can cause physical or psychological symptoms. According to Parvez (2018), the symptoms include epilepsy, multiple sclerosis, neuro infections, Alzheimer's disease, Parkinson's disease, cerebrovascular diseases and insomnia.

There is proof that GABA can enhance memory and cognitive abilities while reducing neurodegeneration. According to the research conducted by Kayahara and Tsukahara (2001), the germination of brown rice can inhibit the protylendopetidase enzyme effectively, which is related to Alzheimer's disease. Similarly, Okada *et al*. (2000) examined, the effects of GABA enriched rice on depression, insomnia and autonomic disorder. The results indicated that, more than 65 per cent of patients with the most prevalent mental symptoms associated with menopause and the pre-senile period, including depression, somnipathy, and sleeplessness, were noticeably improved. On the other hand, GBR demonstrated neuroprotective properties by controlling the metabolism of transcriptional regulation of amyloid precursor protein (APP). Several phenolic compounds and γ-oryzanol were present in the GBR extract and changed the chemical composition of beta-amyloid Aβ (1-42) shows an anti amyloidogenic activity. Additionally, as indicated by decreased lactate dehydrogenase (LDH) release and ROS generation in intracellular cells, GBR was helpful in attenuating the oxidative properties of hydroxyl radicals. The management of complex etiological factors that lead to Alzheimer's disease pathogenesis will be significantly impacted by GBR (Azmi *et al*., 2015).

Furthermore, Chompoopong *et al.* (2016) evaluated the neuroprotective effects of brown rice after germination (GBR) and stated that, GBR consumption

significantly improved motor activity and decreased the number of apoptosis cells (79.32 %).

Additionally, Mabunga *et al.* (2015) pointed out, the consumption of GABA from fermented rice germ (10, 30, or 100 mg/kg), reduces caffeine induced sleep disturbance without altering the spontaneous locomotor activity and coordination of motor activity. Yoshida *et al*. (2015) assessed the anti stress effect of GABA enriched rice and identified that, consuming GABA enriched rice on a daily basis for eight weeks was proven to enhance mental health and sleep, reducing stress in people.

2.3.2. Anti hypertensive effect

High blood pressure is related to hypertension, which leads to various heart conditions like myocardial infarction, hemorrhagic and ischemic stroke, and kidney and heart failure (Schellack and Naicker, 2015).

Furthermore, Murray and FitzGerald (2007) reported that, the potential vasoconstrictor angiotensin II is produced by the angiotensin I converting enzyme (ACE), which has been linked to a vital role in blood pressure regulation. As a result, interaction between the angiotensin II and GABA may be involved in central BP regulation and the pathogenesis of hypertension (Yao *et al*., 2008). Gamma aminobutyric acid (GABA) has shown promise in lowering blood pressure through its ability to inhibit ACE through the blockage of angiotensin II production (Tu *et al*., 2022; Guiyun *et al*., 2022).

According to Matsubara *et al.* (2002), morning systolic and diastolic blood pressure was significantly decreased by 5 and 10 mmHg ($p< 0.05$) in the GABA treatment group after the administration of 80 mg GABA. The administration of rice grains enriched with GABA (0.1 to 0.5 mg/kg) in rats with hypertension for 6 weeks was found to lower blood pressure (Akama *et al*., 2009). Similar results were observed in spontaneously hypertensive rats conducted by Ebizuka *et al*. (2009), where GABA rich brown rice significantly decreased serum cholesterol levels and blood pressure. According to Nishimura *et al.* (2016), compared with the placebo rice, eating GABA rice can lower morning blood pressure. According to Kawakami *et al*.

(2018) and Chen *et al.* (2021), after administering GABA enriched brown rice (GEBR) for a duration of 4 weeks, a noteworthy inhibitory effect on blood pressure elevation was reported in the GEBR group.

2.3.3. Anti diabetic effect

Diabetes mellitus is an endocrine condition characterised by abnormal carbohydrate metabolism, insufficient insulin secretion or action, and the development of chronic hyperglycemia (Kharroubi and Darwish, 2015). The fermentation of foods and rice germination are accompanied by elevation in GABA content (Sivamaruthi *et al*., 2018). Studies have demonstrated the effectiveness of GABA and naturally occurring products enhanced with GABA in decreasing blood sugar levels, reducing insulin intolerance, enhancing the release of insulin and guarding against pancreatic injury.

According to Bansal *et al*. (2011), GABA has been observed to increase the release of insulin in pancreatic INS-1 β-cells. As per the findings of Tian *et al.* (2011), oral administration of GABA significantly reduced fasting blood sugar levels (FBS), inhibited weight gain and increased insulin intolerance and glucose tolerance in high fat diet fed mice. GABA inhibits the nervous related diabetic complications by suppressing the fast and cerebral cortex's mitochondrial-dependent apoptotic pathway (Huang *et al*., 2014). Meanwhile, Liu *et al.* (2017) revealed that, oral GABA administration decreased the baseline level of blood sugar and enhanced the pace of glucose excursion in mice induced with streptozotocin.

The benefits of germinated rice in controlling diabetes and its complications were highly appreciated. According to Hagiwara *et al.* (2004), the administration of a meal mostly consisting of pre-germinated brown rice to rats with diabetes resulted in a considerable reduction in blood glucose levels, as well as a drop in the quantity of the adipocytokine PAI-1 and plasma lipid peroxidation. Similarly, Usuki *et al.* (2007) pointed that, with the intake of pre germinated brown rice (GBR), the blood sugar and lipid content in rats with streptozotocin-induced diabetes were seen to decrease. Simultaneously, Hsu *et al*. (2008) and Hayakawa *et al*. (2009) have suggested that, in

adult onset diabetes mellitus, pre germinated brown rice was found to lower hypercholesterolemia and blood glucose. Moreover, pre germinated brown rice prevents diabetic complications by lowering adipocytokine (TNF-α, PAI-1) concentration and HbA(1c) and elevating the adiponectin level in type2 diabetic mice (Torimitsu *et al*., 2010). Clinical trials, conducted by Ito *et al.* (2015) also found that pre germinated brown rice intake was found to be helpful in decreasing postprandial blood glucose concentration (PPBS) without elevating the secretion of insulin. Furthermore, Adamu *et al.* (2016) observed an increase in adiponectin levels, as well as a reduction in insulin and insulin resistance, leptin, and oxidative stress in the offspring of diabetic pregnant rats that were exposed to a high-fat diet. This effect was achieved by administering germinated brown rice for a duration of eight weeks, and the measurements were conducted using a homeostasis model. As per the findings of Si *et al.* (2018), the bran of GABA enriched rice supplementation to overweight rats showed effective results in reducing serum body lipids, an indicator of insulin resistance. Similarly, giant embryonic blackish purple rice consumption for 8 weeks dramatically reduced ovariectomized rats' adipokine levels, blood glucose and, plasma insulin levels and hepatic glucose regulating enzyme activity (Chung *et al*., 2018). Similar results are reported by Lee *et al.* (2013) that, for eight weeks a C57BLKS/J-db/db mouse model receiving oral treatment of the germinated rough rice extract dramatically boosted insulin levels and decreased blood glucose levels.

2.3.4. Anti cancer effect

Cancer is an uncontrolled growth of cells, inhibition of cell death, invasion, and malignancy (Ribas *et al*., 2016). In this sense, GABA has shown promise as a metabolite that can control cancer growth and spread by causing apoptosis and inhibiting proliferation.

In contrast to white rice cultivars, pigmented cultivars have higher concentrations of cyanidin-3-O-D-glucopyranoside, which has multiple functions including, anti neurodegenerative activity, anti cytotoxicity activity, glycogen phosphorylase inhibition, antioxidant and scavenging activity (Oki *et al*., 2005). As per Mohan *et al*. (2014), brown rice contains a good number of vitamins, minerals,

and phytochemicals like polyphenols, oryzanol, phytosterols, tocotrienols, and tocopherols. Due to the pigment anthocyanins, which have therapeutic qualities such as anticarcinogenic activities, anti-inflammatory and antioxidant activities, red and black rice cultivars are regarded as antimutagenic foods (Bhat and Riar, 2015).

According to Geethakutty *et al*. (2004), *Njavara* is a good antioxidant, having good anticancer activity. According to Rao *et al*. (2010), total phenolic content and total flavonoid content (TFC) of the rice extract (methanolic) were markedly higher in *Njavara* compared with *Jyothi* as noticed in quercetin equivalents. According to the results of Kim *et al*. (2012), the phenol content in germinated black rice and germinated red rice was more than non germinated rice. The presence of the anticancer protein known as Bowman-Birk trypsin inhibitor protein has been documented in *Njavara* rice (Deepa *et al*., 2012). It is rich in tannin, flavones, phenolics, tocols, sterols, γoryzanols, amino acids, and essential oils, among other phytochemicals that support human health by lowering the body's level of free radicals (Thomas *et al*., 2023).

The production rates of specific leukemia cells is decreased and their apoptosis is enhanced by GABA enriched brown rice extract (Oh and Oh, 2004). The findings indicate that higher amounts of GABA in brown rice extracts stimulate the death of cancer cells while inhibiting the growth of leukemia cells. In a study by Latifah *et al.* (2010), colon cancer cells are inhibited by germinated brown rice. According to Kim *et al.* (2012), ethanol extract of rough rice at 4 days shown a greater anticancer impact than raw rough rice extract on the stomach cancer (AGS) cell line and colon cancer (HCT-116) cell line. Similarly, Li *et al.* (2019) recently reported that lactobacillus acidophilus-fermented germinated brown rice with a higher GABA content demonstrated potential as a dietary supplement for colorectal cancer treatment (Radulovic *et al*., 2007).

2.3.5. Antioxidant effect

Aerobic cells use oxygen, which results in the production of free radicals. According to Lobo *et al.* (2010), consuming natural products with strong antioxidant

effects is beneficial for preventing diseases brought on by free radicals. Antioxidants are substances that are present in low concentrations and significantly inhibit the rate of target oxidation within the biological systems (Lai *et al*., 2010).

Many research have demonstrated GABA's antioxidant properties and the significant impact of germinated brown rice (GBR) supplementation leads to boosting enzyme activity of antioxidants, enhancing vitamin E levels, and lowering peroxidation of lipids in hypercholesterolemic rabbits in the aversion of atherosclerotic plaque formation (Mohd *et al.*, 2013). According to Zamri *et al.* (2014), the GABA enriched germinated brown rice extract significantly scavenged thiobarbituric acid reactive and hydroxyl radical compounds, indicating its radical scavenging capacity. A novel product, brew germinated pigmented rice vinegar, has recently emerged as a suggested option due to its notable antioxidant properties (Phuapaiboon, 2017). Furthermore, Chung *et al*. (2018) found that, extracts from the germinated Keunnunjami rice have potential therapeutic benefits against oxidative stress, gastric cancer, and cervical cancer.

2.3.6. Anti-inflammatory effect

GABA has shown to be an anti-inflammatory agent by lowering the synthesis of pro inflammatory mediators and relieving inflammatory symptoms.

The study put forth by Han *et al.* (2007) showed that, GABA has anti-inflammatory properties by decreasing the expression. As a result, it may aid in reducing the whole healing time and speed up the initial stages of wound healing. Similarly, the synthesis of inflammatory cytokines is reduced by GABA (Prud'homme *et al*., 2013).

Recently, germinated brown rice enriched with GABA prevented ROS (Tuntipopipat *et al*., 2015). According to Wunjuntuk *et al*. (2016) germinated brown rice after parboiling (PGBR) reduced liver fibrosis and inflammation. A study conducted by Ngo *et al*. (2022), GABA-enriched products from *Lactobacillus fermentum* fermented rice bran water solution were found to lower the levels of inducible nitric oxide synthase expression and cyclooxygenase2 enzymes.

Additionally, pretreatment with GABA enriched products lowered the production of tumour necrosis factor.

2.3.7. Antimicrobial effect

GABA may improve the virulence of *Pseudomonas aeruginosa* and also promote the action of the bacteria with the host proteins (Dagorn *et al*., 2013). Recently, the importance of GABA in antimicrobial host defences was explained by Kim *et al.* (2018) and reported that, GABAergic activation led to improved antimicrobial responses against *Mycobacteria, Salmonella*, and *Listeria* infections *in vitro* and *in vivo*.

2.3.8. Anti allergic effect

Allergy is an immunological disorder characterized by an excessive immune response to innocuous chemicals present in the surroundings. Kawasaki *et al.* (2014) found that GABA reduced basophil and mast cell degranulation *via* the GABA B receptor on the surface of the cell. They also observed that GABA had an inhibitory effect on histamine produced from *in vitro* activated mast cells.

2.3.9. Hepatoprotective effect

In humans, prolonged ethanol usage can result in liver damage and unhealthy lipid profiles. According to Oh *et al.* (2003), mice fed with brown rice extract for 30 days showed lower amounts of liver aspartate aminotransferase, liver alanine aminotransferase and blood low density lipoprotein (LDL) cholesterol. Additionally, the liver triglyceride and total cholesterol levels were decreased while the liver and serum high density lipoprotein (HDL) and cholesterol levels were both markedly elevated by the brown rice extract. As per the observations of Wunjuntuk *et al*. (2016), germinated brown rice (GBR) after parboiling (PGBR) of Khao Dawk Mali 105 variety reduced CCl_4 caused liver oxidative stress and injury by increasing antioxidant capabilities through the complex interactions of γ oryzanol, phenolic acids, GABA and tocotrienol. As per the findings of Wangpradit *et al*. (2020) the degrees of fat degradation and lipid accumulation in hepatic tissues was enhanced by feeding

with 0.5 per cent germinated Sang-Yod rice per kg per day. Germinated Sang-Yod rice had significant abilities to improve serum lipid profiles and deposition in the liver of obese mice.

2.3.10. Renoprotective effect

Renoprotective agents derived from natural sources play a crucial role in the prevention and management of kidney-related ailments. Research conducted by Kim *et al.* (2004) stated that the administration of oral GABA effectively mitigated the physiological changes associated with acute renal failure (ARF) in rats. These changes included an increase in body and kidney weight, elevated levels of urea creatinine and nitrogen, reduced creatinine clearance, decreased urine osmolarity and sodium FE(Na) secretion. Furthermore, Sasaki *et al.* (2006) reported that GABA exhibited a reduction in nephrectomy induced oxidative stress in rats. This was achieved through an increase in levels of catalase and superoxide dismutase, as well as a decrease in lipid peroxidation. Administration of GABA to rats with an acute tubular necrosis model dramatically reduced the nephrotoxicity caused by cisplatin (Ali *et al*., 2015). In a study conducted by Talebi *et al.* (2016), it was observed that GABA demonstrated a preventive effect on kidney injury caused by renal ischemia reperfusion in ovariectomized rats. This was demonstrated by a decline in serum levels of creatinine and blood urea nitrogen, together with a reduction in kidney weight and amelioration of renal tissue damage.

2.3.11. Intestinal protective effect

As per the findings of Xie *et al.* (2017) who estimated the impact of GABA on the colon health of mice and reported that, the quantities of propionate, acetate, total short chain fatty acids, and butyrate as well as the pH value in the caecal and colonic contents, were found to be increased. Furthermore, Kubota *et al.* (2018) observed that GABA administration resulted in an upregulation of alpha-defensin-5 expression, enhanced IgA secretion, and increased superoxide dismutase activity inside the small intestine (SI) of rats. These effects were shown to mitigate the alterations in intestinal immunity generated by ischemia reperfusion. In a recent study

conducted by Jiang *et al.* (2018), the researchers presented empirical support for the protective effects of GABA in ameliorating alcohol and 2,4,6-trinitrobenzene sulfonic acid induced colitis related damage to the intestinal mucosal barrier.

2.3.12. Other pharmaceutical properties

In a study conducted by Yang *et al*. (2019) examined whether GABA may reduce fluoride-induced thyroid damage and the adult male mice were given 30 days of exposure to sodium fluoride (NaF) (50 mg/kg) to conduct the model of hypothyroidism. It is interesting to note that GABA supplementation significantly increased thyroid peroxidase, thyroid thyroglobulin and sodium/iodide symporter production. Additionally, it enhanced liver metabolic protection, thyroid function associated gene expression, and thyroid redox state. These results showed that GABA has the potential to treat hypothyroidism.

In terms of growth hormone, oral GABA treatment has been shown to increase human post exercise and resting concentrations of immunofunctional and immunoreactive growth hormone (Powers *et al*., 2003). According to the research conducted by Tujioka *et al.* (2007, 2009) and Radulovic *et al.* (2007), it is anticipated that the administration of GABA will result in increased levels of growth hormone (GH) and an elevated rate of protein synthesis in the rat brain. Instead, Sakashita *et al.* (2019) stated the impact of GABA in the development of muscle hypertrophy and they discovered that taking GABA and whey protein together significantly increased the amount of body fat free mass, hence promoting muscular hypertrophy induced by exercise.

Meanwhile, Yang *et al.* (2014) examined the regulatory influence of gamma-aminobutyric acid (GABA) on molecules associated with the metabolism of cholesterol in macrophages generated from human monocytes (HMDMs) by inhibiting the expression of scavenger receptors. GABA inhibits the growth of foam cells produced from human macrophages which implies its role in preventing atherosclerotic lesions.

It is also reported that, due to enhanced GABA signalling in the brain stem, respiratory depression (slow breathing) can be caused by overdosing on GABA modulating medications and foods (Brousse *et al*., 2012).

2.4. Factors affecting the production of GABA in rice

In rice, GABA content is affected by many factors including cultivar, harvest, pH, temperature, moisture, soaking time, germination time, drying, cooking, stress and microorganisms. Each of the factors is discussed in detail below.

2.4.1 Effect of cultivar and harvest on GABA content

GABA is naturally present in rice and the rice cultivar mainly influences its composition. Studies have shown that, GABA content in rice varies according to the cultivar, with some cultivars exhibiting higher GABA content than others.

A study by Zhang *et al.* (2014) evaluated the GABA content in various rice cultivars grown in Korea. Findings of the research showed that the GABA content ranges from 0.02 to 5.74 mg/g, with japonica cultivars having higher GABA content than indica cultivars. Similarly, Chen *et al.* (2015) identified that japonica cultivars had significantly higher GABA content than indica rice cultivars in China. The study also revealed that GABA content varied significantly among different cultivars within each subspecies.

As per the findings of Lee *et al.* (2019a) tested the GABA content in different rice cultivars grown in Korea and Japan and stated that GABA content was positively correlated to the levels of expression of the enzyme accountable for the synthesis of gamma-aminobutyric acid (GABA) - glutamate decarboxylase in rice. The level of GABA accumulation in rice grains exhibited variability based on the particular cultivar (Thomas *et al.,* 2023).

In conclusion, the content of GABA in rice is significantly influenced by the rice cultivar, with japonica rice cultivars generally having higher GABA content than indica rice cultivars. Therefore, selecting and cultivating rice with high amount of

GABA could be an effective strategy to enrich the GABA content in rice. As per the results of Thomas *et al.* (2023), *Njavara* has significant genetic diversity and offers a substantial protein content, potentially contributing to its notable concentration of gamma aminobutyric acid (GABA). The findings of this study have implications for the advancement of rice varieties that offer enhanced health advantages. Furthermore, these results have the potential to support the endorsement of GABA enriched rice as a functional food.

The GABA content in rice is an essential nutritional quality that is also influenced by several factors, including the stage and method of harvesting. Harvesting is a crucial process in rice production that determines the quality and quantity of the GABA content in rice.

As per the findings of Kim *et al.* (2012), the method of harvesting has a significant role in the GABA content in rice. The study showed that hand harvesting resulted in higher GABA content than machine harvesting. In another study by Kim *et al.* (2016), the *Chuchung* variety rice's GABA content enhanced at the maturation stages of pre harvesting and then decreased markedly at the mature stage. Simultaneously, Poojary *et al*. (2017) pointed out that the GABA in rice grains enhanced during the process of maturation, reaching a maximum at the full ripe stage. The study showed that harvesting rice at the fully ripe stage resulted in higher GABA content than harvesting rice at other stages. Additionally, Lee *et al.* (2019b) found out that delaying the harvest time by two weeks leads to a significant hike in GABA content in rice grains. The study revealed that the delayed harvest also showed a decline in the total amino acid content of the rice.

Similarly, Tumpanuvatr *et al.* (2018) investigated that the GABA in rice grains found to be increased significantly when the plants were exposed to a low temperature treatment before harvest. The study stated that the low temperature treatment also increased the gene expression related to the GABA production in rice plants. A study put forth by Liu *et al.* (2018) identified that GABA content in rice was greater in rice grains harvested in the morning than in the evening. And the amount of GABA in rice decreased significantly after midday due to the activity of GABA transaminase, an

enzyme that breaks down GABA in rice. According to Xie *et al.* (2021), 2-acetyl-1-pyrroline (2AP) synthesised under low light circumstances, which increased GABA yield in rice.

In conclusion, the amount of GABA in rice is significantly influenced by timing and method of harvesting. Harvesting rice at the full ripe stage or 40 days after heading, and hand harvesting are recommended to obtain rice with higher GABA content. Delaying harvest by two weeks and low temperature treatment before harvest can also increase GABA content in rice. These findings can be come up with an outcome of implications for the development of strategies to optimize the GABA content in rice for improved health benefits.

2.4.2. Effect of pH and temperature on GABA content

The pH level can affect the GABA synthesis and accumulation in rice grains, through growth and maturation. In a study by Streeter and Thompson (1972), GABA accumulation is high in a slightly acidic environment and low level of accumulation in a basic condition which is reliable with the pH response observed for the GABA pyruvate transaminase (optimum pH of 8.9) and glutamate decarboxylase (optimum pH of 5.9).

Hayat *et al*. (2014) found that the best reaction of GABA was seen in a basic medium with a pH range of 6 to 8, and the maximum GABA content is observed at pH 8. This finding was put forth by Zhang *et al.* (2014), GABA content ranged from pH 5.6 to pH 8.4, except germination durations for short periods of time (6 or 12 h), where pH 7 yielded the lower GABA levels. The pH study was examined by Hayat *et al.* (2015) using buffers with a pH range of 1-10 and discovered that, pH 8.5 was the best suitable condition for maximum GABA synthesis. As per Caceres *et al*. (2017), the pH effect on GABA accumulation in germinating brown rice. The study found that the highest GABA content was resulted at pH 5.5, while the lower content was at pH 7.5. Authors suggested that the optimum pH for GABA synthesis in rice may be between pH 5 and 6 and pH has a significant effect on GABA synthesis during the germination process also.

As per Techo *et al.* (2019), the GABA content was significantly higher at pH 6 compared to pH 5 and 7. The study also revealed that the expression of GABA synthesis related genes was upregulated at pH 6.0, suggesting that pH can affect GABA synthesis in rice by regulating gene expression. Zhang *et al*. (2019) stated that the high GABA was observed at a pH of 6, while the low GABA was at a pH of 5. The authors suggested that pH can affect GABA accumulation in rice by regulating enzyme activity.

The GABA content in rice was assessed under different pH levels of 5.5, 6.5, and 7.5 and the highest GABA content was reported at pH 6.5, while the lowest was at pH 5.5. This suggested that pH can influence GABA synthesis in rice (Oh *et al*., 2019).

Sarasa *et al*. (2020) evaluated the effect of pH on GABA metabolism and expression of genes in rice seedlings. The findings showed that GABA accumulation was significantly enhanced at pH 6 compared to pH 5 and 7. The study also revealed that the expression of GABA shunt pathway genes was significantly upregulated at pH 6, the critical role of pH in regulating GABA metabolism in rice (Zhou *et al*., 2023).

Overall, these results indicated that pH plays an important role in GABA synthesis and accumulation regulation in rice. The optimum pH for GABA synthesis in rice may be between pH 6 and 8, depending on the experimental conditions and the specific rice cultivar.

The effect of temperature on GABA content in rice germ was evaluated by Komatzuaki *et al.* (2007). The GABA was found to be increased significantly with a temperature of 35°C and was found to be decreased at higher temperatures. The findings stated that, the expression of GABA shunt pathway genes was upregulated at high temperatures, suggesting that temperature can affect GABA metabolism in rice by regulating gene expression. Oh *et al.* (2007) investigated the effect of temperature on the accumulation of GABA in germinated brown rice. The GABA content increased with temperature up to 40°C and then decreased at higher

temperatures. The same result was stated by Ng *et al.* (2013), and the study also revealed that, the activity of GABA transaminase, an enzyme involved in GABA catabolism, was inhibited at high temperatures, contributing to the GABA content increase.

Nikmaram *et al*. (2017) found that, the GABA content found to be significantly increased with temperatures up to 30°C and then at higher temperatures GABA decreased. As per Li *et al.* (2018) investigated the role of temperature on accumulation of GABA in germinated brown rice. The results stated that GABA content was found to be increased with temperatures up to 45°C and then decreased. The authors suggested that 45°C could be the optimum temperature for GABA production in germinated rice.

Toyoizumi *et al.* (2021) evaluated the role of temperature on GABA accumulation and found that until 60°C, the GABA content rose with temperature; beyond that, it declined. The authors suggested that moderate temperature drying (40-60 °C) could be used to enhance GABA content in rice.

These findings demonstrated that an appropriate temperature was beneficial for the production of GABA in rice, while an extremely high temperature was detrimental (Tung *et al*., 2011). The optimal temperature for GABA synthesis in rice may be between 30°C- 40°C and varies depending on the experimental conditions and the specific rice cultivar.

2.4.3. Effect of moisture on GABA content

Studies have shown that the moisture content during germination can significantly affect the GABA content in rice. Because of the seed coat and pericarp, water does not enter immediately into the inner endosperm (Bello *et al*., 2004; Thakur and Gupta, 2006). The percentage and quality of germination are close to the moisture content of the rice. After soaking for 12–60 hours or at a moisture content of 18–40 per cent, paddy may begin to germinate. There are variations in soaking time and moisture content in different paddy rice cultivars (Puangwerakul, 2007). By diffusing through the endosperm, water enters the ventral site of the embryo first during

germination (Hwang *et al*., 2009). Maisont and Narkrugsa (2010) reported that the moisture of brown rice, husk, and paddy rice achieved equilibrium at 33.25 per cent, 22.75 per cent, and 32.32 per cent on a wet basis, respectively, after a soaking time exceeding 35 hours.

A study by Choi *et al.* (2014) reported that higher moisture content during germination led to increase in GABA accumulation in brown rice after germination. Another study by Cho and Lim (2016) found that an optimum moisture content of 40 per cent during germination resulted in the highest GABA content in brown rice after germination.

However, excessive moisture content during germination can lead to a decrease in GABA content (Lee *et al*., 2009). The rice could encounter conditions of water stress or anoxia due to the amount of moisture in the paddy, which promotes the accumulation of GABA content (Maisont and Narkrugsa, 2010). These findings suggest that controlling the moisture content during germination is critical in achieving a greater amount of GABA content in rice.

In conclusion, moisture content is a crucial factor that can significantly affect the GABA content in rice. The optimal moisture content during germination can lead to higher GABA accumulation, while excessive moisture can lead to a decrease in GABA content. Therefore, controlling moisture content at the optimal level is essential in achieving higher GABA content in rice and maximising its potential health benefits.

2.4.4. Effect of soaking and germination on GABA content

As grains are soaked, imbibition begins, and within 6 to 12 hours, respiration speeds up, which further stimulates the metabolism of amino acids, resulting in the formation of enzyme systems. Additionally, amino acids such as GABA are synthesised, and their accelerated increase is detectable after 12 hours of incubation. The acceleration is still rapid between 12 and 24 hours, when GABA levels are at their highest, and reaches its peak after 24 hours of incubation (Karladee and Suriyong,

2012). The initial stage of preparing GABA rice involves soaking, which triggers the grain's metabolism before it germinates (Thomas *et al*., 2023).

The effect of soaking on the GABA content in rice is reported in many research studies. Excessive soaking time and temperature can lead to lower GABA content (Zhang *et al*., 2020). After soaking, the enzyme glutamate decarboxylase (GAD), which catalyses the conversion of L-glutamic acid to carbon dioxide and GABA, is activated, resulting in an increase in GABA concentration and a decrease in glutamic acid levels (Thomas *et al*., 2023). Significant elevation in GABA content was also noticed by Thomas *et al*. (2023), which was in line with the longer soaking durations.

Lee *et al.* (2007) found that soaking japonica and indica rice cultivars for 12 hours increased the GABA content by 1.2-2.2 folds. Meanwhile, Seo *et al.* (2010) identified soaking japonica and indica rice cultivars at 30°C for 24 hours increased the GABA content by up to 2 fold. The GABA content went up a lot after being soaked in distilled water for 24 hours. They also made sprouted brown rice with 68.21 mg/100g of GABA by soaking (water) Japanese *Koshihikari* rice for 72 hours at 30ºC. Six types of Thai rice that were soaked for four hours almost 2 fold increase was observed in the the amount of GABA in the rice germ. It was also found that *Haiminori* sprouted brown rice that had been soaked for 24 hours had 10.1 mg/100 g of GABA (Thomas *et al*., 2023). After 4 hours of soaking at 40oC, the amount of GABA in Thai cultivars rose by up to 3.5 times. Following a 24 hour soaking at room temperature, 21 varieties of purple and modern white rice showed GABA levels ranging from 13.65 mg to 23.48 mg per 100 g of GBR. (Thomas *et al*., 2023).

In contrast to this flow, GABA content was higher at 36 hours and then decreased over time, reaching its lowest level at 48 hours of treatment. After soaking for 48 hours, the GABA concentration of IET-25451 increased to 66.86 mg/100g, however it decreased to 42.10 mg/100g after 72 hours (Thomas *et al*., 2023).

It has been suggested that soaking can activate endogenous enzymes that convert glutamate to GABA. Additionally, soaking may also increase the availability of precursors for GABA biosynthesis (Komatsuzaki *et al*., 2007).

It is important that the optimal soaking time and temperature may vary based on the particular rice variety. For example, Ding *et al.* (2018) stated that the increase in GABA during soaking was higher in japonica cultivars than in indica rice cultivars. Therefore, the soaking conditions should be carefully controlled to achieve the desired GABA content. Soaking can also cause tissue stress and lack of oxygen, which may help GABA build up over time (Thomas *et al*., 2023).

Soaking is an effective approach for increasing the content of GABA in rice. The GABA content increase during soaking is mainly because of the activation of endogenous enzymes and the availability of precursors for GABA biosynthesis. Conditions and media of soaking might also affect GABA accumulation. Soaking rice in acidic electrolysed water can result in higher accumulation of GABA (Thomas *et al*., 2023). However, the optimal soaking time and temperature depend on the rice variety and should be carefully controlled to achieve the desired GABA content. Instead, Tipkanon and Abdallah (2014) found that the rice germ had the most GABA when it was soaked at 40°C for eight hours (307.10 mg/100g of rice grain, dry base).

The stored nutrients within the grain will be depleted throughout the process of germination. These nutrients are needed for breathing and for the formation of new cells that make up the embryo, which changes the nutritional and biochemical constitution. One reason for the rise in GABA during sprouting could be that the seed is growing and developing. Germination of seeds for 24 hours in oxygen-free circumstances led to the production of GABA in both the root and the shoot. The sprouting time changed for different types of rice varieties in response to the buildup of GABA (Thomas *et al*., 2023).

The best suitable time for soaking water (neutral pH) was 36 hours. The GABA content of Thai waxy paddy rice rose from 80 mg/100 g FW to 220 mg/100 g FW after 12 to 60 hours of germination. The GABA content rose after 35ºC and 40ºC germination for 20 hours for both coloured and plain rice cultivars (Thomas *et al*., 2023). In a study conducted by Hayat *et al.* (2015), it was observed that the highest level of GABA synthesis occurred following a germination period of 96 hours.

According to Ohtsubo *et al.* (2005), the content of GABA the GBR increased noticeably and GABA seemed to be 11.5 times greater than the brown rice after 72 h. Simultaneously, Komatsuzaki *et al.* (2007) and Ding *et al.* (2018) assessed the effects of germination time and ultrasound treatment on colour of germinated brown rice and red rice flour, and observed that the concentration of gamma-aminobutyric acid (GABA) exhibited an increase subsequent to a germination period of 72 hours. In a study conducted by Thomas *et al.* (2023), it was observed that various rice cultivars exhibited an increase in GABA content following a 72 h germination period.

Numerous research works have documented the impact of diverse therapeutic approaches on augmenting the GABA concentration in rice. At the same time, Thomas *et al.* (2023) found that making GBR in a membrane reactor increased the amount of GABA (variety-*IPB3S*) by 4.5 times, while the manual way only increased it by 2.3 times.

2.4.5. Effect of drying on GABA content

Drying is a process that can affect the GABA content in rice. Drying can activate endogenous enzymes that convert L-glutamate to GABA (Xu *et al*., 2010). Additionally, drying may also reduce the water content in the rice, which may increase the concentration of GABA.

Numerous drying methods, such as fluidized bed drying (Sivakumar *et al*., 2016), sun drying (Caceres *et al*., 2017) and hot-air drying (Yu *et al*., 2021), were used for drying GBR. However, the main disadvantage of hot-air and sun or shade drying is their extended drying time and inconsistent quality of the product (Zhou *et al*., 2019).

In comparison to hot-air drying, fluidized-bed drying allows the entire product surface to make complete touch with the drying medium, such as hot air or superheated steam (Chatchavanthatri *et al*., 2021). This method is frequently used to dry GBR due to its efficiency for high drying and acceptable quality. The feasibility of microwave drying for the drying of GBR has been established by Zheng *et al.*

(2017). This technology has notable advantages in terms of high energy efficiency, cost-effectiveness, and ease of control, as indicated by Shen *et al.* (2021).

Several studies have analysed the effect of drying on the GABA in rice. For example, a study by Oh *et al.* (2007) stated that, that hot-air drying at a temperature of 70°C increased the GABA in brown rice, whereas drying at 50°C and 60°C did not change the level of GABA content. Another study conducted by Jannoey *et al.* (2010) evaluated the effect of different drying methods on GABA content in glutinous rice. The study found that sun drying and oven drying at 50°C resulted in higher GABA content compared to oven drying at 80°C.

It is clear that, the optimal drying method and temperature may change depending on the rice variety. For example, Chungcharoen *et al.* (2015) stated the drying medium effect on GABA content and stated that, at the same drying temperature of 130ºC in a hot air dryer, the GABA content of *Chai Nat 1* was found to be significantly higher than that of *Phitsanulok 2*. Therefore, the drying conditions should be carefully controlled to achieve the desired GABA content.

The moisture content during drying can also impact the GABA in rice (Thitinunsomboon *et al*., 2013). The studies show that, germinated grains were dried till obtained a moisture of 12 per cent (Thitinunsomboon *et al*., 2013). As germinated brown rice, the grains were threshed at a moisture content of 13 to 14 percent, according to Karladee and Suriyong (2012). And Chen *et al.* (2021) reported that excessive moisture content during drying lead to drastic decrease in the GABA content of rice. This suggests that proper drying techniques that maintain the optimal moisture content can help to preserve the GABA content in rice.

2.4.6. Effect of cooking on GABA content

One of the common ways to prepare rice, including GBR, in Asia is to boil it in a rice cooker (Kim, 2013).

Although a rice cooker typically cooks food at a temperature of about 100°C, pressure cookers or earthenware pans may cook food at a temperature of about 130°C.

Contineanu *et al.* (2010) pointed that, the degradation of GABA is mainly due to the elimination of water molecules followed by ring closing and gamma butyrolactone. Additionally, currently created rice cookers with steam systems for superheating have been employed to improve rice texture when heating at 130°C or higher (Toyoizumi *et al*., 2021).

Various research has reported the cooking effect on GABA content of rice and Khan *et al*. (2015) identified that, GABA is easily destroyed, when *Monascus*-Fermented Rice **(**MFR) is heated to a temperature of 121ºC. By keeping the heat treatment of MFR to within 80ºC, more than 70 per cent of the GABA content could be preserved. Meanwhile, the reports of Tiansawang *et al.* (2016) stated that, the cooking process, like boiling, open pan roasting, steaming and microwave cooking decreased the GABA content in germinated cereals and legumes. A study, put forth by Toyoizumi *et al*. (2021) stated that, a cooking temperature above 105ºC, leads to a drastic decrease in GABA in germinated brown rice owing to thermal decomposition. Similarly, the study put forth by Yu *et al.* (2021) pointed out that, about 15.8–48.30 per cent of GABA loss in cooked brown rice.

In conclusion, cooking time and temperature can affect GABA content of rice. From the studies reported, it was clear that, the increased cooking temperature can lead to a decrease in GABA content. However, the cooking temperature and time should be carefully controlled to achieve the desired GABA content.

2.4.7. Effect of environmental stress on GABA content

Environmental stress can affect the GABA content in rice. Stress can activate endogenous enzymes that convert glutamate to GABA (Khwanchai *et al*., 2014). Additionally, stress may also increase the availability of precursors for GABA biosynthesis.

Several studies have assessed the effect of abiotic stress on the GABA in rice. Bown and Shelp (1997), revealed that, anoxia, cold shock, mechanical stimulation, plant development, water stress and cytosolic acidification are some of the stress factors that enhance GABA in GBR. For example, a study by Zhang *et al.* (2014)

stated that water stress increased the GABA content in GBR. The type and intensity of stress may affect the GABA content in rice. Cáceres *et al*. (2014) investigated, the GABA enhancement was higher in germinated brown rice subjected to severe water stress compared to mild water stress. Meanwhile, Bao *et al.* (2018) pointed that salt stress increased the GABA in brown rice.

In conclusion, environmental stress can play an important role in the GABA content in rice. The GABA content increase may be because of the endogenous enzymes activation and the increased availability of precursors for GABA biosynthesis. However, the type and intensity of stress should be carefully controlled to achieve the desired GABA content.

2.4.8. Effect of microbes on GABA content

Microorganisms are known to produce GABA, and thus, the presence of microorganisms in rice may affect the GABA content. It has been suggested that the production of GABA by microorganisms may be because of the presence of glutamate decarboxylase (GAD) (Chung *et al*., 2009; Shi *et al*., 2014). Additionally, microorganisms may also increase the availability of precursors for GABA biosynthesis.

Rather than bacteria, GABA was also reported in many fungi, molds and yeast. Schmit and Brody (1975) observed the GABA pool during the initial stages of spore germination of *Neurospora crassa*. Additionally, *Aspergillus niger (*filamentous fungi) and *Aspergillus nidulans* also contained GABA. A number of microorganisms of bacteria and fungi are producing GABA (Li *et al*., 2008). Shi *et al.* (2016) reported that the isolation of GABA was initially achieved through the treatment of yeast extracts with acid. Additionally, the presence of GABA was identified in the amino acid composition of red yeast, specifically *Rhodotorula glutinis*.

Numerous studies have evaluated the effect of microorganisms on GABA in rice. Kim *et al.* (2016) stated that the inoculation of lactic acid bacteria (LAB) increased the GABA in brown rice (Yu *et al*. 2021).

Meanwhile, the inoculation of *Bacillus subtilis* increased the GABA in germinated brown rice (Thitinunsomboon *et al*., 2013; Gan *et al.*, 2017).

It is clear that, the type and population of microorganisms may affect the GABA in rice. For example, Sasimer *et al.* (2016) stated that the GABA content increase was higher in brown rice inoculated with *Lactobacillus brevis* than with *Lactobacillus plantarum*.

According to Nomura *et al*. (1998), the *Lc. lactis* spp. *Lactococcus* strains 01-7, 01-4, 53-1, and 53-7 were carefully examined and chosen from a pool of cheese starters based on their superior capacity for GABA synthesis. Instead, Chamba and Irlinger (2004) reported, *P. pentosaceus, Pediococcusacidilactici, E. durans, E. faecium*, *Leuconostocs*, *E. faecalis* are other bacteria that produce GABA. Similarly, *Lb. plantarum*, *Lb. paracasei and Lb. delbrueckii* subsp. *bulgaricus* were isolated from traditional fermented fish (Komatsuzaki *et al*., 2005). The most used bacterial group for the production of GABA is LAB. The laboratory has multiple strains of *Lactococcus (Lc.). Lactobacillus brevis* and *Lactobacillus (Lb.)* strains have been obtained using isolation procedures from several fermented food sources, including fermented vegetable kimchi from Korea, traditional paocai from China, alcohol distillery lees, fresh milk, and black raspberry juice (Dhakal *et al*., 2012). The best GABA producing strains, *Lb. delbrueckii* subsp. *bulgaricus* PR1, *Lb. lactis* PU1, *Lb. paracasei* PF6 and *Lb. brevis* PM17, was isolated from the highest GABA containing cheeses, Pecorino di Filiano, Pecorino del Reatino, Pecorino Marchigiano and Pecorino Umbro (Yavarzadeh *et al*., 2023).

In conclusion, microorganisms can affect the GABA in rice. The increase in GABA may be because of the production of GABA by microbes and the increased availability of precursors for GABA biosynthesis. However, the type and concentration of microorganisms should be carefully selected to achieve the desired GABA content.

From these studies, it was clear that, to obtain higher content of GABA, apart from cultivar, factors such as pH, temperature, moisture, soaking and germination, drying, cooking time should be optimised effectively.

2.5. GABA enriched functional foods

The studies have revealed the fact that, the increasing popularity of formulating functional meals enhanced with GABA can be attributed to the several health benefits associated with its use (Diez-Gutierrez *et al*., 2020).

GABA enriched food products have been developed and commercialised. According to Quilez and Diana (2017), these GABA enhanced foods come in a variety of forms, including cereal-based goods like bread, quinoa flakes, brown rice, fermented oats, and wheat sourdough; dairy items like cheese, milk, and yogurt; and legume-based goods like black soybeans, adzuki beans, and fermented soybeans that resemble tempeh. Simultaneously Ngo and Vo (2019) and Hinton and Johnston (2020) identified that, GABA enhanced foods include honey, chocolate, barley, cucumber, soybean products, rice, yoghurt, tea and mung bean. GABA enriched cereal products, yoghurts, probiotic drinks, snacks, baked items, wine *etc* have been developed by various researchers. Pulse based GABA foods, dairy and vegetable based GABA enriched foods were also done by various types of research reviewed in this study.

2.5.1. Cereal based products

GABA enriched products from rice are listed in Table 1.

a) GABA enriched yogurt from rice

The findings of a study conducted by Park and Oh (2005) stated that, concentrated, germinated and fermented brown rice milk with high GABA synthesising strain contained 137.17µg/g of GABA. Meanwhile, Anawachkul and Jiamyangyuen (2009), developed yogurt (GABA enriched) from germinated red rice (*Munpu rice*) and recorded that, the enriched yoghurt had 4.09 mg/100 g of GABA, which was much

greater than the control yoghurt, which did not contain GABA. Similarly, Anawachkul and Jiamyangyuen *et al*. (2009) developed GABA enriched yogurt from germinated red rice and identified that, the enriched yogurt's level of GABA, was noticeably higher than that of commercially available yoghurt.

b) Probiotic drink from fermented rice milk

Likewise, Kittibunchakul *et al*. (2021) developed a probiotic drink using GABA enriched brown rice that was fermented by *Lactobacillus pentosus* 9D3, a GABA producing probiotic organism. The strain found to be significantly increased GABA in brown rice milk fortified with extract of yeast, pyridoxine hydrochloride and isolated soy protein resulting in a beverage with a high amount of GABA (14.3 mg/100 mL) and a large number of probiotic (8.6 log CFU/mL) cells (Kittibunchakul *et al*., 2021). The results of the study promised that, the probiotic drink was high in total phenols and has antioxidant activities. The beverage might block important enzymes connected to diabetes and obesity.

c) GABA rice snacks

Furthermore, Ohtsubo *et al.* (2005) discovered that puffed pregerminated brown rice had higher levels of GABA, total dietary fibres, inositol, oryzanol, and total ferulic acid than unpuffed polished rice. Additionally, Wassanai and Royintarat (2012), developed rice crackers with chili paste flavour, puffed rice cake with tomyum flavour, rice snack bars with chili paste flavour and fried rice pastry with pepper flavour from GABA rice. The GABA enriched germinated rice flakes produced from the rice slurry, which was put forth by Zhao *et al.* (2019).

d) Sour dough bread

Studies have pinpointed the fact that, bread's flavour, texture, shelf life, nutritional value, and general quality are all enhanced by sourdough fermentation. Similar to this, Rizzello *et al.* (2008) found that wholemeal wheat sourdough produced with LAB had higher GABA production (258.7 mg/kg) than other rye and white wheat flours. Lamberts *et al.* (2012) examined the exogenous supplementation of

recombinantly produced glutamic acid decarboxylase (GAD) derived from Yersinia intermedia. This supplementation led to bread production with enriched gamma-aminobutyric acid (GABA) levels, specifically at a concentration of 115 parts per million (ppm). The findings put forth by Bahnwar *et al.* (2013) a high GABA content of 226.22 mg/100g was recorded in Bathura sourdough bread. Meanwhile, Diana *et al.* (2014a) revealed 24.2mg/100 g of GABA in LAB fermented sourdough bread (98.2mg GABA/100mL). GABA enriched bread can be made by utilising the unique process that yeast and lactic acid bacteria produce through their metabolism. The five species of *Lactobacilli* that are found in sourdough *are L. farciminis*, *L. plantarum*, *L. sanfranciscensis*, *L. brevis* and *L. rossiae*. Eighteen lactobacilli strains were isolated and analysed for GABA production; the results showed that *L. farciminis* and *L. brevis* produced high quantities of GABA. Bread inoculated with lactobacilli was prepared and tested. Comparing sourdough loaves to yeast fermented bread, the sourdough bread had a 350 per cent greater GABA concentration (Venturi *et al*., 2019).

Table 1. GABA enriched products from rice

Type of products	**Grain ingredients**
High GABA germinated brown rice	Brown rice
High GABA germinated brown rice tea	Brown rice
High GABA germinated rice milk	Brown rice
Nutritional rice flour (infant use)	Brown rice
Rice vinegar	Brown rice
Rice beverage	Brown rice
Rice crisp	Brown rice
Germinated brown rice cake	Brown rice
Selenium-enriched germinated rice	Brown rice
Antioxidant peptide (Extracted)	Brown rice
Nutritional supplement	Brown rice, millet, barley
Healthcare flour	Brown rice, buckwheat
Healthcare coarse grain food	Black rice, red rice
Yam-rice soup	Brown rice, oat, black wheat, glutinous

Convenient coffee	Millet
Nutritious rice oatmeal	Brown rice, buckwheat, oats
Instant porridge	Rice, Sorghum
Nutritional germinated cereals mix GBR	Tartary buckwheat, brown rice, oats
(GABA enriched)	Brown rice
GABA-enriched cookie	Brown rice
GABA-enriched crispy pancake	Brown rice
Nutritional powder	Wheat germ and germinated brown rice
Nutritional instant rice	Germinated brown rice
GBR yoghourt	Germinated brown rice
GABA-enriched fermented food	Sprouted brown rice bran
A GABA-enriched brown rice product	Giant germ rice, brown rice
Alcoholic drinks (GABA)	Germinated brown rice
Germinated brown rice (GBR)	Germinated 50% polished rice

(Zhao *et al*., 2019)

e) Rice wine

According to the findings put forth by Liu *et al.* (2012) and Gong *et al.* (2019), when the storage time of rice wine increases, GABA concentration is also found to be increased. During primary fermentation, the amount gradually increased during the brewing process. GABA levels in the wine were found to be lower early in the post fermentation. However, a significant rise in GABA content was seen in wine at the final stage of post fermentation.

2.5.2. Pulse based products

a) GABA soya yogurt

Yogurt with high levels of isoflavones, free amino acids, and GABA was developed using germinated soybean extract and lactic acid bacteria (LAB). GABA concentration in GABA soya yoghurt generated with substrate and starter was 424.67 µg/g DW, but GABA concentration in fermented milk prepared conventionally was

less than 1.5 µg/g DW. When compared to other conventional yoghurts, the GABA soya yoghurt had significantly higher quantities of isoflavones and free amino acids (Park and Oh, 2007). Similarly, Ko *et al*. (2013) stated that, fermented black soybean milk enhanced with GABA is considered as a promising therapy option for depression.

b) Tempeh

Tempeh is a conventional soy based food product that undergoes fermentation native to Indonesia's Central Java. Tempeh is made using salt free aerobic fermentation with Rhizopus. Likewise, Watanabe *et al*. (2007) investigated that fermented soybean is similar to tempeh. Soybean was first incubated aerobically, followed by anaerobic incubation, leading to a significant increase in GABA content. Other peptides and free amino acids were seen to be considerably greater in GABA tempeh than in ordinary tempeh.

2.5.3. Dairy based products

a) Cheese

The GABA concentration ranged from 0.26 mg/kg to 391 mg/kg in 22 Italian cheeses. GABA synthesis can be facilitated by a total of twelve distinct LAB strains that are usually present in various Italian cheese varieties. According to Siragusa *et al.* (2007), the strains *Lactobacillus delbrueckii subsp. bulgaricus PR1, Lactobacillus paracasei PF6, Lactobacillus brevis PM17, Lactococcus lactis PU1 and Lactobacillus plantarum C48* were found to generate the highest quantities of GABA during the fermentation process of reconstituted skim milk. Furthermore, Wang *et al.* (2010) collected that, the GABA is higher in probiotic strain containing cheddar cheese than control cheese. Simultaneously Dhakal *et al*. (2012) proved that, *Lactobacillus delbruickii* subsp. *bulgaricus* and *Lactobacillus paracasei* are GABA producing bacteria in cheese. Venturi *et al.* (2019) isolated *Lactococcus lactis*, *Lactobacillus brevis* and *Lactobacillus plantarum* from cheese and used them for the production of GABA enriched bread. Naturally, cheese is enriched in GABA (16mg GABA/50 g cheese) by the *L. lactis* ssp. *lactis* strain, reduced hypertension. Likewise,

discovered high concentrations of GABA in a variety of Spanish artisanal cheeses, meanwhile ripened cheese from sheep milk has higher GABA content (0.98 g/kg) (Saidi *et al*., 2020).

b) Yoghurt

Research findings have demonstrated that yogurt enhanced with gamma aminobutyric acid (GABA) is a newly developed and beneficial fermented dairy product that is currently accessible in the consumer market. According to Chen *et al.* (2016), it has been suggested that yogurt with a high concentration of GABA may possess potential efficacy in enhancing the management of type 1 diabetes (T1D) and mitigating the advancement of diabetes and its associated problems.

c) Yoghurt sake

Yoghurt sake is a smooth and creamy sake based liqueur brewed with yogurt made from 100% fresh raw cow milk. Yoghurt sake includes a high quantity of GABA and in their study, Ohmori *et al.* (2018) successfully isolated lactic acid bacteria (LAB) capable of synthesising gamma aminobutyric acid (GABA) in liquor. Specifically, they identified *Streptococcus thermophilus* Hp as a particularly potent organism for GABA synthesis. The findings of the study indicate that LAB possessing highly active glutamate decarboxylase enzymes play a crucial role in facilitating GABA synthesis in yoghurt.

d) Whey based beverage

Meanwhile, Cunha *et al.* (2022) discovered a natural whey based beverage enhanced with GABA content using *Enterococcus malodoratus SJC25*. Additionally, it was noted that the content of gamma-aminobutyric acid (GABA) in whey based beverages fell within the range of 250-300 mg per 100 mL, a comparable amount to that of commercially available GABA supplements.

e) Fermented milk

Streptococcus thermophilus was chosen by Han *et al.* (2020) to make naturally GABA enriched fermented milk. Following a fermentation period of 48 hours, the examined strain exhibited a GABA concentration of 2.8 g/L. Additionally, it was observed that the GABA dosage achieved through the fermentation of milk was comparable to the dosages seen in commercially accessible GABA supplements. In addition, the utilisation of *S. thermophilus* for the production of GABA enriched fermented milk demonstrated affordability.

2.5.4. Vegetable based products

a) Tomato

Studies have pointed out the fact that, among fresh vegetables, tomatoes have one of the highest natural GABA concentrations. Compared to modern cultivars, the concentration seems to be larger in wild genotypes. This may be due to the desired umami flavour, related to the GABA precursor glutamate. The highest GABA content ever reported in a tomato wild type, *Solanum pennellii* has an approximate 200 mg/100 gFW level. However, during fruit ripening, the GABA level rapidly decreases and Phenotypes exhibiting distinct chemical compositions have the potential to manifest dysfunctionality (Gramazio *et al*., 2020).

2.5.5. Beverages

Gamma aminobutyric acid (GABA) tea was reported as a popular tea, and contains a high amount of GABA (>1.5 mg/g). In 100g of GABA tea have at least 150mg of GABA. According to the findings of Tsushida and Murai (1987) started processing tea in anaerobic conditions in the 1980s, which raised the natural amounts of GABA. Meanwhile, Yasuhiko *et al*. (1995), a GABA rich tea was produced through the process of fermenting freshly harvested green tea leaves in an environment enriched with nitrogen gas and reported that, GABA rich tea helps to lower existing high blood pressure also prevents chances of developing hypertension. Instead, Zhao *et al*. (2011) indicated that the GABA content of puerh teas was lower than that of

oolong, green, white and black tea. GABA tea has become well liked in various Asian countries due to its beneficial effects on health. (Lin *et al*., 2012). Leaves of Tea subjected to various kind of stress showed GABA accumulation which is controlled by two different processes combined mechanical damage and anoxic stress, which causes CsGAD1 and CsGAD2 to become more active, which increases the GABA content (Mei *et al*. 2016). GABA was reported in all types of tea; white tea, yellow tea, green tea, oolong tea, black tea and puerh tea (Hinton and Johnston, 2020). Simultaneously, Mei *et al*. (2020) reported that, two active glutamate decarboxylase (GAD) isoforms have been reported in *Camellia Sinensis* tea. The finding put forth by Dai *et al*. (2020) stated that, the anaerobic treatment in GABA tea induced considerable alterations in the levels of TCA cycle metabolites, lipids, dimeric flavanols, bases/nucleosides, and the majority of amino acids, in addition to a 16 fold rise in GABA content.

As per Cataldo *et al.* (2020), it was determined that strawberry juice exhibits notable potential as a vegetable matrix for the development of fermented functional meals with enhanced levels of GABA.

According to Cagno *et al.* (2010), the grape must which have been fermented with *L. plantarum DSM19463* to increase its GABA content was thought to be a health focused product with potent antihypertensive and skin protective properties.

As per Wang *et al.* (2021), it was stated that the fermentation of litchi juice using *Lactobacillus plantarum HU-C2W* at a temperature of 37°C for a duration of 40 hours resulted in a significant increase in the concentration of gamma aminobutyric acid (GABA), reaching 134 mg per 100 mL. Additionally, this fermentation process led to a notable reduction of 27 per cent in the overall carbohydrate content of the juice.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study entitled 'Medicinal properties and process optimisation for GABA enrichment in rice' was carried out to assess the medicinal properties and to optimise conditions for Gamma Amino Butyric Acid (GABA) enrichment in rice varieties and also to assess the effect of processing on GABA content in rice.

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

3.1. Collection of rice varieties

3.2. Optimisation of conditions for GABA enrichment

3.2.1. Optimisation of soaking and germination

3.2.2. Quantification of GABA content

3.2.3. Total antioxidant activity

3.2.4. Organoleptic evaluation of table rice

3.2.5. Selection of most suitable conditions for GABA

3.2.6. Parboiling of rice varieties

3.3. Quality evaluation of rice varieties

3.3.1. Chemical and nutritional qualities

3.3.2. Organoleptic qualities

3.4. Assessment of antioxidant and antiproliferative properties of GABA enriched rice

3.4.1. Antioxidant properties

3.4.2. Antiproliferatory activities

3.5. Storage qualities

3.5.1. Nutritional qualities

3.5.2. Organoleptic qualities

3.5.3. Enumeration of microbial population

3.5.4. Insect infestation

3.6. Processing GABA enriched rice for preparation of rice products

3.6.1. Organoleptic evaluation of GABA enriched rice products

3.6.2. Quantification of GABA content

3.6.3. Total antioxidant activity of GABA enriched rice products

3.7. Statistical analysis

3.1. Collection of rice varieties

The popular rice cultivar *Jyothi*, medicinal rice cultivar *Njavara* (yellow glumed), and traditional rice cultivar *Chitteni* were purposively selected for the study (Plate 1).

Jyothi (PTB 39) is a short duration (110-115 days) high yielding variety released from RARS, Pattambi of Kerala Agricultural University in 1974. It has red, long bold grains with good cooking quality. It is a popular rice variety of Kerala, which is photosensitive in nature and recommended for all seasons.

Jyothi ***Njavara*** ***Chitteni***

Plate 1. Rice varieties selected for the study

Njavara (Navara) is a traditional unique medicinal rice variety of Kerala. There are two types of *Njavara*; black and yellow glumed and it is also a short duration (70-90 days) rice variety. The seed coat is red in colour and the grains are not scented. Compared with other varieties, *Njavara* possesses higher calcium and phosphorous. It is known for its therapeutic potential and is mainly used in ayurvedic treatments like panchakarma and to treat rheumatism, neurological problems, rejuvenation of muscles *etc*.

Chitteni is a traditional rice variety of Kerala which is released as PTB 20 from RARS, Pattambi of Kerala Agricultural University. It is also found to be photosensitive and short duration (125-130 days) rice variety still under cultivation.

The *Jyothi* variety was collected from the Agricultural Research Station (ARS), Mannuthy. *Chitteni* and *Njavara* were procured directly from the farmer's field and identified by plant breeders from Kerala Agricultural University. The paddy grains were dehulled in a rubber roll sheller, and all the quality evaluation studies were conducted on the whole rice grains.

3.2. Optimisation of conditions for GABA enrichment

3.2.1. Optimisation of soaking and germination

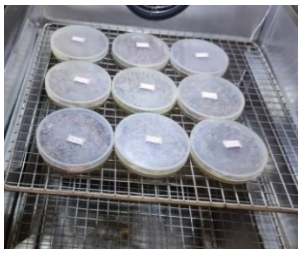
The germination of the grain samples was conducted using the methodology employed by Komatzuaki *et al.* (2007). Grains weighing 100 g were subjected to a soaking process in 500 ml of water with a neutral pH at a temperature of 30ºC. The soaking duration varied, with three various time intervals of 24 h, 48 h, and 72 h. The water used for soaking was replaced every 24 hours. The details of the soaking process are presented in Table 2. Following the process of soaking, each of the rice grains was separately enclosed within a Petri dish, which was subsequently covered with a lid. The Petri dishes were subjected to incubation at a temperature of 35ºC for durations of 24 h, 48 h and 72 h respectively (Thomas *et al*., 2023).

Table 2. Soaking and germination duration of rice varieties

Grains of *Jyothi, Njavara* and *Chitteni*	**Soaking at 30ºC (h)**	**Germination at 35ºC (h)**		
	24	24	48	72
	48	24	48	72
	72	24	48	72

Whole rice grains
(100 g)

Soaked in 500 ml for 24, 48
and 72 h water at 30°C



Rice grains of different
varieties seperately packed
in petridish with lid
and incubated at 35°C

Shade dried germinated grains
to obtain 10-12 per cent
moisture content

The dried seeds husk removed
and used for quantification of
GABA

Figure 2. Flow chart for soaking and germination of rice varieties for GABA quantification

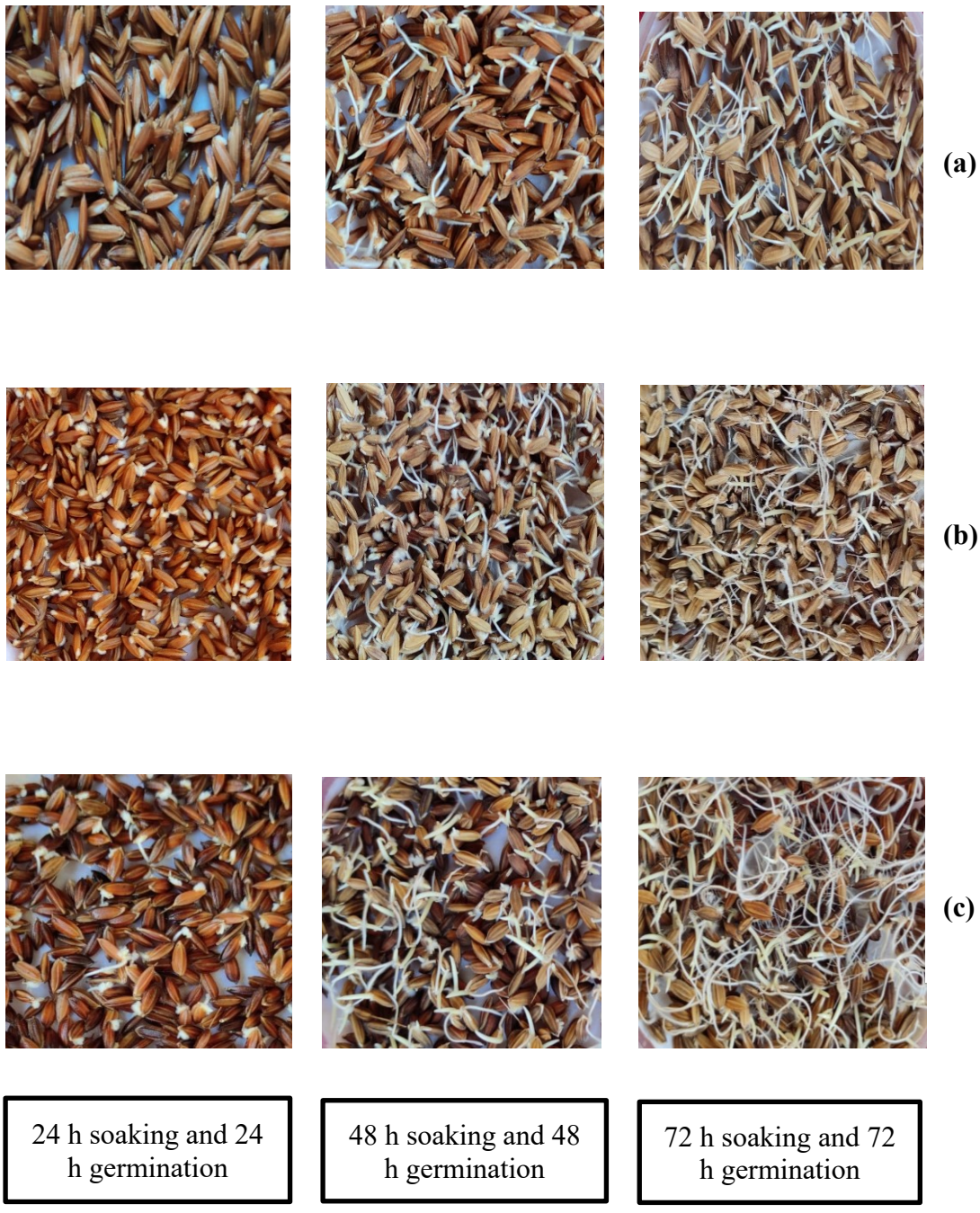


Plate 2. (a) *Jyothi* (b) *Njavara* and (c) *Chitteni* after various soaking and germination conditions

After being shade dried, the moisture content of these sprouted seeds was reduced to 10 to 12 per cent. After dehusking the seeds (dried), they were kept in airtight jars between 4 and 6°C until needed (Thomas *et al*., 2023) (Figure 2).

3.2.2. Quantification of GABA content

a) Preparation of standard solutions and derivatisation procedure

The methodology explained by Hayat *et al.* (2015) was employed to perform the preparation of standard solutions and derivatisation procedure. A standard stock solution with a concentration of 0.5 mg/ml was made by dissolving 50 mg of GABA, which had a purity of 99 per cent, in a volumetric flask (50 ml). The resulting solution was stored in the dark at a temperature of 4°C (Thomas *et al*., 2023).

A series of eight standard solutions of GABA were generated by diluting the stock solution with double distilled water to achieve concentrations of 5µg/ml, 10µg/ml, 20µg/ml, 40µg/ml, 80µg/ml, 100µg/ml, 125µg/ml, and 150µg/ml. These solutions were then subjected to the derivatisation procedure. A 1 ml aliquot of each working solution was subjected to treatment with 1 ml of 2-hydroxynaphthaldehyde (2% concentration in methanol) and afterward combined with 0.5 ml of boric acid-NaOH buffer (pH 8) in a 5 ml volumetric flask. The mixture was subjected to heating in a water bath for a duration of 15 minutes at a temperature of 85°C. Following the process of cooling the solution to ambient temperature, the volume was subsequently modified to 5 ml using methanol. The spectrum of absorption was measured utilising High Performance Liquid Chromatography (HPLC) in comparison to a reagent blank that was made using the same process but without the inclusion of any standards (Thomas *et al*., 2023).

b) Extraction and derivatisation of rice samples

The extraction of samples was conducted using the methodology described by Komatsuzaki *et al*. (2007). A 15 mL falcon tube was used in this experiment. The tube contained one gram of pulverised rice and 5 mL of ethanol solution with a concentration of 80% (v/v). The tube was subjected to agitation on a vortex mixer for a duration of 5 minutes. The sample underwent centrifugation at 5000 rpm speed per

minute for 10 minutes at a temperature of 4°C. Subsequently, the supernatant was passed through a Millipore filter paper with 0.4 micrometers pore size. The filtrate was collected into a vial. Following the repetition of the extraction process twice, the resulting extracts were subsequently blended. The resulting extracts were subjected to drying using a rotary evaporator till the ethanol was completely evaporated. The resulting dried residue was then dissolved in 1 ml of water for derivatisation, followed by further analysis using high-performance liquid chromatography (HPLC) (Thomas *et al*., 2023).

c) High performance liquid chromatography (HPLC) analysis of GABA

The analysis of gamma-aminobutyric acid (GABA) using high performance liquid chromatography (HPLC) was conducted following the procedure described by Hayat *et al.* (2015). The extraction techniques were applied to all the rice samples, including the control and germinated samples, and each sample was analysed three times. The data obtained was noted as the average value of three separate analyses conducted on each sample, together with the corresponding standard deviation. 1 ml aliquots of rice extract subjected to treatment with the derivatisation reagents, following the procedure described in section 3.2.2 a. The standards and samples underwent high-performance liquid chromatography (HPLC) studies using an Agilent 1260 series instrument equipped with a gradient system consisting of solvent A (water) and solvent B (methanol). The gradient elution protocol consisted of a mixture of 50% A and 50% B at the start (0 min), followed by a transition to 60% A and 40% B at 2 min, then to 30% A and 70% B at 5 min, further transitioning to 20% A and 80% B at 8 min, then to 10% A and 90% B at 10 min, and finally returning to a mixture of 50% A and 50% B at 12 min. The process of separation was conducted for a duration of 12 minutes using a reversed phase column (Agilent Technologies, Eclipse Plus C-18, 4.6 x 250mm, 5 μm). Detection of the peak was observed at a wavelength of 230 nm (Thomas *et al*., 2023).

3.2.3. Total antioxidant activity

Antioxidant properties like total antioxidant activity (TAA) of all the germinated rice varieties *Jyothi*, *Njavara* and *Chitteni* were analysed.

a) Preparation of extract

The rice germs were sieved, dried, and then ground into a powder using a grinder. The 5 g sieved particles were extracted three times using 100 ml methanol in a 24 h period at 40°C in an electronic shaker. A rotary evaporator (Heidolph, Germany) was used to evaporate the extracts under vacuum after they had been filtered through Whatman No. 1 filter paper. The remaining crude methanolic extracts were weighed, diluted in dimethyl sulphoxide (DMSO), and then filtered through a 0.45 µm nylon membrane filter before being stored at -20°C for further analysis (Thomas *et al*., 2023).

b) Total antioxidant activity analysis

The total antioxidant activity (TAA) of extracts of rice was analysed by using phosphomolybdate assay developed by Prieto *et al.* (1999). The standard used in the experiment was ascorbic acid. A solution containing ascorbic acid at a concentration of 10 mg/ml was generated using distilled water. Subsequent dilutions were then performed using this stock solution. In a test tube, a volume of 300 µl of rice extracts was combined with 3 ml of a phosphomolybdate reagent. This reagent consisted of a solution containing 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The test tube was enveloped with aluminum foil and subjected to incubation at 95°C for a duration of 90 minutes. Subsequently, the combination was let to equilibrate to ambient temperature, at which point the absorbance of the solution was measured at a wavelength of 765 nm. The blank was conducted using an identical approach, with the exception that an equivalent volume of methanol was substituted for the samples. The measurement of antioxidant capacity was expressed in micrograms of ascorbic acid equivalents (AAE) per milliliter.

3.2.4. Organoleptic evaluation of table rice

The properties of the starch mostly determine the cooking characteristics of rice. Hence, the cooking qualities of germinated whole rice grains were evaluated.

a) Preparation of cooked rice

Rice varieties were cooked by using the straining method that Saleh and Meullenet (2007) suggested. Rice was measured and washed with cold water repeatedly and strained. Put a fairly large quantity of water in a big pan and bring to the boil. Add the known quantity of rice to the boiling water. After cooking, the rice water was drained.

b) Selection of judges

A set of organoleptic experiments were conducted using a basic triangle test at the laboratory scale. Jellenick (1985) conducted an organoleptic examination by enlisting a panel of fifteen judges within the age ranging from 18 to 35 years.

c) Preparation of score card

For the organoleptic evaluation of prepared products, a score card was prepared to contain six sensory quality parameters colour, appearance, flavour, taste, texture, and overall acceptability. The evaluation of each sensory quality attribute was conducted using a nine-point hedonic scale. The overall acceptability was calculated by independently averaging the five quality qualities described above. The score card used for the selection process is mentioned in Appendix I.

3.2.5. Selection of most suitable conditions for GABA

The most suitable conditions for GABA enrichment in each variety were identified based on GABA content, total antioxidant activity and organoleptic qualities.

a) Normalisation scale

Based on GABA content, total antioxidant activity and organoleptic qualities, the most suitable conditions for GABA enrichment in each variety were identified by using a normalisation scale (Dodge, 2003). The process of normalisation involves the adjustment of data that are measured on disparate scales to a theoretically common scale, before the computation of an average. And scale down the values of features

between 0 to 1 which is mentioned in Appendix II. The methodology used for normalisation scale is mentioned in Figure 3.

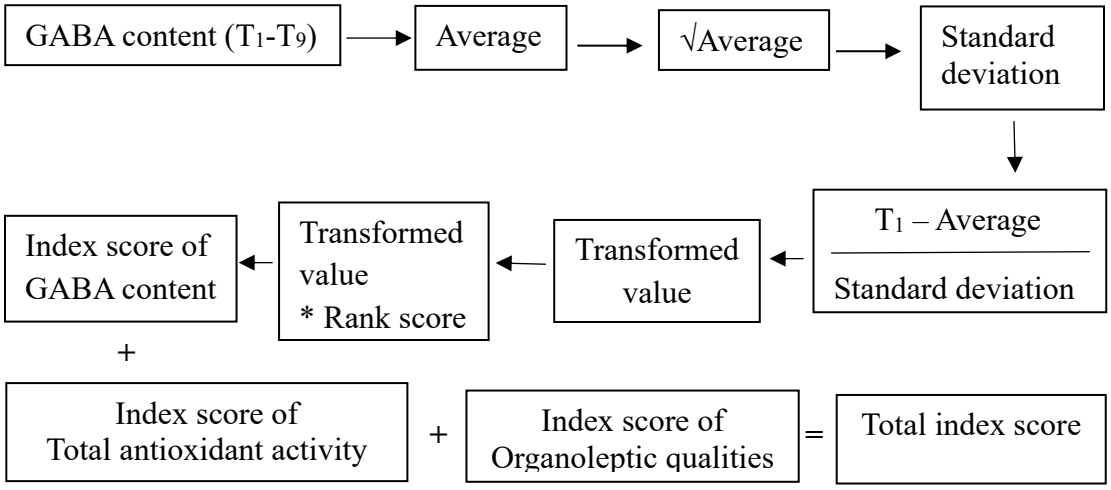


Figure 3. Methodology of normalisation scale

3.2.6. Parboiling of rice varieties

The selected GABA enriched paddy samples were parboiled by the hot water soaking process developed by CFTRI (1969). After soaking and germinated for 72 h, the water was drained and the paddy was transferred to boiling water and continued steaming until it started to broke and then sundried until a moisture content of 13 per cent was obtained. The parboiled paddy is dehusked and used to determine GABA content and is compared with non-parboiled germinated grains.

3.3. Quality evaluation of rice varieties

Whole rice grains with bran obtained by dehulling were cleaned and various quality parameters like biochemical, nutritional, organoleptic and storage qualities of the selected rice cultivars were evaluated.

3.3.1. Chemical and nutritional qualities

The GABA enriched rice varieties were dehusked and the biochemical and nutritional qualities like moisture, starch, carbohydrate, protein, total fat, energy, calcium, zinc, iron and phosphorus, thiamine, riboflavin, vitamin E were assessed.

The starch *in vitro* digestibility and *in vitro* availability of calcium, zinc, phosphorus and iron were also evaluated using standard procedures. The raw rice of the selected varieties was kept as control. All the analyses was carried out in triplicate samples for the following parameters and is discussed below.

a) Moisture

The moisture content of rice was determined using the A.O.A.C (2012) method. Samples weighing 5 grams were collected and placed on a Petri dish. Subsequently, the samples were subjected to a drying process in a hot air oven, maintained at a temperature range of 60°C-70°C. After the drying process, the samples were allowed to drop down to room temperature before being weighed. The process was repeated till a consistent weight was observed. The moisture content was quantified as a percentage and determined from the weight loss during the drying process.

$$\text{Moisture (\%)} = \frac{\text{(Initial weight) – (Final dry weight)}}{\text{Weight of the sample}} \times 100$$

b) Starch

The starch content was colorimetrically estimated using anthrone reagent (A.O.A.C, 2012). The rice grains were ground into a fine powder, and then, the resulting powders (0.5g) were subjected to extraction using 80 per cent ethanol. The residue which consisted of 5 ml of water and 6.5 ml of perchloric acid with a concentration of 52 per cent was subjected to drying in a water bath. Subsequently, the residue was extracted once more at a temperature of 5°C for a duration of 20 minutes. The supernatant was gathered and diluted to a volume of 100 ml using a standard flask, after which a 0.2 ml supernatant was extracted using a pipette. In this, a mixture consisting of 1 ml of water and 4 ml of anthrone reagent was prepared and subjected to heating for a duration of eight minutes. The measurement of color intensity was conducted using a spectrophotometer set at a wavelength of 630 nm.

c) Amylose content

The quantification of amylose content was conducted following the procedure of Sadasivam and Manikkam (1992). A pulverised rice sample weighing 100 mg was subjected to a treatment involving the addition of 1 ml of 1 N NaOH. The mixture was allowed to stand overnight, after that the volume was adjusted to 100 ml. In this experiment, a volume of 2.5 ml of extract was combined with 20 ml of distilled water and phenolphthalein indicator (3 drops) was added. Subsequently, a solution of 0.1 N hydrochloric acid (HCl) was incrementally introduced, drop by drop, until the observed pink colour was disappeared. A volume of 1 ml of iodine reagent was added into the solution, which was subsequently diluted to a total volume of 50 ml within a standard flask. The measurement of colour intensity was assessed at a wavelength of 590 nm using a spectrophotometer.

d) Carbohydrate

The quantification of carbohydrates was conducted by a colorimetric method employing anthrone reagent, as detailed by Sadasivam and Manikkam (1992). Samples of pulverised rice grain weighing 0.1 g were subjected to hydrolysis using 5 ml of 2.5 N hydrochloric acid (HCl). After cooling, the resulting residue was neutralized by adding solid sodium carbonate. The sample was aliquoted into a standard flask with a volume of 100 ml and thereafter subjected to centrifugation. A volume of 0.1 ml of the supernatant was transferred using a pipette, followed by 1 ml of distilled water and 4 ml of anthrone reagent. The contents were subjected to heating for a duration of eight minutes. Subsequently, on cooling the intensity of the color transition, ranging from green to dark green, was measured at 630 nm wavelength. The quantification of the total carbohydrate content in the sample was determined using a standard graph and is denoted in grams.

e) Protein

The protein content was determined using the A.O.A.C. (2012) technique. In order to turn the rice (0.2 g) colour to green, it was digested in a digestion flask using 0.5 g of CuSO4, 6 ml of Con H2SO4, and 3.5 g of K2SO4. To determine the nitrogen content, it was diluted with water and 25 ml of NaOH after digestion, and titrated with

0.2 N HCl. The protein content was determined by multiplying the obtained nitrogen content by 6.25 factor. The value is articulated in percentage.

f) Total fat

Rice's fat content was assessed using the A.O.A.C. (2012) method. Five grams of materials were ground into powder and extracted using petroleum ether in a Soxhlet apparatus, which was gently heated for six hours. After the extraction flask had cooled, the ether was removed by heating and the weight was recorded. The proportion of fat content was stated in percentages.

g) Energy

The energy content was worked out from the total carbohydrate, protein and fat exist in the sample. Total carbohydrate, protein and fat were analysed by the method as described in 3.3.1.d, 3.3.1.e and 3.3.1.f. Finally the total carbohydrate, protein, and fat content should be multiplied by factors of 4, 4, and 9, respectively. Subsequently, the outcomes are aggregated to obtain the total energy. Energy content was expressed as kilo calorie (Kcal).

$$\text{Energy (Kcal)} = (\text{CHO} \times 4) + (\text{Protein} \times 4) + (\text{Fat} \times 9)$$

h) Calcium

Calcium content was estimated with the diacid extract prepared from the sample using an atomic spectrophotometer (Sadasivam and Manickam, 1992). The diacid was prepared by mixing 70 per cent perchloric acid in a ratio 9:4. Two grams of rice sample was digested in this diacid and the extract of rice was made up to 100 ml. This solution was analysed directly in an atomic absorption spectrophotometer. Calcium was expressed in mg per 100 g of the sample.

i) Zinc

The estimation of zinc content in the sample was conducted using the atomic absorption spectrophotometric method, as described by Sadasivam and Manickam (1992). The diacid solution was measured using an atomic absorption

spectrophotometer. The concentration of zinc is quantified as milligrams per 100 grams of the sample.

j) Iron

The estimation of iron concentration in the sample was conducted using the atomic absorption spectrophotometric method, specifically utilizing the diacid extract derived from the sample as described by Sadasivam and Manickam in 1992. The diacid solution was examined using an atomic absorption spectrophotometer, and the results are reported as milligrams per 100 grams of the sample.

k) Phosphorus

The estimation of phosphorus content was conducted using the methodology proposed by Jackson (1973), which involves the utilisation of a nitric acid vandate molybdate reagent to produce a yellow coloration. A volume of 5 ml of a pre digested sample was combined with 5 ml of a reagent consisting of nitric acid and vanadate molybdate. The resultant mixture was then diluted to a final volume of 50 ml using distilled water. The optical density (OD) was measured at 420 nm wavelength after a duration of 10 minutes. The phosphorous concentration in the sample was determined by analysing a standard graph that was constructed using a series of dilutions of a standard phosphorous solution. The resulting concentration was then represented as milligrams per 100 grams.

l) Thiamine

The estimation of thiamine content was conducted using the methodology proposed by Sadasivam and Manikam (1992). A rice sample weighing five grams was meticulously pulverised and placed into a conical flask with a volume capacity of 250 ml. A volume of 100 milliliters of 0.1 normal (N) sulphuric acid was gradually introduced into the solution without agitation, and afterwards allowed to stand undisturbed for a period of one night. Following vigorous shaking, the solution was subsequently filtered using a Whatman No.1 filter paper, with the initial 10 to 15 ml filtrate being discarded. A volume of 10 ml of the extract was transferred using a pipette into separating funnels with a capacity of 100 ml. A volume of 10 ml of the

working standard was extracted using a pipette, and afterward, 3 ml of a 15 per cent NaOH solution was introduced into each separating funnel without delay. This was promptly followed by the addition of four drops (equivalent to 0.2 ml) of ferricyanide solution. Following a gentle shaking for 30 seconds, the rapid addition of 15 ml of isobutanol was performed using a fat delivery burette. The process was promptly halted, and vigorous shaking was performed for a duration of 60 seconds, after which the distinct layers were allowed to separate. The lower layer was carefully decanted, and a single spatula of sodium sulphate was introduced directly into the separating funnel. The funnel was then stopped and gently swirled to facilitate the clarification of the extract. The transparent sample was carefully transferred from the upper portion into a sterile and desiccated test tube. Subsequently, the sample was subjected to analysis using a spectrofluorometer, with 365 nm of excitation wavelength and 435 nm of emission wavelength. The excitation and emission band passes were set at 10 nm, and the sensitivity of the instrument was adjusted to 500 V. The quantification of thiamine was reported in milligrams per 100 grams of the analysed sample.

m) Riboflavin

The riboflavin of the rice samples was analysed using the methodology proposed by Sadasivam and Manickam (1992). A quantity of two grams of the rice sample were carefully transferred into a conical flask with a volume of 250 ml. The material was then thoroughly combined with 75 ml of a 0.1 normal solution of H_2SO_4. Subsequently, the specimen underwent autoclaving at a pressure of 15 pounds per square inch for a duration of 30 minutes. The flasks were allowed to cool until room temperature by being shaken every five minutes. Subsequently, a volume of 5 milliliters of sodium acetate solution with a concentration of 2.5 molar was introduced into the mixture.

Table 3. Details of oxidation for the assessment of riboflavin content

	Low/Blank Tube A	**Sample Tube B**	**High/Blank Tube A**	**Sample Tube B**
Sample solution(ml)	10	10	10	10
Standard solution(ml)	1	-	1	-
Water (ml)	1	2	-	1
*$KMnO_4$ (4%) (ml)	0.5	0.5	1	1
Time laps (min)	2	2	4	4
**Hydrogen peroxide (ml)	0.5	0.5	1	1

*Stir samples after addition of permanganate
**Shake after adding peroxide until foaming is negligible

The resulting solution was thoroughly mixed and left undisturbed for a duration of one hour. The pH of the above mentioned solution was modified to a value of 4.5. Subsequently, the solution was put into a volumetric flask with a capacity of 100 ml and the volume was adjusted by adding distilled water. After passing through a Whatman filter paper No.2, the initial 10 to 15 ml were discarded. Oxidation experiments were conducted in test tubes labeled A and B, each having a diameter of one inch, and equipped with stirring bars, as indicated in Table 3.

The fluorometer was calibrated, and used to measure the fluorescence of solutions A and B. In the cuvette, a solution of 20 mg sodium hydrosulfite was introduced, followed by stirring, and the subsequent recording of the blank fluorescence (C). The riboflavin content (%) was calculated by the formula:

$$\text{Riboflavin } \mu g/100\ g = \frac{B - C}{A - C} \times \frac{R}{S} \times \frac{V}{V_1} \times 100$$

Where,

A= Reading of the sample plus riboflavin standard

B= Reading of sample plus water

C= Reading after adding sodium hydrosulphite

R= Standard riboflavin added = µg/V1 of sample solution

V= Original volume of sample solution in ml

V_1= Volume of sample solution taken for measurement (10 ml)

S= Sample weight (g)

In this solution R =1, V=100 and V1=10

n) Vitamin E

The estimation of vitamin E in the rice samples was carried out using the methodology proposed by Sadasivam and Manickam (1992). Using a pipette, extract 1.5 ml of standard solution, 1.5 ml of tissue extract and 1.5 ml of water into the relevant centrifuge tubes designated for the test, standard, and blank samples. Proceed to securely seal the tubes. A volume of 1.5 milliliters of water was introduced to the standard, while 1.5 milliliters of ethanol was added to both the blank test tubes. The resulting mixture was subjected to centrifugation. One milliliter of the xylene layer was carefully transferred into a separate stopper tube, ensuring that no ethanol or protein was included. A volume of 1 milliliter of the 2,2-dipyridyl reagent was added to each tube, which was then stopped and stirred. Using a pipette, extract a volume of 1.5 ml from the mixes and transfer it into a cuveatte. Proceed to measure the absorbance in both the test and standard samples at 460 nm, relative to the blank. Subsequently, the experimental procedure commenced by sequentially introducing 0.33 milliliters of ferric chloride solution into the designated receptacle. The mixture was thoroughly agitated, and precisely after a duration of 15 minutes, the test and standard samples were assessed against the blank at a wavelength of 520 nm.

Amount of tocopherol in µg/g of tissues

$$= \frac{\text{Reading of test at 520 nm- reading of test at 460 nm x 0.29x15}}{\text{Reading of standard at 520 nm}} \times \frac{\text{Total volume of homogenate}}{\text{Volume used x Weight of the tissue}}$$

o) *in vitro* digestibility of starch

The estimation of starch digestibility followed the procedure proposed by Satterlee *et al.* (1979). A rice sample weighing one gram was pulverised and subjected to gelatinisation in 100 ml of water. The mixture was then subjected to boiling for a duration of one hour and subsequently filtered. A volume of 1 mL of gelatinised solution was extracted and combined with 1 mL of enzyme solution (saliva diluted with an equal volume of water). The solution was subjected to incubation at a temperature of 37°C for a duration of 1 to 2 hours. The reaction was halted by adding 1 milliliter of sodium hydroxide (NaOH). Subsequently, the estimation of glucose was conducted using the methodology proposed by Somoygi in 1952, and the IVSD was calculated.

p) *in vitro* availability of minerals

HCl extractability

The rice sample was subjected to extraction using a hydrochloric acid solution with a concentration of 0.03N. The extraction process involved shaking the mixture at a temperature of 37^{o}C for a duration of 3 hours. The filtrate produced after filtration using Whatman No.42 filter paper was subjected to oven drying at a temperature of 100^{o}C and thereafter underwent wet acid digestion. The quantification of HCl extractable calcium, zinc, iron, and phosphorus in the digested sample was conducted using the mineral estimation methods mentioned by Sadasivam and Manickam (2017).

$$\text{Mineral extractability} = \frac{\text{Mineral extractability in 0.03N HCl} \times 100}{\text{Total mineral content}}$$

3.3.2. Organoleptic qualities

The sensory qualities of cooked whole rice grains were evaluated as per the procedure mentioned in 3.2.4.

a) Preparation of cooked rice

Rice varieties were cooked by straining method as per the procedure indicated in 3.2.4.a

b) Selection of judges

A sequence of sensory trials was done as per the procedure disclosed in 3.2.4.b

c) Preparation of score card

The score card prepared as per the procedure mentioned in 3.2.4.c

d) Cooking time

The method described by Juliano and Bechtel (1985) was utilised to estimate the optimal cooking time. Approximately 100 ml of distilled water was heated to a temperature of 98 ± 1°C in a 250 ml beaker. Subsequently, a quantity of 10 g of head rice sample was introduced into the heated water. The commencement of the measurement of cooking duration occurred promptly. Following an initial 10-minute interval, and subsequently every minute thereafter, a quantity of one or two rice grains was extracted and subsequently compressed between two glass plates that had been well cleaned. The cooking duration was measured at the point when a minimum of 90 percent of the grains exhibited the absence of opaque cores or undercooked centres. Subsequently, the rice was simmered for an additional duration of approximately two minutes, so ensuring the complete gelatinization of the innermost portion of each grain. The optimal cooking duration encompassed an additional duration of two minutes dedicated to simmering.

3.4. Assessment of antioxidant and antiproliferative properties of GABA enriched rice

Antioxidant properties like free radical scavenging activities, and antiproliferatory studies in controlled and GABA enriched *Jyothi, Njavara* and *Chitteni* rice varieties were analysed.

3.4.1. Antioxidant properties

The study evaluates the antioxidant capabilities of control and GABA-enriched rice in scavenging free radicals. The current study aimed to evaluate the antioxidant properties of rice extracts by various assays, including DPPH (1,1-diphenyl 1-2-picrylhydrazyl) radical scavenging activity, nitric oxide (NO) scavenging activity, reducing power (RP) assay, superoxide and hydroxyl scavenging activity, and total antioxidant activity (Thomas *et al*., 2023). The total flavonoid content (TFC) and total phenol content (TPC) were also analysed.

a) Preparation of the rice extract

The preparation of the extracts was done as per the procedure disclosed in 3.2.3.a.

b) DPPH radical scavenging assay

The scavenging ability of the methanolic extract of rice against the stable free radical DPPH (2, 2-diphenyl-1- picrylhydrazyl) was assessed using the methodology described by (Aquino *et al.*, 2001). The radical form of DPPH exhibits an absorption band with a wavelength of 517nm, which becomes disappears upon its reduction (Thomas *et al*., 2023).

In connection with the findings of Deepa *et al.* (2012), varying concentrations of methanolic extracts from *Jyothi, Njavara*, and *Chitteni* were individually introduced to 0.375 ml of freshly made DPPH solution in methanol. The volume was adjusted to 2 mL by adding methanol. The reaction mixture underwent incubation under dark conditions for a duration of 20 minutes, following which the absorbance was quantified at a wavelength of 517nm, as reported by Shawky *et al.* (2019). The percentage of inhibition was analysed and the concentration required to achieve the half-maximal inhibitory concentration (IC_{50}) was estimated.

c) Superoxide radical scavenging assay

The determination of the superoxide scavenging activity of the rice extract was conducted using the method of nitro blue tetrazolium (NBT) reduction, as described

by McCord and Fridovich in 1969. The outcome is contingent upon the light-induced production of superoxide by riboflavin and the subsequent reduction of NBT, as described by Vijayan *et al.* (2007). Different amounts of the rice extract were introduced into the reaction mixture, which comprised 0.3 mM NaCN, 0.1 M EDTA, 0.12 mM riboflavin, 0.067 M phosphate buffer, and 1.5 mM NBT. The total volume of the mixture was adjusted to 3 mL. The tubes were subjected to homogeneous illumination using an incandescent lamp for a duration of 15 minutes. The optical density (OD) at a wavelength of 560 nm was measured both before and after the illumination period. The assessment of superoxide production inhibition was conducted by comparing the control and experimental tubes' absorbance values.

d) Estimation of total flavonoid content (TFC)

The quantification of flavonoids in the methanolic extracts of *Jyothi, Njavara,* and *Chitteni* was analysed using the methodology stated by Chang *et al.* (2002). Approximately 0.5 milliliters of rice extracts were combined with 1.5 milliliters of methanol, 0.1 milliliters of a 10 percent solution of aluminium chloride, 0.1 milliliters of a 1 molar solution of potassium acetate, and 2.8 milliliters of distilled water. The reaction mixture was subjected to incubation at ambient temperature for a duration of 30 to 35 minutes, following which the absorbance of the reaction mixture was quantified at a wavelength of 415nm. The calibration curve was generated by employing quercetin at concentrations ranging from 50 to 250 µg/ml dissolved in methanol. The quantification of flavonoid concentration was conducted by expressing it as µg/mg Quercetin equivalent (QE) in the dry extracts.

e) Estimation of total phenolic content (TPC)

The quantification of the total phenolic content in the methanolic extracts of *Jyothi, Njavara*, and *Chitteni* was conducted using the Folin-Ciocalteu reagent, employing a slightly modified procedure based on Ainsworth's method (2007). A reference standard of Gallic acid, ranging from 20 to 100 µg/ml, was employed to construct the calibration curve. A 0.5 ml sample of rice extracts with a concentration of 100 µg/ml was combined with 2 ml of the Folin-Ciocalteu reagent, which had been diluted 1:10 with de-ionized water. The resulting mixture was then neutralized by

adding 4 ml of a sodium carbonate solution with a concentration of 7.5% (w/v). The reaction mixture was subjected to incubation at ambient temperature for a duration of 30 minutes, during which occasional shaking was employed to facilitate the development of color. The measurement of absorbance for the resultant blue colour was conducted at a wavelength of 765 nm with a double-beam UV-VIS spectrophotometer. The quantification of total phenolic contents was conducted by employing a linear equation derived from a standard curve constructed using gallic acid. The phenolic component concentration was quantified and reported in units of μg/mg gallic acid equivalent (GAE) in the dry extracts.

f) Hydroxyl radical scavenging activity

The production of hydroxyl radical occurs in the Fe3+-ascorbate – EDTA-H_2O_2 system (Fenton reaction) degrading 2-deoxyribose the product of which condense with Thiobarbituric acid Reacting Substances (TBAR's) forming pink coloured complex (Elizebeth *et al*., 1990). The assessment of the effectiveness of test compounds in disrupting the reaction is conducted. The reaction mixture was composed of several components in a final volume of 1.0 ml. These components included 2-deoxy-2-ribose at a concentration of 2.8 mM, a KH_2PO_4-KOH buffer at a concentration of 20 mM with a pH of 7.4, $FeCl_3$ at a concentration of 0.1 mM, EDTA at a concentration of 0.1 mM, H_2O_2 at a concentration of 0.1 mM, and ascorbic acid at concentrations ranging from 0.25 to 4.0 μg/ml, derived from rice extracts. Following incubation for a duration of 1 hour at a temperature of 37°C, a volume of 0.4 ml from the reaction mixture was subsequently transferred into test tubes. To this, 1.5 ml of a 0.8 per cent solution of TBA, 1.5 ml of a 20% solution of acetic acid at a pH of 3.5, and 200 μl of an 8.1 per cent solution of SDS were added. The resultant volume was adjusted to 4.0 ml using distilled water. The incubation process was conducted at a temperature of 100°C for a duration of 1 hour. Following the chilling process, the absorbance was quantified at a wavelength of 532 nm in comparison to a suitable blank solution. In this test, vitamin C was used as a positive control.

g) Ferric reducing antioxidant power assay (FRAP)

The method by Pulido (2000) was followed with minor modifications to determine the antioxidant capacity of rice extracts. The ferric-reducing ability (FRAP) is measured using this method (Thomas *et al*., 2023). The shift in absorption at 595 nm can be used to track the reduction of a ferric tripyridyl-s-triazine (Fe3+ TpTz) complex to ferrous (Fe2) form at low pH, which exhibits a strong blue color. A freshly generated 900 µl of FRAP reagent (25 ml acetate buffer (300 mmol/l), pH 3.6; 2.5 ml TpTz (2,4,6-tripyridyl-s-triazine) (10 mmol/l) in 40 mmol HCl and 2.5 ml $FeCl_3$.6H2O solution) was combined with various quantities of rice extracts, and the volume was increased to 1.0 ml by the addition of distilled water. After 15 minutes of incubation at 37°C, the tubes were read at 595 nm against distilled water. The effective concentration was calculated by comparing the absorbance values of the control and experimental tubes, (Thomas *et al*., 2023).

h) Nitric oxide scavenging activity

In a 100 ml volumetric flask, various concentrations of the sample solution were prepared. This was mixed with 0.1489 g of sodium nitroprusside (final concentration 5 mM) and allowed to incubate. 5.6 ml was taken at various times, 0.2 mL of reagent A was added, and the mixture was incubated for 10 minutes at 30°C. Following incubation, 0.2 ml of Griess reagent was added, and the absorbance at 542 nm was recorded in comparison to a blank. The experiment was then incubated for 20 minutes at 30°C.

i) Total antioxidant activity

The total antioxidant activity (TAA) of extracts of rice was analysed by using phosphomolybdate assay developed by Prieto *et al.* (1999). The standard used in the experiment was ascorbic acid. A solution containing ascorbic acid at a concentration of 10 mg/ml was generated using distilled water. Subsequent dilutions were then performed using this stock solution. In a test tube, a volume of 300 µl of rice extracts was combined with 3 ml of a phosphomolybdate reagent. This reagent consisted of a solution containing 28 mM sodium phosphate, 0.6 M sulfuric acid, and 4 mM

ammonium molybdate. The test tube was enveloped with aluminum foil and subjected to incubation at a temperature of 95°C for a duration of 90 minutes. Subsequently, the combination was let to equilibrate to ambient temperature, at which point the absorbance of the solution was measured at a wavelength of 765 nm. The blank was conducted using an identical approach, with the exception that an equivalent volume of methanol was substituted for the samples. The measurement of antioxidant capacity was articulated in micrograms of ascorbic acid equivalents (AAE) per milliliter.

3.4.2. Antiproliferatory activities

In human hepatic cell lines (HepG2), an antiproliferative activity was conducted using MTT [3- (4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide] assay in control and GABA enriched rice *Jyothi*, *Njavara* and *Chitteni*.

a) Cell line and maintenance

The National Centre for Cell Sciences, Pune, was the supplier of the human hepatic cell line, which was kept in Dulbecco's Modified Eagle Media (DMEM), with 10% FBS and 100 U/L of standard concentrations of penicillin and streptomycin.

b) Cytotoxicity analysis

Approximately, $1x10^6$ cells/ml of HepG2 cells were seeded in flat bottomed 48 well plates containing 0.25 mL DMEM. The cells at 80 to 90 per cent confluency were incubated with different concentrations of rice extracts (25 to 125 µg/ml) for 48 h. Following the completion of the treatment, a volume of 20 microliters of a solution containing 5 mg/mL of MTT (3-(4, 5 – dimethyl-2-thiazoyl)-2, 5 – diphenyl – 2 H - tetrazolium bromide) was introduced into each well. The samples were then incubated for a duration of 4 hours. The formazan crystals that formed were solubilized by the addition of 500 µl of dimethyl sulphoxide, which served as a solubilizing reagent. The coloured products' absorbance was measured at 570 nm. Cell viability was then calculated by comparing it with the control well absorbance (Mosmann, 1983). The plates of the cell lines were taken using an inverted microscope in 200x magnification.

3.5. Storage qualities

3.5.1. Nutritional qualities

The nutritional qualities of GABA enriched rice varieties were analysed by the procedures mentioned in 3.3.1.

3.5.2. Organoleptic qualities

The organoleptic qualities of GABA enriched rice varieties were done as per the procedure mentioned in 3.2.4.2 and the scorecard was prepared as per the procedure mentioned in 3.2.4.

3.5.3. Enumeration of microbial population

The rice samples were initially evaluated for the presence of bacteria, yeast, and fungi and at three month intervals throughout a period of six months. The methodology proposed by Agarwal and Hasija (1986) involved the utilisation of the microbe's serial dilution and plate count technique. A quantity of 10 grams of the sample was introduced into 90 milliliters of sterile water and subjected to agitation for a duration of 20 minutes. A volume of 1 ml of the solution was transferred into a test tube containing 9 ml of sterile water, resulting in a dilution of 10^{-2}. Similarly, dilutions of 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} were prepared using the same method. The enumeration of the entire microflora was conducted by employing nutritional agar media for bacterial growth, Potato dextrose agar media for fungal growth, and Sabouraud's dextrose agar media for yeast growth. The dilution factor used for bacteria was 10^{-5}, while for yeast and fungi, it was 10^{-3}.

3.5.4. Insect infestation

The rice grains were examined under a microscope to determine whether storage insects were present. First, samples were put through a 60 BL sieve and examined under a microscope. The degree of insect infestation in samples that were stored was first evaluated visually, and then again at three-month intervals during a six-month period.

3.6. Processing GABA enriched rice for preparation of rice products

The rice varieties with maximum GABA content and medicinal properties were subjected to processing conditions like cooking, pulverizing, flaking, and puffing. Rice flakes and puffed rice were prepared from paddy, and dehusked rice was used for cooking and pulverising by standard procedure. The procedures adopted for the preparation of GABA enriched rice products are given in Appendix III.

3.6.1. Organoleptic evaluation of GABA enriched rice products

Organoleptic evaluation of GABA enriched rice products was carried out by standard procedures by preparing rice flakes, puffed rice, porridge, and cooked rice. The products prepared from each cultivar were compared with similar products prepared from other rice cultivars and the most suitable processing method for each variety will be determined. The organoleptic evaluation was analysed in the morning using score card (Appendix IV) by a selected panel of ten judges as mentioned in 3.2. 4.b and 3.2.4.c.

3.6.2. Quantification of GABA content

The GABA content of processed rice products was estimated as per the procedure indicated in 3.2.2.

3.6.3. Total antioxidant activity of GABA enriched rice products

The total antioxidant activity of processed rice products was analysed as per the procedure mentioned in 3.2.3.

3.7. Statistical analysis

The data were analysed by using a completely randomised design (CRD). The maximum GABA content, total antioxidant activity and organoleptic qualities were analysed by three factorial analysis. Based on the GABA content, total antioxidant activity, and organoleptic evaluation, the best treatment was selected using a normalisation scale. The quality evaluation of control and GABA enriched rice, antioxidant and antiproliferative properties were compared based on two sample t test.

The organoleptic evaluation of control and cooked (GABA enriched) rice varieties was assessed using Kendall's Coefficient of Concordance (W), and the total index score was worked out.

RESULTS

4. RESULTS

The results of the study entitled 'Medicinal properties and process optimisation for GABA enrichment in rice' are presented under the following heads.

4.1. Optimisation of conditions for GABA enrichment

4.1.1. Quantification of GABA content

4.1.2. Total antioxidant activity

4.1.3. Organoleptic evaluation of table rice

4.1.4. Parboiling of rice varieties

4.2. Quality evaluation of GABA enriched rice varieties

4.2.1. Chemical and nutritional qualities

4.2.2. Organoleptic qualities

4.3. Assessment of antioxidant and antiproliferative properties of GABA enriched rice

4.3.1. Antioxidant properties

4.3.2. Antiproliferatory activities

4.4. Storage qualities

4.4.1. Nutritional qualities

4.4.2. Organoleptic qualities

4.4.3. Enumeration of microbial population

4.4.4. Insect infestation

4.5. Processing GABA enriched rice for preparation of rice products

4.5.1. Organoleptic evaluation of GABA enriched rice products

4.5.2. Quantification of GABA content

4.5.3. Total antioxidant activity of GABA enriched rice products

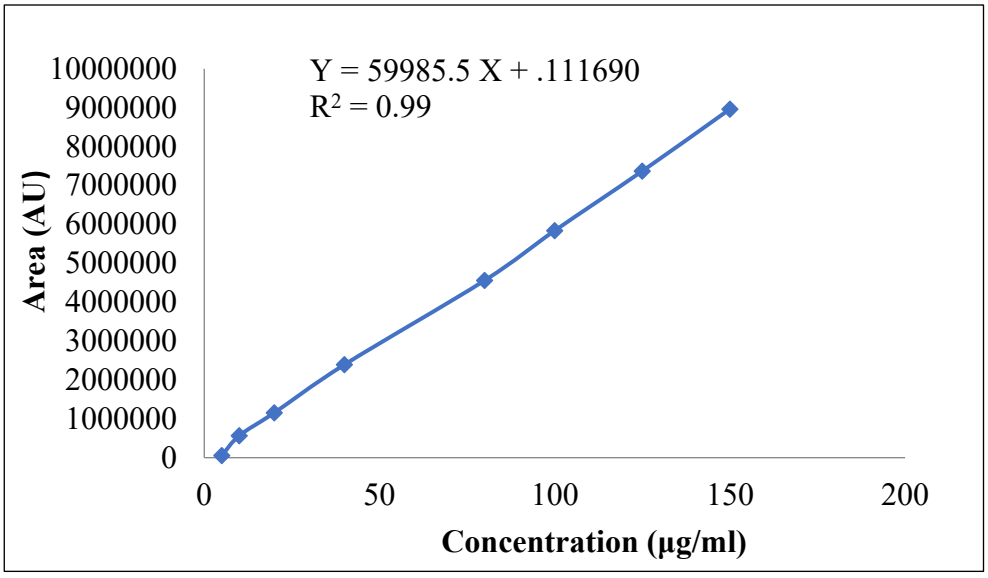
4.1. Optimisation of conditions for GABA enrichment

The popular high yielding cultivar *Jyothi*, medicinal rice cultivar *Njavara* (yellow glumed) and traditional rice cultivar *Chitteni* were purposively selected and subjected to soaking and germination as per the procedure mentioned in 3.2.1

a) Quantification of GABA content

As per the results of high-performance liquid chromatography (HPLC), the maximum response of GABA was obtained at a retention time of 7.65 minutes and at a wavelength of 230 nm in *Jyothi*, *Njavara* and *Chitteni*, respectively (Appendix V).

For quantitative analysis of GABA, a calibration curve was plotted after analysing a series of standard GABA solutions having concentrations between 5µg/ml to 150µg/ml, and the results showed that, the coefficient of determination (R^2) of the standard curve was 0.99, indicating the linearity of the curve (Figure 4).



Y = Peak area (AU)and X = Concentration of GABA (µg/ml)

Figure 4. Calibration curve for standard GABA

b) Effect of cultivar, soaking and germination time on GABA enrichment

The GABA content of the rice varieties in terms of various soaking and germination time (24, 48 and 72 h) is presented in Table 4.

In the three rice cultivars, as per contrast analysis, the mean GABA levels in control (0h soaking and 0h germination) samples were 19.18 mg/kg, 28.63 mg/kg, and 26.67 mg/kg ($P \leq 0.000$) in *Jyothi, Njavara* and *Chitteni,* respectively. The cultivar

Njavara had the highest content of GABA, followed by the indigenous variety *Chitteni* and the commonly used rice variety *Jyothi* without soaking and germination.

The GABA content in the rice cultivar *Jyothi* increased from 19.18 mg/kg to 116.89 mg/kg after 72 h of soaking and 72 h of germination. In 24 h soaking, highest GABA content was 91.78 mg/kg (72 h germination), followed by 67.31 mg/kg and 69.72 mg/kg after 48h and 24 h germination time respectively. A gradual increase in GABA was found after 48 h soaking 59.89 mg/kg, 75.71 mg/kg and 98.44 mg/kg after 24 h, 48 h and 72 h germination time. After 72 h soaking, highest GABA content of 116.89 mg/kg was observed in *Jyothi* germinated for 72 h, followed by 100.99 mg/kg (48 h) and 55.82 mg/kg (24h), respectively. From this, it can be concluded that, a maximum increase (6.09 fold) in GABA content with respect to control (Table 5) was observed with *Jyothi* variety soaked and germinated for 72 h.

The cultivar *Njavara* had the highest GABA content (28.63 mg/kg) before soaking and germination treatment which gradually increased from 24 h (74.85 mg/kg) of soaking and germination to 72 h of soaking and germination (130.29 mg/kg). The GABA content in *Njavara* increased by 4.55 fold after 72 h soaking and 72 h germination (Table 5). A gradual increase in GABA content, 74.85 mg/kg, 106.36 mg/kg, and 102.64 mg/kg was observed after 24 h, 48 h, and 72 h of germination. After 48 h of soaking, GABA content increased from 85.19 mg/kg to 112.36 mg/kg and then to 111.64 mg/kg when germinated for 24 h, 48 h and 72 h. The highest GABA content (130.29 mg/kg) was observed in *Njavara* rice samples soaked and germinated for 72 h, followed by rice samples soaked for 72 h and germinated for 48 h (88.36 mg/kg) and samples soaked for 72 h and germinated for 24 h (78.89 mg/kg).

In the traditional cultivar *Chitteni*, the GABA content was the lowest among the three cultivars after 24 h of soaking and germination (49.97 mg/kg), but it increased to 127.97 mg/kg after 72 hours of soaking and germination (4.79 fold). A gradual increase in GABA content was observed in 24 h, 48 h and 72 h of incubation, and maximum response was recorded after 72 h of soaking and 72 h of germination.

Table 4. GABA content of *Jyothi, Njavara* and *Chitteni* rice cultivars in various soaking and germination conditions

Treatment	Soaking (h)		Germination (h)		
		0	24	48	72
			GABA (mg/kg)		
Jyothi	0	19.18			
	24		69.72^{r}	67.31^{s}	91.78^{j}
	48		59.89^{t}	75.71^{o}	98.44^{i}
	72		55.82^{u}	100.99^{h}	116.89^{c}
Njavara	0	28.63			
	24		74.85^{op}	106.36^{f}	102.64^{g}
	48		85.19^{m}	112.36^{e}	111.64^{e}
	72		78.89^{n}	88.36^{l}	130.29^{a}
Chitteni	0	26.67			
	24		49.97^{v}	89.87^{k}	103.01^{g}
	48		71.37^{q}	87.37^{l}	98.55^{i}
	72		74.38^{p}	114.07^{d}	127.97^{b}

Results shown as mean of three injections (n=3) for each sample
Values with same alphabet represented in each column form a homogenous group

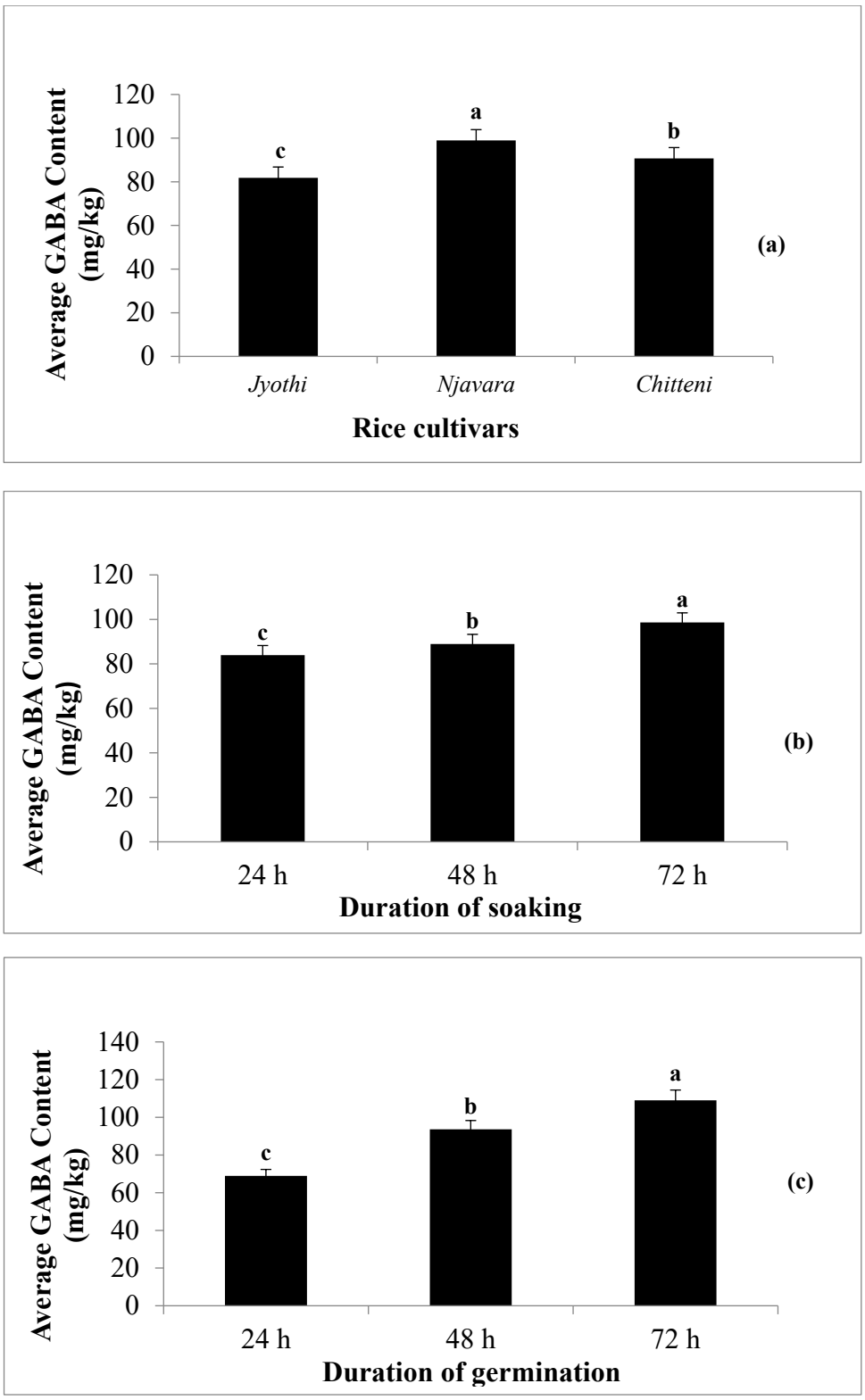
After three factorial analysis, it was observed that, cultivar, soaking time and germination time have a significant impact on GABA enhancement, and comparing all three rice cultivars, the average GABA content was significantly highest in *Njavara* (98.95 mg/kg) followed by *Chitteni* (90.73 mg/kg) and *Jyothi* (81.84 mg/kg) (Figure 5a). So, the results revealed that the amount of GABA accumulation in rice germ varied according to the cultivar.

Table 5. Fold increase in GABA content of *Jyothi, Njavara* and *Chitteni* rice cultivars in various soaking and germination conditions

Treatment	Soaking (h)	Germination (h)		
		24	48	72
		GABA fold increase		
Jyothi	24	3.63	3.50	4.78
	48	3.12	3.94	5.13
	72	2.91	5,26	6.09
Njavara	24	2.61	3.71	3.58
	48	2.97	3.92	3.89
	72	2.75	3.08	4.55
Chitteni	24	1.87	3.36	3.86
	48	2.67	3.27	3.69
	72	2.78	4.27	4.79

The results indicated that the soaking conditions significantly affected the accumulation of GABA during germination. The three factorial analysis shows that, GABA content increased significantly with soaking time, and the highest amount of GABA was at 72 h (98.63 mg/kg), followed by 48 h (88.95 mg/kg) and 24 h (83.95 mg/kg) (Figure 5b).

It was found that, the GABA content increased continuously with germination time in all three rice cultivars. After three factorial analysis, GABA content increased considerably to 109.02 mg/kg after 72 h of germination, 93.60 mg/kg after 48h of germination and 68.90 mg/kg after 24 h of germination respectively (Figure 5c).



Results shown as mean of three injections (n=3) for each sample

Figure 5. Effect of cultivar (a), soaking duration (b) and germination duration (c) on GABA content

A significant three-way interaction between cultivar ($P \leq 0.05$), soaking time ($P \leq 0.05$), and germination time ($P \leq 0.05$) on GABA content was observed. Our results show that soaking *Njavara* rice grains for 72 h followed by germination for 72 hours in neutral soaking water resulted in the highest amount of GABA.

4.1.2. Total antioxidant activity of GABA enriched rice varieties

Total antioxidant activity is based on the reducing phosphomolybdic acid to phosphomolybdenum blue complex.

The total antioxidant activity (EC_{50}) in the three rice cultivars, as per contrast analysis, without soaking and germination was found to be 7.61 µg/ml, 7.24 µg/ml and 8.83 µg/ml ($P \leq 0.000$) in *Jyothi, Njavara* and *Chitteni,* respectively (Table 6). The cultivar *Njavara* showed the highest total antioxidant activity, followed by *Jyothi* and *Chitteni*, even without soaking and germination.

Germinated brown rice (GBR) extracts showed total antioxidant activity effectively by scavenging the free radicals and the results are presented in Table 6.

The total antioxidant activity of germinated brown rice extracts was dose dependent and inversely proportional to the EC_{50} value. The methanolic extracts of germinated brown rice showed a significant change in the absorbance value from 0 to 10 µg/ml.

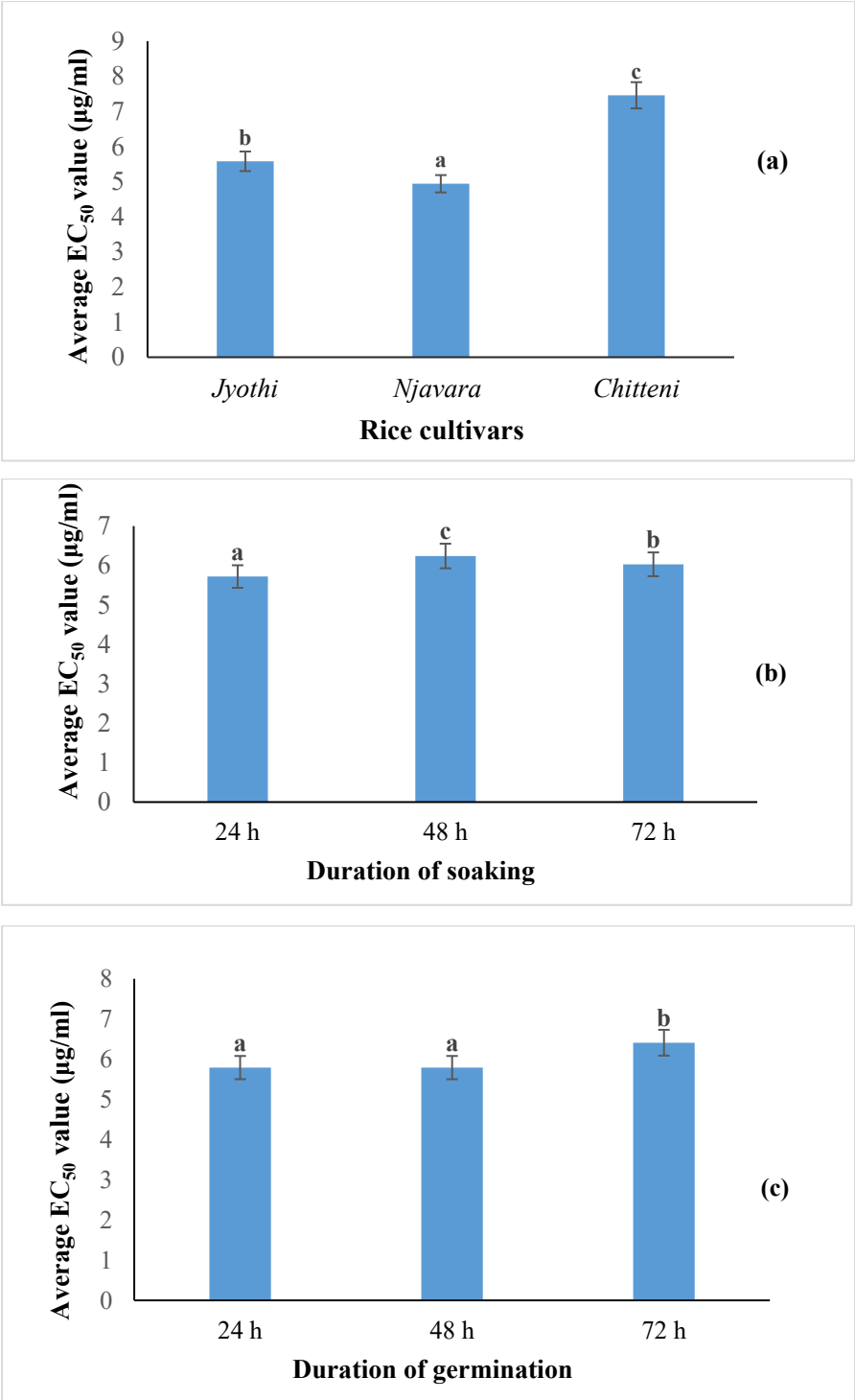
Even though all the rice varieties have shown an increase in the total antioxidant activity with increasing concentration, 48 h soaked and 24 h germinated rice samples of variety *Jyothi*, highest antioxidant activity with the projected EC_{50} value of 4.79 µg/ml. In 24 h soaking, highest total antioxidant activity was obtained as 5.19 µg/ml followed by 6.39 µg/ml and 6.88 µg/ml after 24 h, 48 h, and 72 h germination respectively. A gradual increase in total antioxidant activity was found after 48 h soaking like 5.49 µg/ml, 5.32 µg/ml and 4.79 µg/ml after 72 h, 48 h, and 24 h germination time, respectively. After 72 h soaking, the maximum total antioxidant activity observed was 5.23 µg/ml followed by 5.39 µg/ml and 5.41 µg/ml in 72 h, 24 h, and 48 h of germination.

Table 6. Total antioxidant activity of *Jyothi, Njavara* and *Chitteni* cultivars in various soaking and germination conditions

Treatment	Soaking (h)		Germination (h)		
		0	24	48	72
			Total antioxidant activity EC_{50} value (µg/ml)		
Jyothi	0	7.61			
	24		5.19^{k}	6.39^{h}	6.88^{fg}
	48		4.79^{l}	5.32^{ijk}	5.49^{i}
	72		5.39^{ijk}	5.41^{ij}	5.23^{jk}
Njavara	0	7.24			
	24		4.33^{m}	4.79^{l}	4.69^{l}
	48		4.49^{lm}	4.3^{m}	6.98^{f}
	72		4.84^{l}	4.28^{m}	5.47^{i}
Chitteni	0	8.83			
	24		7.5^{e}	5.45^{i}	6.32^{h}
	48		8.57^{a}	7.72^{d}	8.01^{c}
	72		6.74^{g}	8.29^{b}	8.62^{a}

Results are shown as the mean of three injections (n=3) for each sample
Values with same alphabet represented in each column form a homogenous group

The total antioxidant activity was the highest in *Njavara* with the projected EC_{50} value of 4.28 µg/ml after 72 h soaking and 48 h germination. In 24 h soaking, the total antioxidant activity was observed as, 4.33 µg/ml, 4.79 µg/ml and 4.69 µg/ml in 24 h, 48 h and 72 h of germination. After 48 h of soaking, total antioxidant activity increased from to 4.49 µg/ml to 6.98 µg/ml on varying germination times (24 h and 72 h), respectively. In 72 h soaking, the highest total antioxidant activity was noted as 4.28 µg/ml in 48 h of germination followed by, 4.84 µg/ml in 24 h and 5.47 µg/ml in 72 h germination.



Results shown as mean of three injections (n=3) for each sample

Figure 6. Effect of cultivar (a), soaking duration (b) and germination duration (c) on total antioxidant activity

In the traditional rice cultivar *Chitteni*, though the total antioxidant content was the lowest among the three cultivars, highest antioxidant activity (EC_{50} = 5.45 µg/ml) was reported after 24 h soaking and 48 h germination. So, the results revealed that, the antioxidant activity of *Chitteni* can be improved by adopting a soaking and germination process.

a) Effect of cultivar, soaking and germination time on total antioxidant activity

After three factorial analysis, a significant three-way interaction was observed between cultivar ($P \leq 0.05$), soaking time ($P \leq 0.05$), and germination time ($P \leq 0.05$) on total antioxidant activity and comparing all three rice cultivars, the average total antioxidant activity was significantly highest in *Njavara* (4.94 µg/ml) followed by *Jyothi* (5.58 µg/ml) and *Chitteni* (7.46 µg/ml) (Figure 6a). So, the results revealed that the antioxidant activity varied according to the cultivar.

It was found that, the soaking conditions significantly affected the total antioxidant activity. The three factorial statistical analysis shows that, the highest total antioxidant activity was found at 24 h (5.72 µg/ml), followed by 72 h (6.03 µg/ml) and 48 h (6.24 µg/ml) (Figure 6b).

In the case of germination, the research findings after three factorial analysis indicated that, the highest total antioxidant activity was reported with an average EC_{50} value of 5.79 µg/ml, 5.79 µg/ml, and 6.41 µg/ml after 48 h, 24 h, and 72 h of germination, respectively (Figure 6c).

4.1.3 Organoleptic evaluation of table rice

The prepared cooked rice from germinated brown rice of *Jyothi, Njavara* and *Chitteni* at various soaking and germination times was evaluated organoleptically using score card for different quality attributes like appearance, colour, flavour, texture, taste and overall acceptability. Each cooked rice was ranked for all quality attributes based on their mean rank scores using Kendall's coefficient (W) test which is presented in Appendix VI. The average mean score is tabulated and mentioned in Table 7 to determine the best table rice for maximum GABA enhancement.

The average mean score in the three rice cultivars, as per contrast analysis, in control (0h soaking and 0h germination) samples were found to be 7.5, 7.36, and 7.42 ($P \leq 0.000$) in *Jyothi, Njavara* and *Chitteni,* respectively (Table 7). The cultivar *Jyothi* showed the highest average mean score, followed by *Chitteni* and *Njavara* even without soaking and germination.

Table 7. Average mean score of *Jyothi*, *Njavara* and *Chitteni* table rice

Treatment	Soaking (h)		Germination (h)		
		0	24	48	72
			Average mean score		
Jyothi	0	7.50			
	24		7.53^{m}	7.42^{q}	7.65^{h}
	48		7.49^{o}	7.63^{i}	7.71^{f}
	72		7.74^{e}	8.15^{b}	8.34^{a}
Njavara	0	7.36			
	24		7.44^{p}	7.40^{r}	7.51^{n}
	48		7.49^{o}	7.58^{k}	7.65^{h}
	72		7.67^{g}	7.99^{c}	8.16^{b}
Chitteni	0	7.42			
	24		7.56^{l}	7.49^{o}	7.56^{l}
	48		7.51^{n}	7.61^{j}	7.63^{i}
	72		7.73^{e}	7.95^{d}	8.00^{c}

Results shown is mean of three injections (n=3) for each sample
Values with same alphabet represented in each column form a homogenous

The average mean score obtained for *Jyothi*, *Njavara* and *Chitteni* were considered for determining the suitability of selected varieties for maximum GABA enhancement for table rice (Table 7). In cultivar *Jyothi*, after 24 h soaking, the maximum mean score obtained was 7.65 followed by 7.53 and 7.42 on germination up to 72 h, 24 h, and 48 h, respectively. A gradual increase in mean score was found after 48 h soaking from 7.49 to 7.63 and to 7.71 after 24 h, 48 h and 72 h of germination time, respectively. After 72 h soaking, the maximum mean score of 8.34

was observed, followed by 8.15 and 7.74 in 72 h, 48 h, and 24 h of germination, respectively.

The results revealed that, in *Njavara* cultivar, after 24 h soaking, the highest mean score of 7.51 was obtained, followed by the mean score of 7.44 and 7.40 after 72 h, 24 h and 48 h of germination. After 48 h of soaking, the average mean score increased from 7.49 to 7.65 after 24 h, and 72 h of germination. The highest average mean score was obtained after 72 h of soaking, was 7.67 in 24 h, 7.99 in 48 h and 8.16 in 72 h of germination.

In the cultivar, *Chitteni*, the highest total mean scores were reported after 72 h of germination. The maximum response of the total mean score was recorded as 8.00, after 72 h of soaking and 72 h of germination.

a) Effect of cultivar, soaking and germination time on organoleptic qualities

After three factorial analysis, it was discovered that the three factors such as cultivar, soaking time, and germination time significantly impact on organoleptic qualities. When comparing the average total mean score of the three rice cultivars, after different treatments *Jyothi*, *Chitteni* and *Njavara* scored 7.74, 7.67 and 7.65 respectively (Figure 7a). As per the findings, the organoleptic qualities of rice cultivars significantly differed from one another.

The results showed that the soaking conditions substantially impacted on the organoleptic qualities of cultivars. The organoleptic qualities increased considerably with soaking time, with the maximum mean score at 72 h (7.96), followed by 48 h (7.58) and 24 h (7.50) in selected cultivars after different treatments (Figure 7b).

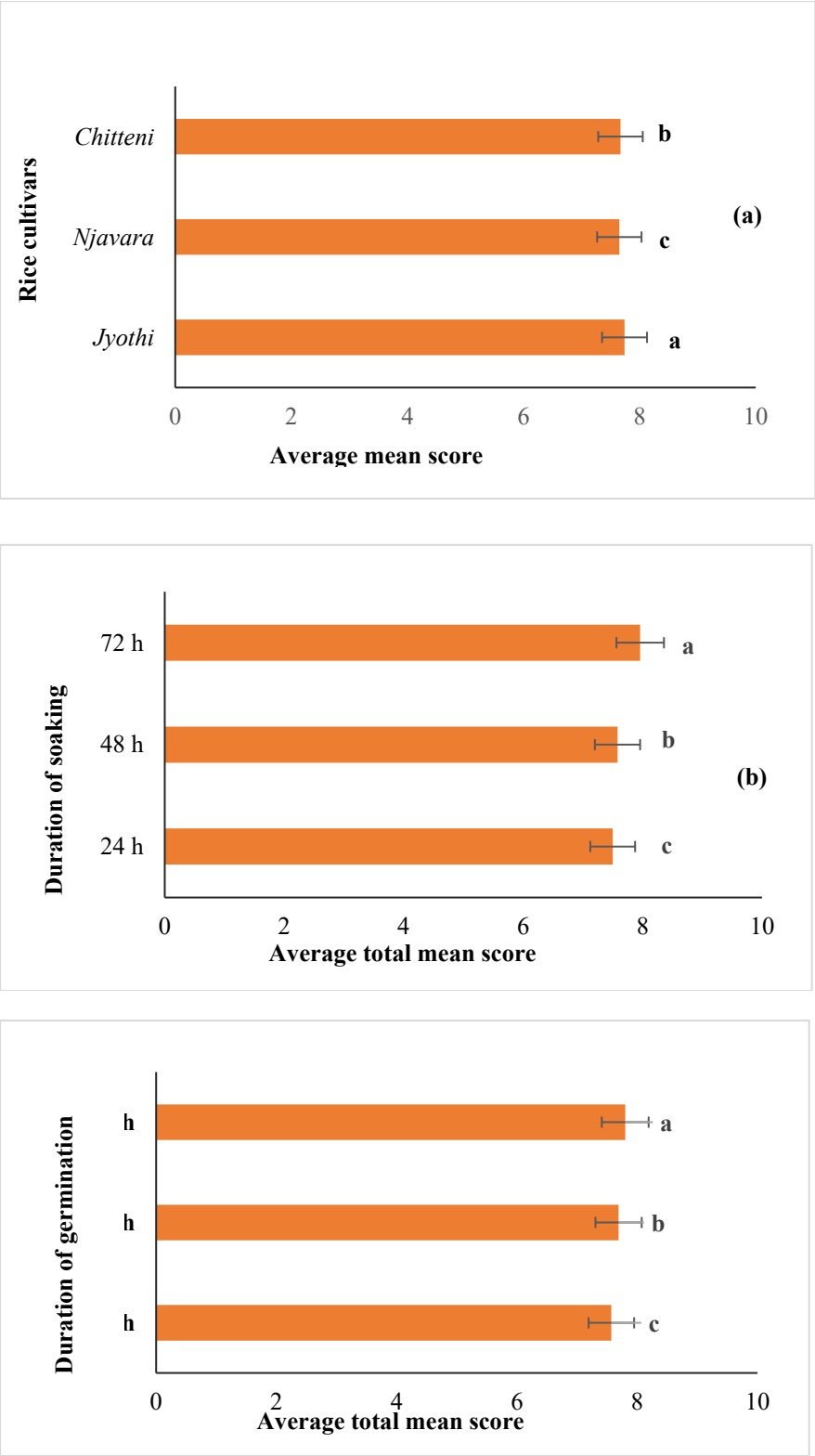


Figure 7. Effect of cultivar (a), soaking duration (b) and germination duration (c) on organoleptic qualities

The organoleptic qualities of all three rice cultivars, increased steadily with germination time. The average mean score increased to 7.80 after 72 h of germination, which were 7.69 after 48 h of germination, and 7.57 after 24 h of germination, respectively (Figure 7c). It is clear from the results that, all the rice varieties have obtained an average index score greater than 7, indicating they are organoleptically acceptable, and 72 h soaking and germination was found to be the most suitable condition for the preparing cooked germinated brown rice.

Based on GABA content, total antioxidant activity and organoleptic qualities, the most suitable conditions for GABA enrichment in each variety were identified by using normalisation scale (Appendix II). The total index score obtained for each variety with respect to GABA content, total antioxidant activity and organoleptic qualities are presented in Table 8.

Table 8. Total index score of *Jyothi, Njavara* and *Chitteni* after various soaking and germination conditions

Treatment	Soaking (h)	Germination (h)		
		24	48	72
		Total index score		
Jyothi	24	-0.36^{n}	-0.91^{r}	-0.28^{m}
	48	-0.49^{op}	-0.16^{kl}	0.36^{f}
	72	-0.53^{p}	0.88^{d}	**1.50^{a}**
Njavara	24	-0.74^{q}	-0.09^{j}	-0.03^{i}
	48	-0.47^{o}	0.40^{f}	-0.17^{kl}
	72	-0.49^{op}	0.28^{g}	**1.29^{b}**
Chitteni	24	-1.00^{s}	0.09^{h}	0.28^{g}
	48	-0.86^{r}	-0.21^{l}	-0.01^{i}
	72	-0.1^{jk}	0.78^{e}	**1.07^{c}**

Results shown as mean of three injections (n=3) for each sample
Values with same alphabet represented in each column form a homogenous group

After using the normalisation scale, the cultivar *Jyothi* obtained the highest total index score of 1.5 among the three cultivars and after 24 h soaking, the index

score was obtained as -0.28, -0.36, and -0.91 after 72 h, 24 h and 48 h of germination time. In 48 h of soaking, the maximum mean score of 0.36, 0.16, and 0.49 was obtained after 72 h, 48 h and 24 h of germination respectively. A gradual increase in index score was found after 72 h of soaking a and a gradual increase in index score was observed as 0.53, 0.88 and 1.5 after 24 h, 48 h and 72 h of germination (Table 8).

The results revealed that, in *Njavara* cultivar, after 24 h soaking, the index score was obtained as -0.03, -0.09 and -0.74 after 72 h, 48 h and 24 h of germination. After 48 h of soaking, the total index score increased from -0.47, 0.17 and 0.4 after 24 h, 72 h and 48 h of germination respectively. The maximum index score was observed after 72 h of soaking and germination as 1.29 followed by 48 h (0.28) and 24 h (-0.49) of germination.

Chitteni had the highest total index score among the cultivars, which 1.07 after 72 h of soaking and germination. After 24 h of soaking, the maximum response was observed as 0.28, 0.09, and -1.00 after 72 h, 48 h and 24 h of germination. After 48 h of soaking, a gradual increase in total index score was observed from -0.86, -0.21 and -0.01 after 24 h, 48 h and 72 h of germination. After 72 h soaking, the maximum mean score was 1.07 followed by 0.78 and -0.1 in 72 h, 48 h, and 24 h of germination respectively.

The current study's findings demonstrate that 72 hours of soaking and 72 hours of germination was the best suitable condition for maximum GABA enrichment in all three rice cultivars and *Jyothi, Njavara*, and *Chitteni* after 72 h soaking and germination were chosen for further investigations.

4.1.4. Parboiling of rice varieties

The GABA enriched rice from *Jyothi, Njavara* and *Chitteni* was parboiled by standard procedure, and the retention of GABA content was observed and presented in Table 9. The GABA enriched *Jyothi* had a GABA concentration of 116.89 mg/kg, which was reduced to 93.47 mg/kg after parboiling which was significant after t-test, the retention of GABA content after parboiling was significant at 1 per cent level.

Table 9. Per cent retention of GABA after parboiling

GABA enriched rice varieties

Parameters	*Jyothi*	*Njavara*	*Chitteni*
	GABA Content (mg/kg)		
GABA enriched	116.89	130.29	127.97
GABA enriched (Parboiled)	93.47	109.89	105.01
Percentage retention	79.96 %	84.34 %	82.05 %
t value	19.86**	17.25**	10.09**

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

Maximum GABA retention (84.34 %) after the parboiling was observed in the *Njavara* cultivar. The GABA content after enrichment was 130.29 mg/kg, and after parboiling, it dropped to 109.89 mg/kg. The observed percentage retention was 84.34, and the difference was significant at 1per cent level.

The GABA content in *Chitteni* after enrichment was 127.97 mg/kg. On parboiling, the GABA content decreased to 105.01 mg/kg. The decrease was statistically significant with a percentage retention of 82.05 at 1 per cent level.

According to the findings, more than 79 per cent of GABA was retained in all three rice cultivars following parboiling.

4.2. Quality evaluation of GABA enriched rice varieties

The GABA enriched rice varieties were dehusked, and the physicochemical qualities were evaluated. The raw rice of the selected varieties was taken as control.

4.2.1. Chemical and nutritional qualities

The nutritional qualities like moisture, starch, amylose, carbohydrate, protein, total fat, energy, calcium, zinc, iron, phosphorus, thiamine, riboflavin, vitamin E, *in*

vitro digestibility of starch and *in vitro availability* of calcium, zinc, iron and phosphorus of the control and GABA enriched rice varieties of *Jyothi, Njavara* and *Chitteni* were evaluated. The results on the chemical and nutritional qualities of raw and GABA enriched rice are presented in Tables 10 and 11.

a) Moisture

The moisture content of GABA enriched rice, and their control is shown in Table 10. After analysing the data, the control sample of each rice variety reported lower moisture content compared to their GABA enriched counterparts. The moisture content of the GABA enriched *Jyothi* was 13.53 per cent, while the control was 11.81 per cent, and as per the t test, there was a significant difference between the control and GABA enriched rice with respect to moisture content. Meanwhile, the moisture content of the cultivar *Njavara* was 13.19 per cent for GABA enriched rice and for the control, it was 11.63 per cent. The t-test revealed that, significant difference between the control and GABA enriched rice of *Njavara*. The indigenous cultivar *Chitteni* had a moisture content of 13.58 per cent and 11.83 per cent for GABA enriched and the control, respectively, and was found to be significantly different.

b) Starch

The starch content of rice varieties was assessed (Table 10), and the starch content in GABA enriched rice significantly decreased compared to the control. In *Jyothi* rice variety, the starch content was 77.8 per cent and 75.76 per cent in control and GABA enriched rice, respectively. The starch content of *Njavara* cultivar was 74.62 per cent initially and decreased to 71.5 per cent in GABA enriched rice. Similarly, the initial starch content of *Chitteni* was 75.42 per cent, which decreased to 73.16 per cent in GABA enriched rice. In terms of starch, there was no significant difference between the control and GABA enriched rice of three cultivars.

c) Amylose

Amylose content in GABA enriched rice is presented in Table 10. The amylose content was noticed as 25.75 per cent initially and reduced to 23.43 per cent in GABA enriched *Jyothi*. Based on t test, significant variation was not observed in

the amylose content between the control and GABA enriched rice. In the *Njavara* cultivar, initially, the amylose content was 24.21 per cent and in GABA enriched rice, it was 22.88 per cent. The decrease in amylose content was observed in GABA enriched *Njavara*, but it was found to be statistically insignificant. Similarly, the initial amylose content of *Chitteni* (24.66 per cent), decreased to 23.87 per cent in GABA enriched rice. In the case of amylose, there was no significant difference between the control and GABA enriched *Chitteni*.

d) Carbohydrate

The data on carbohydrate content of GABA enriched rice varieties are presented in Table 10. It was found that, the popular rice cultivar *Jyothi*, had a carbohydrate content of 69.14 per cent; after GABA enrichment, it decreased to 63.23 per cent. A significant difference was observed between control and GABA enriched rice with respect to carbohydrate content. Similarly, the cultivar *Njavara* also had a significant difference in carbohydrate content between the control and GABA enriched rice. The obtained carbohydrate content was 66.02 per cent in control and 60.43 per cent in GABA enriched rice. The carbohydrate content recorded for the *Chitteni* cultivar was 67.8 per cent initially, which decreased to 61.16 per cent in GABA enriched rice, and significant variation was found between the control and GABA enriched rice.

Table 10. The nutritional qualities of GABA enriched rice varieties of *Jyothi*, *Njavara* and *Chitteni*

Parameters	*Jyothi*			*Njavara*			*Chitteni*		
	C	T	t value	C	T	t value	C	T	t value
Moisture (%)	11.81	13.53	6.59**	11.63	13.19	9.06**	11.83	13.58	11.09**
Starch (%)	77.80	75.76	2.24NS	74.62	71.52	3.78*	75.42	73.16	2.39*
Amylose (%)	25.75	23.43	2.25NS	24.21	22.88	2.07NS	24.66	23.87	0.43NS
Carbohydrate (%)	69.14	63.23	14.26*	66.02	60.43	4.44*	67.8	61.16	10.46*
Protein (%)	10.62	12.43	14.97*	12.90	14.28	6.01*	11.56	13.01	7.21*
Fat (%)	1.59	2.04	22.59**	1.98	2.81	3.21*	1.75	3.00	3.96*
Energy (Kcal/100g)	333.35	321.01	1.16NS	333.5	324.13	2.05NS	333.19	323.68	1.16NS

Significant at 5 % level, ** Significant at 1% level, NS- non significant

C- Control, T- GABA enriched rice

e) Protein

The protein content of the GABA enriched rice varieties are presented in Table 10. In the case of cultivar *Jyothi*, the control samples had the lowest protein content (10.62 %) than the GABA enriched rice (12.43 %). The protein content of the *Njavara* cultivar was 12.90 per cent initially and 14.28 per cent in GABA enriched rice. Similarly, the initial protein content of *Chitteni* was 11.56 per cent which increased to 13.01 per cent in GABA enriched rice. Regarding protein content, there was a significant increase between the control and GABA enriched rice of three cultivars, of three cultivars namely *Jyothi*, *Njavara* and *Chitteni*. From this, it is clear that soaking and germination increased the protein content in these rice samples.

f) Fat

The fat content of the GABA enriched rice varieties are furnished in Table 10. The fat content noticed was 1.59 per cent initially and increased to 2.54 per cent in GABA enriched *Jyothi*. The increase observed in the fat content between the control and GABA enriched rice is statistically significant at 5 per cent level. In the *Njavara* cultivar, initially, the fat content was 1.98 per cent, and in GABA enriched rice, it was 3.12 per cent. The increase in fat content noticed in GABA enriched *Njavara*, was found to be statistically significant. Similarly, the initial fat content of *Chitteni* (1.75 %), increased to 3.24 per cent in GABA enriched rice. In the case of fat, significant increase was observed between the control and GABA enriched rice in all the three rice cultivars.

g) Energy

The energy content of the GABA enriched rice varieties are presented in Table 10. In the commonly used rice cultivar *Jyothi*, it was found that, the control and GABA enriched rice had an energy content of 332.68 and 324.88 Kcal, respectively. The difference in energy content between the control and GABA enriched rice is statistically insignificant. Soaking and germinating rice samples did not affect their energy content. Similarly, cultivar *Njavara* also reported no significant difference between the control and GABA enriched rice and obtained an energy value of 332.84

Kcal and 326.30 Kcal for the control and GABA enriched rice, consecutively. The energy content determined for *Chitteni* cultivar was 332.54 Kcal and 325.23 Kcal for the control and GABA enriched rice respectively. No significant difference was found between the control and GABA enriched rice.

h) Calcium

Calcium content in the GABA enriched rice varieties is presented in Table 11. It was observed that the control samples of each rice variety had higher calcium levels than the GABA enriched samples. There was a significant difference between the control and GABA enriched rice of *Jyothi* in terms of calcium content. The calcium content of the GABA enriched *Jyothi* was 13.11 mg/100g which is lower when, compared to the control (14.84 mg/100g). For the GABA enriched *Njavara*, calcium content was 11.35 mg/100g, which was lower than the calcium content in the control (12.11 mg/100g). However, the difference is statistically insignificant. The indigenous cultivar *Chitteni* had a calcium content of 12.50 mg/100g and 13.38 mg/100g for GABA enriched and the control rice, respectively, and the difference is not statistically significant.

i) Zinc

The zinc content of the GABA enriched rice varieties is presented in Table 11. It was found that, in the *Jyothi* cultivar, the control and GABA enriched rice had a zinc content of 2.91 mg/100g and 2.33 mg/100g, respectively. The observed difference in zinc content between the control and GABA enriched rice is statistically significant (1 % level). Similarly, in cultivar *Njavara* the zinc content was found to be 3.28 mg/100g and 2.81 mg/100g for the control and GABA enriched rice consecutively. The difference in zinc content was statistically insignificant. The zinc content determined for the *Chitteni* cultivar was initially 3.78 mg/100g and decreased to 3.37 mg/100g in GABA enriched rice. The decrease in zinc content was statistically significant.

Table 11. Mineral and vitamin contents in GABA enriched rice varieties of *Jyothi*, *Njavara* and *Chitteni*

Parameters	*Jyothi*			*Njavara*			*Chitteni*		
	C	T	t value	C	T	t value	C	T	t value
Calcium (mg/100g)	14.84	13.11	3.51*	12.11	11.35	1.53NS	13.38	12.50	1.52NS
Zinc (mg/100g)	2.91	2.33	5.44**	3.28	2.81	1.22NS	3.78	3.37	3.22*
Iron (mg/100g)	3.60	3.12	1.55NS	2.62	2.45	0.48NS	2.41	2.25	0.94NS
Phosphorous (mg/100g)	328.95	321.66	8.42**	359.15	300.77	60.30**	338.11	332.58	6.69**
Thiamine (mg/100g)	0.61	0.77	12.44**	0.68	0.80	0.47NS	0.52	0. 64	8.13**
Riboflavin (mg/100g)	0.14	0.15	0.54NS	0.16	0.18	2.50*	0.11	0.13	1.68NS
Vitamin E (µg/g)	3.57	4.68	3.44*	4.19	4.61	1.23NS	2.45	5.11	6.30**

* Significant at 5 % level, ** Significant at 1% level, NS -non significant

C- Control, T- GABA enriched rice

j) Iron

The iron content of the GABA enriched rice varieties are presented in Table 11. The highest iron content was recorded in the cultivar *Jyothi*, followed by *Njavara* and *Chitteni*. In the *Jyothi* cultivar, the control (3.60 mg/100g) had the highest iron content than the GABA enriched rice (3.12 mg/100g). The *Njavara* cultivar initially had an iron content of 2.62 mg/100g and 2.45 mg/100g for GABA enriched rice. Similarly, the initial iron content of *Chitteni* was 2.41 mg/100g, which decreased to 2.25 mg/100g in GABA enriched rice. With respect to iron, there was no significant difference between the control and GABA enriched rice of three cultivars, namely *Jyothi, Njavara* and *Chitteni*.

k) Phosphorous

Phosphorous content in GABA enriched rice is presented in Table 11. The phosphorous content was initially noticed to be 328.95 mg/100g which decreased to 321.66 mg/100g in GABA enriched samples of *Jyothi*. In the *Njavara* cultivar, the phosphorous content was initially 359.15 mg/100g and while in GABA enriched rice, it was 300.77 mg/100g. Similarly, the initial phosphorous content of *Chitteni* (338.11 mg/100g), decreased to 332.58 mg/100g in GABA enriched rice. The difference in phosphorous content in *Jyothi*, *Njavara* and *Chitteni* was statistically significant (1 % level).

l) Thiamine

The thiamine content of the GABA enriched rice varieties is presented in Table 11. It was found that, in the *Jyothi* rice cultivar, the control and GABA enriched rice had a thiamine content of 0.61 mg/100g and 0.77 mg/100g, respectively. There was a significant difference between control and GABA enriched rice with respect to thiamine content. In the cultivar *Njavara,* a thiamine content of 0.68 mg/100g and 0.80 mg/100g was reported for the control and GABA enriched rice, consecutively. The thiamine content determined for the *Chitteni* cultivar was initially 0.52 mg/100g and increased to 0.64 mg/100g in GABA enriched rice. The increase in thiamine content was statistically significant (1 % level).

m) Riboflavin

The riboflavin content of the GABA enriched rice varieties was presented in Table 11. It was found that, in the *Jyothi* rice cultivar, the control and GABA enriched rice had a riboflavin content of 0.14 mg/100g and 0.15 mg/100g, respectively and there no significant difference was observed between control and GABA enriched rice. Similarly, cultivar *Njavara* reported significance between the control and GABA enriched rice and obtained a riboflavin content of 0.16 mg/100g and 0.18 mg/100g for the control and GABA enriched rice, consecutively. The riboflavin content determined for the *Chitteni* cultivar was 0.11 mg/100g and 0.13 mg/100g for the control and GABA enriched rice. In the case of riboflavin, the difference observed between the control and GABA enriched counterparts was statistically not significant.

n) Vitamin E

The vitamin E content of the GABA enriched rice varieties are presented in Table 11. It was found that, in the commonly used rice cultivar *Jyothi*, the control and GABA enriched rice had a vitamin E content of 3.57 µg/g and 4.68 µg/g, and significant difference was observed between control and GABA enriched rice. In cultivar *Njavara,* vitamin E content of 4.19 µg/g and 4.61 µg/g for the control and GABA enriched rice, consecutively. The vitamin E content determined for the *Chitteni* cultivar was 2.45 µg/g initially and increased to 5.11 µg/g in GABA enriched rice. In the *Chitteni* rice cultivar, the increase in vitamin E content was statistically significant (1 % level).

o) *in vitro* digestibility of starch

in vitro digestibility of starch and *in vitro* availability of the minerals in different varieties like *Jyothi, Njavara, Chitteni* and their GABA enriched counterparts were evaluated and the results are tabulated in Table 12. The *in vitro* starch digestibility of the GABA enriched rice varieties was evaluated. The starch digestibility of the control and GABA enriched *Jyothi* was 66.25 per cent and 75.13 per cent respectively. Likewise, in the case of *Njavara,* the starch digestibility was only 69.39 per cent for control whereas the enriched *Njavara* had 78.18 per cent digestibility. In the case of

Chitteni, the starch digestibility was only 67.58 per cent for control and for GABA enriched rice, it was 76.04 per cent. GABA enriched *Njavara* had the highest starch digestibility among the three rice varieties. In the case of starch digestibility, there was a significant difference between the control and GABA enriched *Jyothi, Njavara* and *Chitteni*.

p) *in vitro* availability of minerals

in vitro availability of minerals, calcium, zinc, iron and phosphorous was estimated. In the case of mineral availability, the GABA enriched rice had higher availability compared to the control samples of all the three varieties and the difference was found to be statistically significant. The availability of calcium was found to be 35.63 per cent and 45.85 per cent, respectively in control, and GABA enriched *Jyothi*. Similarly, in the case of *Njavara*, the calcium availability was found to be 34.98 per cent for control and 49.55 per cent for GABA enriched *Njavara.* The availability of calcium in *Chitteni* rice was 33.09 per cent, and of its GABA enriched one was 44.09 per cent.

The zinc availability was the highest for the *Njavara* variety compared to *Jyothi* and *Chitteni.* In the case of *Jyothi,* the availability was found to be 22.47 per cent and 36.04 % per cent for control and GABA enriched rice, respectively. The *Njavara* rice had 23.35 per cent zinc availability while its GABA enriched counterpart had 38.14 per cent availability. The *Chitteni* rice had an absorption percentage of 22.97 per cent and 34.54 per cent for its control and its GABA enriched counterpart. The increase in zinc availability was statistically significant in the control and GABA enriched rice of three cultivars.

Table 12. *in vitro* digestibility of starch and *in vitro* availability of minerals in GABA enriched rice varieties of *Jyothi*, *Njavara* and *Chitteni*

Parameters	*Jyothi*			*Njavara*			*Chitteni*		
	C	T	t value	C	T	t value	C	T	t value
In vitro digestibility of starch (%)	66.25	75.13	9.20**	69.39	78.18	7.24**	67.58	76.04	7.22**
In vitro availability of calcium (%)	35.63	45.85	8.30**	34.98	49.55	16.89**	33.09	44.09	13.45**
In vitro availability of zinc (%)	22.47	36.04	8.99**	23.35	38.14	9.60**	22.97	34.54	8.47**
In vitro availability of iron (%)	21.95	30.09	8.60*	23.01	33.24	14.59**	21.18	31.54	16.01**
In vitro availability of phosphorous (%)	32.59	56.01	14.93**	35.18	59.95	25.16**	33.94	55.89	16.49**

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

C- Control, T- GABA enriched rice

The iron content and availability were comparatively lower when compared to that of all other minerals. The iron availability was found to be 21.95 per cent, 23.01 per cent, 21.18 per cent for control and 30.09 per cent, 33.24 per cent, and 31.54 per cent for GABA enriched rice of *Jyothi, Njavara* and *Chitteni* respectively. An increase in *in vitro* availability was observed and was found to be statistically significant.

Phosphorous is another mineral seen in rice; the availability of phosphorous was the highest than the other minerals. The availability of phosphorus was 32.59 per cent in control and 56.01 per cent in GABA enriched *Jyothi*. About 35.18 per cent and 59.95 per cent phosphorous absorption were found in control and GABA enriched *Njavara* cultivar. The availability was 33.94 per cent in control, and 55.89 per cent in GABA enriched *Chitteni* rice.

4.2.2. Organoleptic qualities

The mean scores for several quality attributes of cooked rice of GABA enriched *Jyothi, Njavara* and *Chitteni* were determined (Table 13). Cooked rice was subjected to organoleptic evaluation for sensory parameters such as appearance, colour, flavour, taste, texture and overall acceptability (Appendix VII). The mean values for each parameter were recorded and were compared to understand the sensory quality of both control and GABA enriched rice. The statistical analysis was carried out with the help of t test.

In general, mean scores for all the sensory parameters of GABA enriched rice were higher than that of the control. The mean score for the appearance of *Jyothi* rice was found to be 7.33, while for GABA enriched *Jyothi* rice was 8.38. The mean score for *Njavara* and its GABA enriched rice was found to be 7.45 and 8.40. The control and its GABA enriched *Chitteni* rice had a mean score of 7.54 and 8.14, respectively. The t value estimated for each variety was found to be significant *i.e.* the difference between the mean values of control and GABA enriched rice was statistically significant.

Table 13. Organoleptic qualities of the cooked GABA enriched rice of *Jyothi, Njavara* and *Chitteni*

Parameters	*Jyothi*			*Njavara*			*Chitteni*		
	C	T	t value	C	T	t value	C	T	t value
Appearance	7.33	8.38	15.55*	7.45	8.40	8.34*	7.54	8.14	4.49*
Colour	7.52	8.33	12.02*	7.59	8.23	19.09*	7.66	8.00	2.13*
Flavour	7.38	8.35	8.74*	7.42	8.16	1.42*	7.54	8.23	6.48*
Taste	7.57	8.35	11.00*	7.38	7.90	3.54*	7.54	7.90	2.65*
Texture	7.54	8.38	7.82*	7.42	8.16	11.71*	7.52	7.97	3.21*
Overall acceptability	7.57	8.40	11.06*	7.57	8.16	7.90*	7.61	7.95	2.18*

* Significant at 5 % level

C- Control, T- GABA enriched rice

The colour of the table rice was also evaluated and the mean values recorded were 7.52 and 8.33 for control and GABA enriched *Jyothi.* While for control and GABA enriched *Njavara*, the values were, 7.59 and 8.23. *Chitteni* rice had a mean value of 7.66, and GABA enriched *Chitteni* had 8.00. The mean values were statistically significant from each other.

Flavour was another parameter included in the organoleptic evaluation of table rice. The mean score value for the control was 7.38 for the GABA enriched *Jyothi*, it was 8.35. The *Njavara* rice had a mean score of 7.42, and its GABA enriched rice had a value of 8.16. The mean values of 7.54, and 8.23 were recorded for the control and GABA enriched *Chitteni* rice.

Taste of the table rice indicates that, taste significantly increased between in GABA enriched rice in all the three varieties. The score recorded was 7.57, 7.38, 7.61 for control and 8.35, 7.90, 7.91 for GABA enriched *Jyothi, Njavara,* and *Chitteni* respectively.

The texture of the table rice in all three varieties, in the control and GABA enriched rice was also evaluated. The mean score recorded for *Jyothi* rice was 6.54 and that for GABA enriched rice was 8.38. *Njavara* rice had a mean score of 7.42 and its GABA enriched rice had a mean score of 8.38. The mean score for *Chitteni* rice was 7.54, and for GABA enriched *Chitteni* rice it was 7.97. The mean scores of all three varieties were significantly different from their GABA enriched counterparts.

Based on the overall acceptability, it was found that the organoleptic qualities were better for the control and GABA enriched *Jyothi* than the other two varieties. The control had a value of 7.57 and GABA enriched *Jyothi* had a value of 8.40. The mean score of 7.57 and 8.16 was recorded for the control and GABA enriched *Njavara* respectively, whereas a score of 7.52 and 7.95 were recorded for the control, and GABA enriched *Chitteni* cultivar.

4.2.2.1. Cooking time of GABA enriched rice

The cooking time recorded for GABA enriched *Jyothi, Njavara* and *Chitteni* is given in Table 14.

Table 14. Cooking time of GABA enriched *Jyothi, Njavara* and *Chitteni*

Rice varieties		**Cooking time (min)**
Jyothi	C	31.44
	T	24.02
	t value	15.54**
Njavara	C	32.63
	T	27.15
	t value	16.90**
Chitteni	C	32.1
	T	26.89
	t value	17.40**

** Significant at 1 % level, C- Control, T- GABA enriched rice

The GABA enriched rice reported less time for cooking compared with its control counterparts. It was found that, the popular rice cultivar *Jyothi*, the control and GABA enriched rice found a cooking time of of 31.44 minutes and 24.02 minutes. In cultivar *Njavara,* a cooking time of 32.63 minutes and 27.15 minutes was reported for the control and GABA enriched rice consecutively. The cooking time determined for *Chitteni* cultivar was 32.10 minutes initially and decreased to 26.89 minutes in GABA enriched rice. In all three rice varieties the decrease in cooking time was found to be statistically significant (1 % level).

4.3. Assessment of antioxidant and antiproliferative properties of GABA enriched rice

Antioxidant compounds are considered functional compounds due to the reduction of oxidative stress. Antioxidant and antiproliferative activities were studied

in GABA enhanced rice varieties of *Jyothi, Njavara*, and *Chitteni*. The results are as follows:

4.3.1. Antioxidant properties

Antioxidant activities such as DPPH (1,1-diphenyl 1,2- picrylhydrazyl) radical scavenging activity, reducing power (RP) assay, nitric oxide (NO) scavenging activity, superoxide and hydroxyl scavenging activity were assessed in control and GABA enriched rice of *Jyothi, Njavara* and *Chitteni* (Table 15). The total phenol (TC) and total flavonoid (FC) were also analysed. The results are discussed below.

a) DPPH radical scavenging activity

The free radical scavenging activities of GABA enriched brown rice extracts and the control were assessed by DPPH assay. The free radical scavenging activity was determined by finding the value of IC_{50} (inhibition coefficient). The free radical, DPPH, was effectively scavenged by the control and GABA enriched rice extracts within the concentrations of 0 -100 µg/ml. Ascorbic acid, the positive control, had an IC_{50} value of 3.37 µg/ml. The cultivar *Njavara* showed the highest radical scavenging activity, followed by *Chitteni* and *Jyothi*. Among the rice varieties, both control and GABA enriched brown rice possessed low antioxidant activity by scavenging the free radical with the IC_{50} value of 133.86 µg/ml, 108.38 µg/ml, 128.92 µg/ml for control, and 121.01 µg/ml, 101.38 µg/ml, 119.14 µg/ml for GABA enriched rice of *Jyothi, Njavara*, and *Chitteni* respectively (Table 15).

Table 15. IC_{50} values for antioxidant activities of GABA enriched rice varieties of *Jyothi, Njavara* and *Chitteni*

Parameters	*Jyothi*		*Njavara*		*Chitteni*	
	C	T	C	T	C	T
DPPH radical scavenging activity (µg/ml)	133.86	121.01	108.38	101.38	128.92	119.14
Ferric reducing power activity (µg/ml)	59.50	53.81	51.99	47.30	55.15	52.15
Nitric oxide scavenging activity (µg/ml)	ND	ND	ND	ND	ND	ND
Superoxide scavenging activity (µg/ml)	93.37	88.34	65.51	61.19	85.10	79.38
Hydroxyl scavenging activity (µg/ml)	101.02	94.25	77.51	70.42	93.5	84.07

ND- Not detected

C-Control, T-GABA enriched rice

b) Ferric reducing antioxidant power activity (FRAP)

Antioxidant activity has been linked to reducing power, and the ferric reducing antioxidant power assay measures the reducing capacity of the test compounds. As a result, the antioxidant potential of methanol extracts of control and GABA enriched rice extract was calculated based on their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II). The reducing power of the control and GABA enriched rice extracts increased with the increase in concentration and showed ferric reducing ability within the range of 0–100 µg/ml (Table 15). The reducing power activity was measured with the IC_{50} values in a range of 59.50 µg/ml, 51.99 µg/ml, and 55.15 µg/ml for control and 53.81 µg/ml, 47.30 µg/ml, and 52.15 µg/ml for GABA enriched rice of *Jyothi, Njavara,* and *Chitteni*, consecutively. From the results of the present study, it was clear that *Njavara* and *Chitteni* showed relatively higher ferric ion reducing activity compared to the commonly used staple variety *Jyothi.*

c) Superoxide radical scavenging activity

The capacity of the methanolic extract to prevent superoxide from reducing nitro-blue tetrazolium (NBT), which is produced by the photo reduction of riboflavin, is the basis for superoxide radical scavenging activity. When compared with the control rice extract, GABA enriched rice extract was more effective at scavenging superoxide radicals created by the photo reduction of riboflavin. The extracts added dose-dependent at different concentrations (0 – 100 µg/ml) do not possess an effective scavenging activity. But moderate scavenging activity with the IC_{50} value of 93.37 µg/ml, 65.51µg/ml, 85.10 µg/ml for control samples and 88.34 µg/ml, 61.19 µg/ml, 79.38 µg/ml respectively, for GABA enriched rice samples of *Jyothi, Njavara* and *Chitteni* (Table 15) was observed.

d) Hydroxyl radical scavenging activity

The competition between deoxyribose and the test compounds for hydroxyl radicals generated by Fe^{3+}/ ascorbate/ EDTA/ H_2O_2 systems was studied to determine hydroxyl radical scavenging activity. The hydroxyl radical attacks deoxyribose, eventually forming thiobarbituric acid reaction chemicals (TBARS). The

effectiveness was shown to be dose-dependent at concentrations ranging from 0 to 100 g/ml. The hydroxyl radical scavenging activity was moderate in control and GABA enriched rice with the IC_{50} values of 101.02 µg/ml, 77.51µg/ml, 93.50 µg/ml for control samples, and 94.25 µg/ml, 70.42 µg/ml, 84.07 µg/ml for GABA enriched rice of *Jyothi, Njavara*, and *Chitteni* respectively. Table 15 represents the hydroxyl radical scavenging activity of the control and GABA enriched rice.

e) Total phenol and total flavonoid content

The total phenolic content of methanolic extract of GABA enriched rice was estimated using gallic acid as a standard, and results were expressed as equivalents of gallic acid in µg /g of rice (Table 16). The phenolic content noticed was 15.34 GAE µg/mg initially, which increased to 35.92 GAE µg/mg in GABA enriched *Jyothi*. In the *Njavara* cultivar, phenol content was initially 20.42 GAE µg/mg, while in GABA enriched rice, it was 42.50 GAE µg/mg. Similarly, the initial phenol content of *Chitteni* (17.06 GAE µg/mg), increased to 38.87 GAE µg/mg in GABA enriched rice. In terms of total phenol, a significant difference was observed between the control and GABA enriched rice of three cultivars: *Jyothi*, *Njavara,* and *Chitteni*.

The flavonoid content of the control and GABA enriched rice varieties were analysed and presented in Table 16. Quercetin was used as a standard for total flavonoid content assessment. The highest flavonoid content was recorded in the cultivar *Njavara*, followed by *Chitteni* and *Jyothi*. In the case of the *Jyothi* cultivar, the control (7.8 QE µg/mg) had inferior flavonoid content than the GABA enriched rice (13.8 QE µg/mg). The *Njavara* cultivar initially had a flavonoid content of 9.66 QE µg/mg and 17.5 QE µg/mg for GABA enriched rice.

Table 16. Total phenol and total flavonoid content of GABA enriched rice varieties of *Jyothi, Njavara* and *Chitteni*

*

Parameters	*Jyothi*			*Njavara*			*Chitteni*		
	C	T	t value	C	T	t value	C	T	t value
Total phenol content (GAE µg/mg)	15.34	35.92	14.78**	20.42	42.50	17.01**	17.06	38.87	26.16**
Total flavonoid content (QE µg/mg)	7.8	13.8	6.69**	9.66	17.5	7.98**	8.86	15.97	9.47**

Significant at 5 % level, ** Significant at 1% level, NS non significant

C- Control, T- GABA enriched rice

Similarly, the initial flavonoid content of *Chitteni* was 8.86 QE µg/mg and increased to 15.97 QE µg/mg in GABA enriched rice. With respect to total flavonoid content, there was a significant difference between the control and GABA enriched rice of three cultivars: *Jyothi, Njavara* and *Chitteni*.

4.3.2. Anti proliferative activities

Anti proliferative assay in a hepatic cancer cell line (HepG2) using MTT [3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide] assay was done in control and GABA enriched extract of *Jyothi, Njavara* and *Chitteni.* The results are reported in Figure 8, 9, and 10.

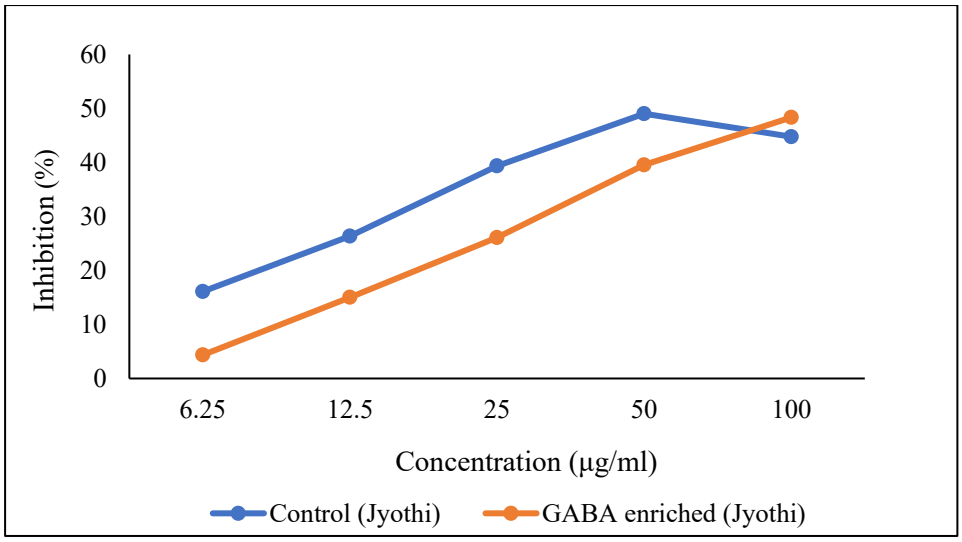


Figure 8. Antiproliferatory activity of the control and GABA enriched *Jyothi*

Antiproliferatory activity was separately studied in the control and GABA enriched rice extracts. Different doses of the control and GABA enriched rice extract (methanol), ranging from 6.25-100 µg/ml, were applied to the hepatic cancer (HepG2) cell lines. The cytotoxicity of the rice extracts was expressed as IC_{50}, which is the concentration causing a 50 per cent inhibition of cell proliferation.

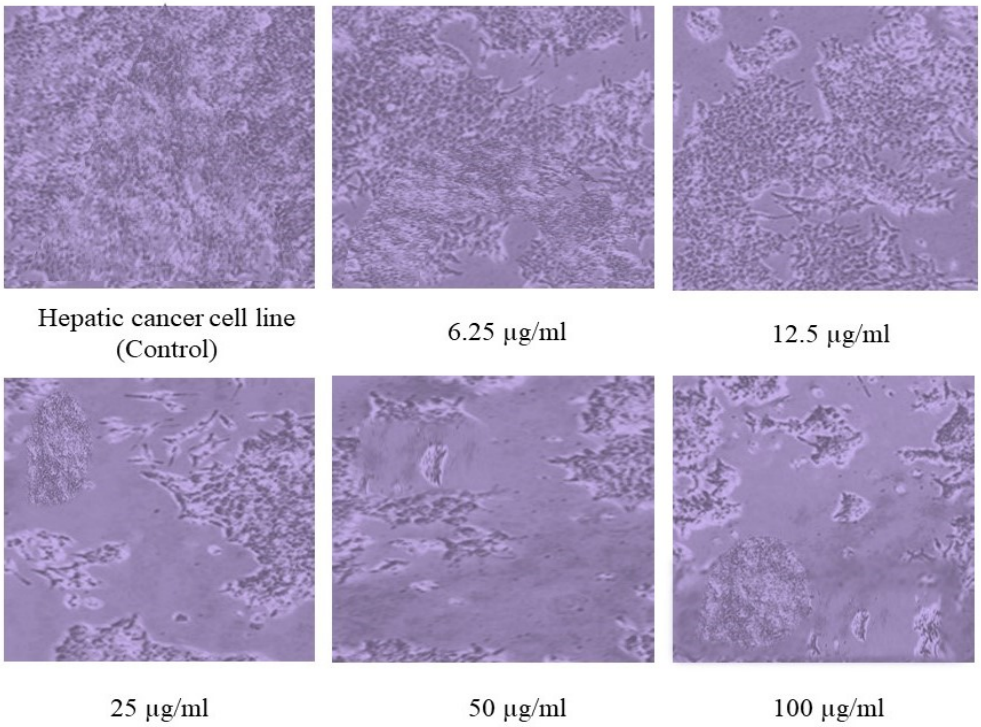


Plate 3. Cell death induced by *Jyothi* (control)

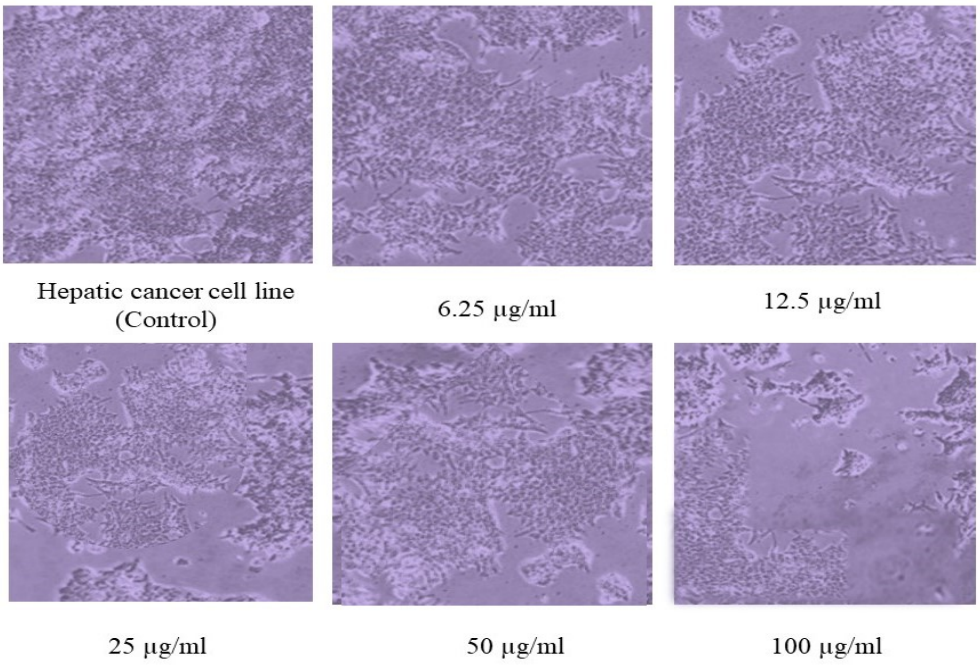


Plate 4. Cell death induced by GABA enriched *Jyothi*

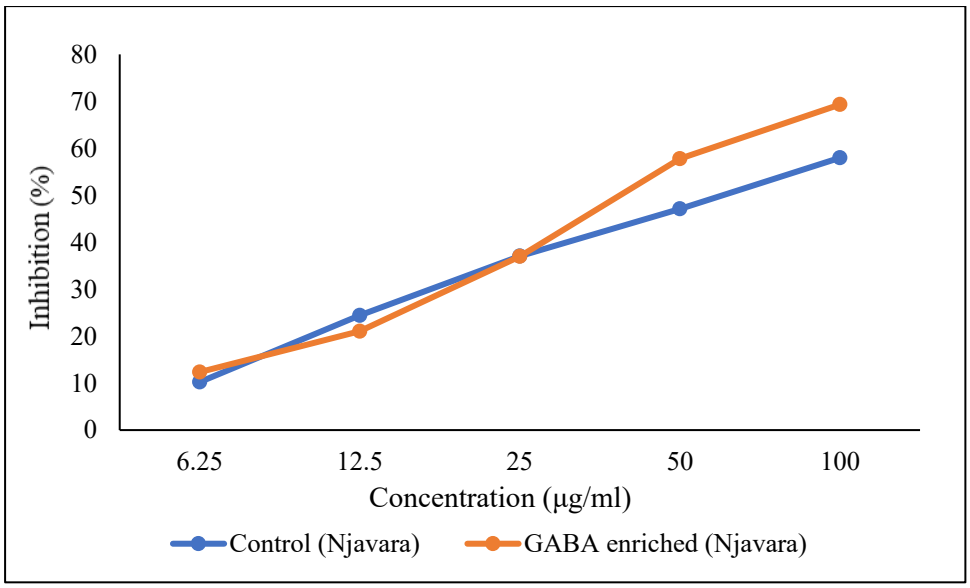


Figure 9.**Antiproliferatory activity of the control and GABA enriched *Njavara***

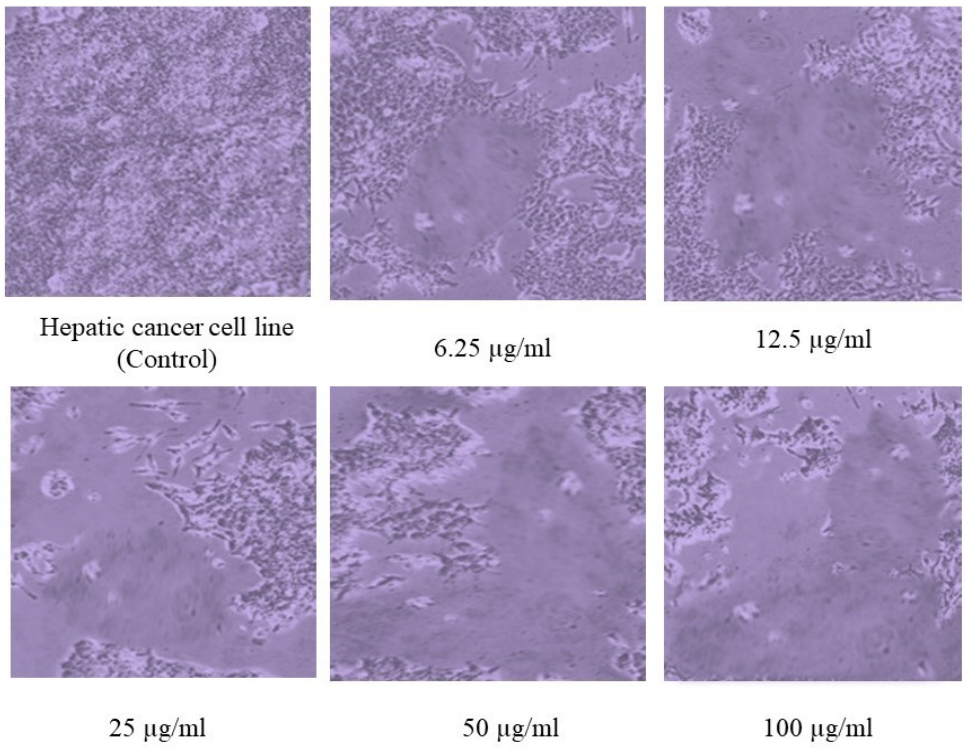


Plate 5. **Cell death induced by *Njavara* (control)**

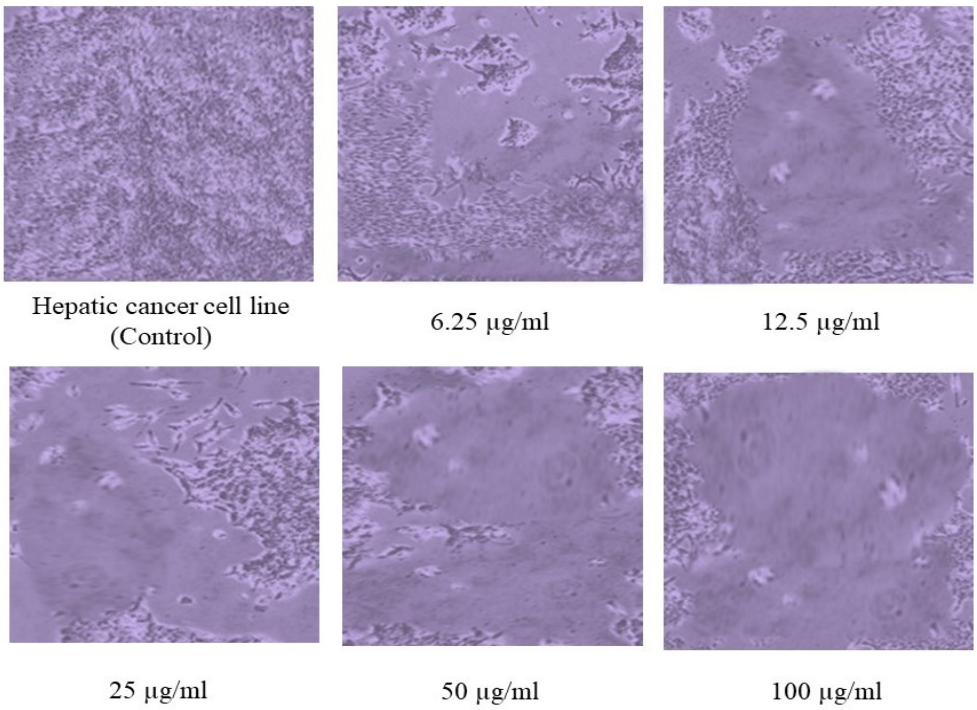


Plate 6. Cell death induced by GABA enriched *Njavara*

In the cytotoxicity analysis towards human hepatic cell line (HepG2), the inhibition was detected in both the control and GABA enriched rice extracts of *Jyothi* with an IC_{50} values of 95.01 µg/ml and 92.75 µg/ml respectively. From this assay, after the concentration of 100 ug/ml, a steady decline in cytotoxicity towards the human hepatic cell line (HepG2) was observed.

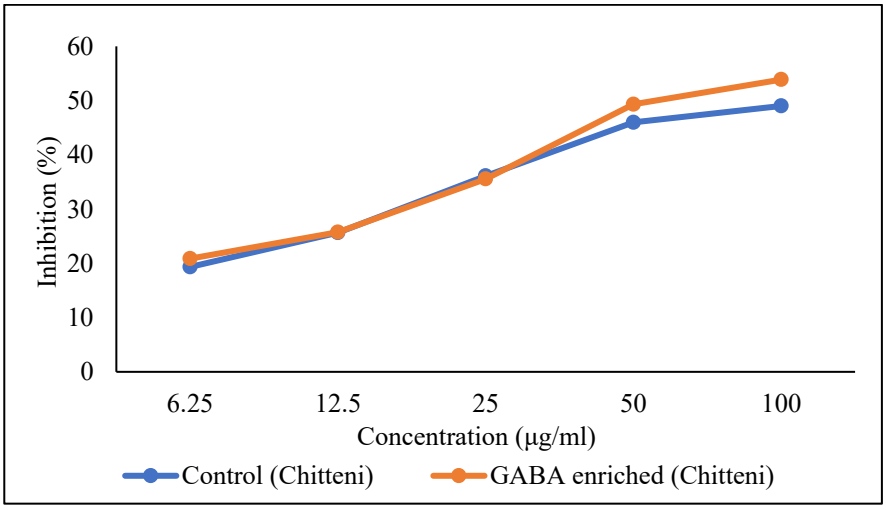


Figure 10. Antiproliferatory activity of the control and GABA enriched *Chitteni*

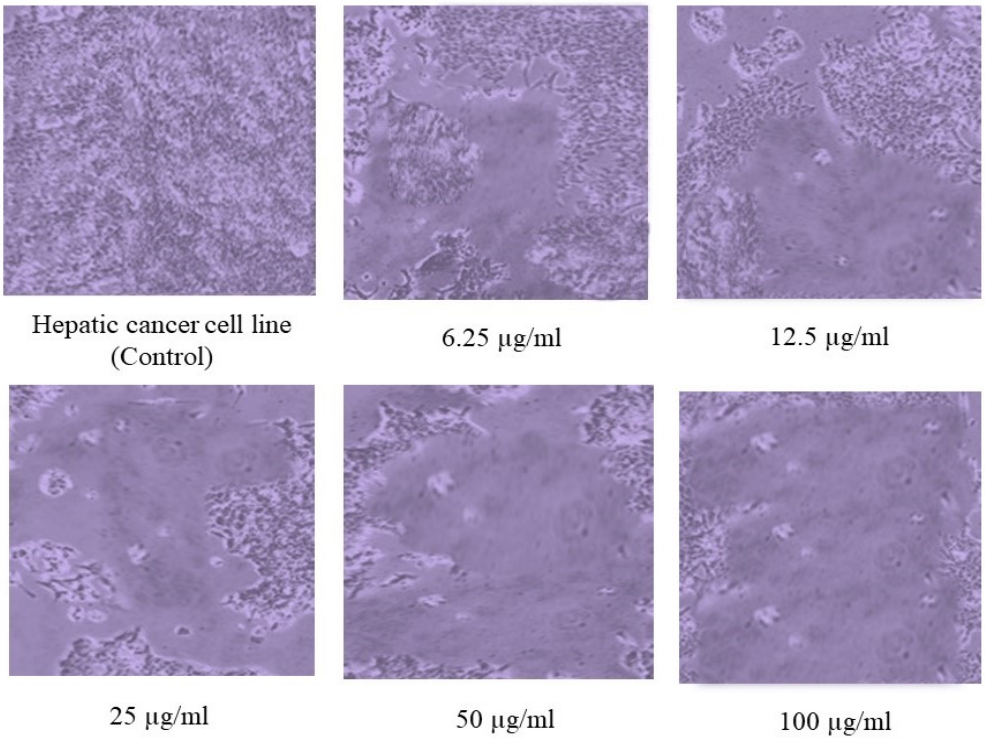


Plate 7. Cell death induced by *Chitteni* (control)

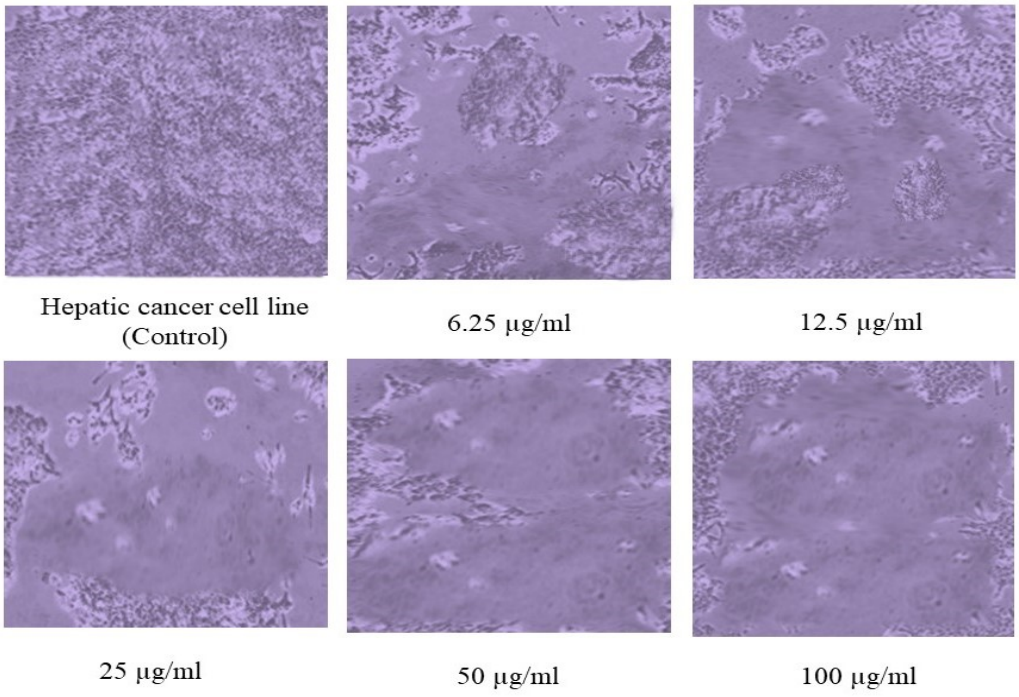
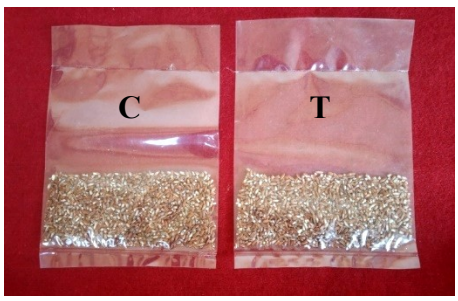


Plate 8. Cell death induced by GABA enriched *Chitteni*

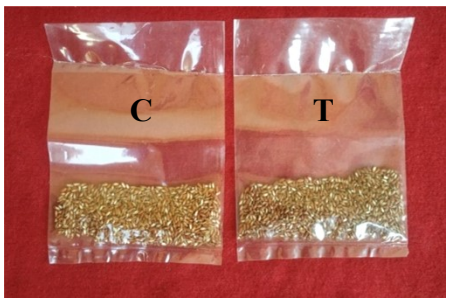
Likewise, in *Njavara*, an inhibition was detected with an IC_{50} value of 71.51 µg/ml, and 56.53 µg/ml in the control and GABA enriched rice of *Njavara.* Meanwhile, in *Chitteni*, the control and GABA enriched rice had inhibition with an IC_{50} value of 88.68 µg/ml and 76.10 µg/ml, consecutively. Antiproliferatory activity was comparatively higher for GABA enriched *Njavara, Chitteni* and *Jyothi* rice than the control. In this study, moderate antiproliferatory activity was observed against the hepatic cell line (HepG2). The control cancer cell line and the cell death induced by rice extract are depicted in Plates 3, 4, 5, 6, 7, and 8 respectively.

4.4. Storage qualities

Storage qualities of GABA enriched rice varieties were studied (Plate 9) and the results are presented below.



Jyothi



Njavara

Chitteni

Plate 9. Stored *Jyothi, Njavara* and *Chitteni*

4.4.1. Nutritional qualities of GABA enriched rice during storage

The GABA enriched rice of *Jyothi, Njavara* and *Chitteni* were evaluated for nutritional qualities like moisture, energy, carbohydrate, protein and fat initially and also at three and six month intervals of storage.

The moisture content of the control samples and GABA enriched samples were evaluated initially, third and six month after storage (Table 17). The results have showed that the moisture absorption in GABA enriched rice was high in all varieties when compared to the control rice varieties of *Jyothi, Njavara* and *Chitteni,* respectively. The increase in the moisture absorption of all rice varieties during storage was statistically significant.

Table 17. Moisture content of GABA enriched rice varieties of *Jyothi, Njavara* and *Chitteni* during storage

Rice varieties		Moisture (%)			
		Initial	3rd month	6th month	CD
Jyothi	C	11.81^{b}	12.24^{ab}	12.64^{a}	0.556
	T	13.53^{b}	14.92^{a}	15.29^{a}	0.505
	t value	6.59**	10.48**	14.65**	
Njavara	C	11.63^{c}	12.57^{b}	13.61^{a}	0.577
	T	13.19^{c}	14.06^{b}	15.03^{a}	0.366
	t value	9.06**	8.60**	5.65**	
Chitteni	C	11.83^{c}	12.65^{b}	13.71^{a}	0.380
	T	13.58^{c}	14.34^{b}	15.65^{a}	0.421
	t value	11.09**	8.22**	11.79**	

** Significant at 1% level, C- Control, T- GABA enriched rice

DMRT row wise comparison

In the case of *Jyothi,* the moisture content on the initial day of storage was recorded as 11.81 per cent, which increased to 12.64 per cent by the end of 6 months after storage. Likewise, the moisture content GABA enriched *Jyothi* rice was 13.53 per cent at the beginning of storage and at the end, it became 15.29 per cent. A

significant increase was observed in the moisture content of GABA enriched rice varieties after 6 months of storage compared to its initial moisture content in all three rice varieties.

In the case of *Njavara*, the moisture content of control was 11.63 per cent, and that of GABA enriched *Njavara* rice was 13.19 per cent. The moisture content increased to 13.61 per cent and 15.03 per cent after six months of storage for the control and GABA enriched *Njavara* respectively. The increase of moisture in each evaluation period was statistically significant.

Moisture content of the control and GABA enriched *Chitteni* were also evaluated. The moisture content increased throughout the storage period. The moisture content was 11.83 per cent and 13.58 per cent initially, and at the end of the sixth month, it was 13.71 per cent and 15.65 per cent respectively, for the control and GABA enriched *Chitteni*. There was a significant increase in the moisture content during the storage period.

Table 18. Energy content of GABA enriched rice varieties of *Jyothi, Njavara* and *Chitteni* during storage

Rice varieties		Energy (Kcal/100g)			
		Initial	3rd month	6th month	CD
Jyothi	C	333.35^{a}	321.22^{b}	306.47^{c}	4.57
	T	321.01^{a}	311.00^{b}	296.90^{c}	2.74
	t value	6.53**	6.04**	6.90**	
Njavara	C	333.5^{a}	322.17^{b}	320.83^{b}	9.13
	T	324.15^{a}	309.25^{b}	298.09^{c}	6.12
	t value	2.00^{NS}	5.77**	7.85**	
Chitteni	C	333.19^{a}	317.99^{b}	308.63^{c}	6.73
	T	323.69^{a}	310.94^{b}	298.16^{c}	6.13
	t value	3.19*	2.90*	4.53**	

* Significant at 5 % level, ** Significant at 1% level, NS-non significant

DMRT row wise comparison, C- Control, T- GABA enriched rice

The energy of all the rice varieties was calculated on the initial day, and the variations in the values throughout the storage were also calculated (Table 18). The energy value depend on its carbohydrate, fat and protein values. The results have shown that there was a significant decrease in the energy value of the rice varieties as the storage period increased. A significant difference is observed between the mean values of control and their GABA enriched counterparts initially and after 3 months and 6 months of storage. The energy value changes throughout each variety's storage period and its GABA enriched counterparts were statistically different.

In the rice variety *Jyothi,* the energy value was 333.35 Kcal during the initial days, which decreased to 306.47 Kcal by the end of the storage period for the control rice sample. The GABA enriched *Jyothi* had an energy value of 321.01 Kcal initially and by the end of 6 months after storage, the energy value became 296.90 Kcal. Significant differences existed in the mean energy values between the control and GABA enriched rice throughout the storage period.

In the *Njavara* cultivar, the control and GABA enriched rice initially had an energy value of 333.50 Kcal and 324.15 Kcal, respectively. The value reached to 320.83 Kcal, and 298.09 Kcal for the control and GABA enriched rice of *Njavara* by the end of the sixth month of storage. There was a significant difference between the energy values of the control and GABA enriched *Njavara*.

The energy value of the control and GABA enriched *Chitteni* decreased from 333.19 Kcal to 308.63 Kcal, and 323.69 Kcal to 298.16 Kcal respectively, by the end of six months of storage.

The data on carbohydrate content of the control and GABA enriched *Jyothi, Njavara* and *Chitteni* showed that, the carbohydrate content of all rice varieties decreased with increase in storage period. In *Jyothi*, the carbohydrate content of the control was 69.14 per cent initially and 65.32 per cent after six months of storage. The GABA enriched *Jyothi* rice had a carbohydrate content of 63.23 per cent initially and 59.46 per cent by the end of six months. A significant difference in the carbohydrate

content between the control and GABA enriched *Jyothi* throughout the storage period of six months was observed.

In the case of *Njavara*, the carbohydrate content ranged from 66.02 per cent to 64.09 per cent in control with the increase in storage period, while in the case of GABA enriched *Njavara*, the content ranged from 60.43 per cent to 57.62 per cent during storage. There was a significant difference in the carbohydrate content between the control and GABA enriched *Njavara*.

Table 19. Carbohydrate content of GABA enriched rice varieties of *Jyothi, Njavara* and *Chitteni* during storage

Rice varieties		Carbohydrate (%)			
		Initial	3rd month	6th month	CD
Jyothi	C	69.14[a]	67.63[b]	65.32[c]	0.807
	T	63.23[a]	61.95[b]	59.46[c]	0.684
	t value	14.26**	19.92**	21.43**	
Njavara	C	66.02	65.17	64.09	NS
	T	60.43[a]	58.79[b]	57.62[b]	1.511
	t value	4.43**	28.41**	38.24**	
Chitteni	C	67.80[a]	66.01[b]	65.22[b]	1.149
	T	61.16[a]	59.24[b]	57.39[c]	0.543
	t value	10.46**	40.77**	37.62**	

** Significant at 1% level, NS - non significant

DMRT row wise comparison, C- Control, T- GABA enriched rice

Chitteni rice variety had a carbohydrate content of 67.80 per cent initially which fell to 65.22 per cent by the end of the six-month storage period. The GABA enriched *Chitteni* had a carbohydrate content of 61.16 per cent initially, which decreased to, 59.24 per cent after a storage of three months and to 57.39 per cent after six months of storage, and the observed decrease was statistically significant.

Protein content in the rice varieties is tabulated in Table 20. The protein content of the control and GABA enriched rice was compared, and there was a significant difference between them in the case of all three rice varieties.

In *Jyothi,* the control and GABA enriched rice had a protein content of 10.62 per cent and 12.43 per cent initially, and the values decreased to 8.95 per cent and 10.62 per cent by the end of six months of storage. There was a significant difference between the control and GABA enriched counterparts during the storage period.

Table 20. Protein content of the GABA enriched rice varieties of *Jyothi, Njavara* and *Chitteni* during storage

Rice varieties		Protein (%)			
		Initial	3rd month	6th month	CD
Jyothi	C	10.62[a]	9.83[b]	8.95[c]	0.428
	T	12.43[a]	11.41[b]	10.62[c]	0.360
	t value	14.97 **	7.82**	8.76**	
Njavara	C	12.90[a]	11.72[b]	10.86[c]	0.391
	T	14.28[a]	12.59[b]	11.93[c]	0.632
	t value	6.01**	4.15 NS	4.74*	
Chitteni	C	11.56[a]	10.13[b]	9.26[c]	0.335
	T	13.01[a]	12.02[b]	11.09[c]	0.444
	t value	7.21**	10.51**	13.68**	

** Significant at 1% level, NS- non significant

DMRT row wise comparison, C – Control, T- GABA enriched rice

The medicinal rice variety *Njavara* had the highest protein content. The initial content was 12.90 per cent and 14.28 per cent for control and GABA enriched rice, respectively. The value decreased drastically as the storage period increased. Statistically, the increase in protein values in GABA enriched *Njavara* was significant. The decrease was observed in control and GABA enriched rice throughout the storage period.

In *Chitteni,* the protein content for control was 11.56 per cent, 10.13 per cent, and 9.26 per cent initially, three months and six months after storage. The protein content of the GABA enriched rice was 13.01 per cent initially, which changed to 12.02 per cent after three months of storage and to 11.09 per cent after six months of storage. In *Chitteni*, it was observed that, the increase in protein content was statistically significant.

Table 21. Fat content of GABA enriched rice varieties of *Jyothi, Njavara* and *Chitteni* during storage

Rice varieties		Fat (%)			
		Initial	3rd month	6th month	CD
Jyothi	C	1.59[a]	1.25[b]	1.03[b]	0. 243
	T	2.04	1.97	1.83	NS
	t value	4.99**	4.58**	8.82**	
Njavara	C	1.98	1.62	1.43	NS
	T	2.81	2.63	2.31	NS
	t value	6.09**	4.00**	4.43**	
Chitteni	C	1.75[a]	1.48[ab]	1.18[b]	0.360
	T	3	2.87	2.69	NS
	t value	17.07**	5.96**	7.99**	

** Significant at 1% level, NS - non significant

DMRT row wise comparison,C- Control, T- GABA enriched rice

The changes in the fat content during storage of the control and GABA enriched rice of *Jyothi, Njavara* and *Chitteni* and are furnished in Table 21. There were no significant changes in the fat content of the rice varieties with the advancement of the duration of the storage. Statistically significant difference between the control and GABA enriched rice varieties can be observed.

The fat content in *Jyothi* was 1.59 per cent, which decreased to 1.03 per cent by the end of six months of storage, while in the GABA enriched rice, the initial value

of 2.04 per cent, which decreased to 1.83 per cent by the end of six months of storage. There was a significant difference in the fat content of the control and GABA enriched rice throughout the storage period.

In the rice variety *Njavara*, the fat content was 1.98 per cent initially and at the end of the storage period, it decreased to 1.43 per cent. In the case of GABA enriched *Njavara,* the fat content changed from 2.81 per cent to 2.31 per cent within six months of the storage period. There was a significant difference in the mean values between the control and its GABA enriched rice, and with an increase in storage period, a significant difference was observed in both control and GABA enriched *Njavara.*

In *Chitteni* also, the control and GABA enriched rice showed a decrease in the fat content. The fat content of *Chitteni* was 1.75 per cent initially which changed to 1.18 per cent by the end of the six months of storage. In the case of GABA enriched *Chitteni*, the fat content varied from 3 per cent to 2.69 per cent during six months of storage. The mean values of the control and GABA enriched rice varied statistically throughout the storage period.

4.4.2. Organoleptic qualities of GABA enriched rice during storage

The control and GABA enriched rice of *Jyothi, Njavara* and *Chitteni* was evaluated for organoleptic qualities like appearance, colour, flavour, taste, texture, and overall acceptability at initial, three ans six months after storage (Appendix VIII). The mean values for each parameter for each rice were recorded and compared to understand the changes in sensory qualities in both control and GABA enriched rice. The statistical analysis was carried out with the help of t test and Duncan multiple range test.

Rice grains were stored for six months and the organoleptic evaluation was done before storage, and in the third month and sixth month after storage using nine point hedonic scale among fifteen judges. The mean values of the scores were calculated and compared (Table 22).

The organoleptic mean scores decreased as the storage period increased. The GABA enriched rice had better scores than the control in all three varieties. According to t-test there was a significant difference between the control and GABA enriched rice initially, and at the third and sixth month after storage. However, no significant difference was observed either in control and GABA enriched rice with advancement of storage period.

Table 22. Organoleptic qualities of the cooked GABA enriched rice of *Jyothi, Njavara and Chitteni* during storage

Rice varieties		Average mean score			
		Initial	3rd month	6th month	CD
Jyothi	C	7.48	7.29	7.06	NS
	T	8.36	8.17	7.95	NS
	t value	4.81*	4.67*	5.09*	
Njavara	C	7.47	7.23	7.03	NS
	T	8.16	8.00	7.83	NS
	t value	5.46*	5.15*	4.67*	
Chitteni	C	7.56	7.35	7.10	NS
	T	8.03	7.86	7.59	NS
	t value	3.85*	4.57*	3.00*	

* Significant at 5 % level, NS - non significant

DMRT row wise comparison, C- Control, T- GABA enriched rice

In the case of *Jyothi*, the mean score obtained for the control was 7.48, which decreased to 7.06 by the end of six months of storage. Whereas in the case of GABA enriched *Jyothi* rice, it was 8.36 at the beginning of storage which decreased to 7.95.

The control and GABA enriched *Njavara* were also evaluated. It was found that the GABA enriched rice had better scores than the control in the other two varieties. The mean scores initially recorded were 7.47 and 8.16 respectively, for the control and GABA enriched *Njavara*. The scores decreased as the storage period increased and became 7.03 and 7.83 by the end of six months of storage.

The *Chitteni* and its GABA enriched rice were also evaluated for 6 months. Initially, the score was 7.56 and 8.03 for *Chitteni* rice and its GABA enriched rice, respectively. Gradually the mean scores decreased to 7.35 and 7.86 after the third month of storage and 7.10 and 7.59 after six months of storage.

4.4.3. Enumeration of microbial population in GABA enriched rice during storage

The microbial count of the control and the GABA enriched rice were evaluated for six months (Table 23). Bacteria were initially detected in all three rice varieties, which increased during storage. Fungal infection was detected only after six months of storage. The yeast infection varied for control and GABA enriched rice.

In the case of *Jyothi* rice, the bacterial count was 0.50 x 10^5 cfu/g initially, which increased to 2 x 10^5cfu/g by the end of six months. In GABA enriched rice, the bacterial count was 0.67 x10^5 cfu/g initially, which increased to 2.40 x 10^5 cfu/g by the end of six months of storage.

In *Njavara* rice variety, initially the bacterial count detected was 0.46 x 10^5 cfu/g, which increased to 2.12 x 10^5 cfu/g by the end of six months of storage. Whereas in GABA enriched *Njavara* rice variety, initially, the bacterial count detected was 0.70 x 10^5 cfu/g which increased to 2.35 x 10^5 cfu/g by the end of six months of storage.

In *Chitteni* rice, initially the bacterial count was 0.40 x 10^5 cfu/g and 0.66 x 10^5 cfu/g in control and its GABA enriched rice respectively. The count increased to 2.10 x 10^5 cfu/g and 2.32 x 10^5 cfu/g by the end of six months of storage.

Fungal infection was more in the GABA enriched rice than in the control. Fungal colonies were not detected initially and after three months of storage. By the end of six months of storage, the emergence of fungal colonies was evident. After six months of storage, in *Jyothi* rice, 1 x 10^3 cfu/g of fungal colonies were detected whereas, for the GABA enriched rice, 1.70 x 10^3 cfu/g were detected. In the case of *Njavara*, 1 x 10^3 cfu/g, and 1.30 x 10^3 cfu/g fungal colonies were detected in the

control and GABA enriched rice, respectively. In *Chitteni* rice, 1 x 10^3 cfu/g and in GABA enriched *Chitteni* rice 1.70 x 10^3 cfu/g of fungal colonies were detected.

Yeast counts were also examined in the rice varieties and their GABA enriched counterparts throughout the storage period. The yeast colonies were not detected initially in any of the rice varieties. After three and six months of storage, GABA enriched *Jyothi* rice and GABA enriched *Chitteni* rice showed yeast colonies.

4.4.4. Insect infestation

Observations on the presence of storage pests and insects were taken in control, and GABA enriched rice initially, at the end of third and sixth month of storage. No storage pests were observed in control, and GABA enriched rice of *Jyothi, Njavara* and *Chitteni* cultivars. Insect infestation was not seen in control and GABA enriched rice throughout the storage period.

Table 23. Microbial population in GABA enriched rice during storage

Rice varieties		Microbial population (cfu/g)								
		Initial			3rd month			6th month		
		Bacteria ($x10^5$)	Fungi ($x10^3$)	Yeast ($x10^3$)	Bacteria ($x10^5$)	Fungi ($x10^3$)	Yeast ($x10^3$)	Bacteria ($x10^5$)	Fungi ($x10^3$)	Yeast ($x10^3$)
Jyothi	C	0.50 (4.69)	ND	ND	1.03 (5.01)	ND	ND	2.00 (5.30)	1.00 (3.00)	ND
	T	0.67 (4.82)	ND	ND	1.53 (5.18)	ND	1.2 (3.07)	2.40 (5.38)	1.70 (3.23)	1.30 (3.11)
Njavara	C	0.46 (4.66)	ND	ND	1.00 (5.00)	ND	ND	2.12 (5.32)	1.00 (3.00)	ND
	T	0.70 (4.84)	ND	ND	1.70 (5.23)	ND	ND	2.35 (5.37)	1.30 (3.11)	ND
Chitteni	C	0.40 (4.60)	ND	ND	1.00 (5.00)	ND	ND	2.10 (5.32)	1.00 (3.00)	ND
	T	0.66 (4.81)	ND	ND	1.65 (5.21)	ND	1.4 (3.14)	2.32 (5.36)	1.70 (3.23)	1.60 (3.20)

Values in the parenthesis are the log value, ND- Not detected, C -Control, T- GABA enriched rice

4.5. Processing GABA enriched rice for preparation of rice products

The rice varieties with maximum GABA content and medicinal properties were subjected to the following processing conditions: cooking, pulverising, flaking, and puffing as presented in Plate 10. The most suitable processing method for each variety was determined based on organoleptic qualities, GABA content, and antioxidant activity.

4.5.1. Organoleptic evaluation of GABA enriched rice products

The processed products from GABA enriched rice of *Jyothi, Njavara*, and *Chitteni* were evaluated organoleptically using scorecard for different quality attributes like appearance, colour, flavour, texture, taste, and overall acceptability. And the organoleptic qualities of pulverised GABA rice were evaluated by preparing porridge. Each processed product was ranked for all quality attributes based on their mean rank scores using Kendall's coefficient (W) test, which is presented in Appendix XI.

Table 24. Average mean score of organoleptic qualities of processed products from GABA enriched rice of *Jyothi, Njavara* and *Chitteni*

Parameters	GABA enriched *Jyothi*	GABA enriched *Njavara*	GABA enriched *Chitteni*	CD
	Average mean score			
Cooked	8.36	8.17	8.03	NS
Pulverised	7.99	7.88	7.75	NS
Flaked	8.01	8.02	7.85	NS
Puffed	8.07	8.1	7.82	NS

NS -non significant

DMRT row wise comparison

Plate 10. (a) Cooked, (b) pulverised, (c) flaked and (d) puffed rice prepared from GABA enriched *Jyothi, Njavara* and *Chitteni* cultivars

After cooking, GABA enriched *Jyothi* received the highest total mean score of 8.36, followed by GABA enriched *Njavara* (8.17) and GABA enriched *Chitteni* (8.03). Likewise, after pulverising, GABA enriched *Jyothi*, obtained the highest mean score of 7.99, followed by GABA enriched *Njavara* (7.88) and GABA enriched *Chitteni* (7.75). Among the three rice varieties, flakes made from GABA enriched *Njavara* received higher mean scores that descended dramatically to 8.01 and 7.85 in GABA enriched *Jyothi* and GABA enriched *Chitteni,* respectively. The puffed rice prepared from GABA enriched *Njavara* had the highest mean score (8.1) in comparison with GABA enriched *Jyothi* (8.07) and GABA enriched *Chitteni* (7.82), and there was no significant difference in organoleptic qualities between the processed products of GABA enriched rice from three cultivars, namely *Jyothi, Njavara*, and *Chitteni.*

4.5.2. Quantification of GABA content in processed rice products

The GABA content of the prepared processed products from *Jyothi, Njavara* and *Chitteni*, cultivars was estimated using the standard procedure reported in 3.2.2. and is presented in Table 25.

Table 25. The GABA content of processed products of *Jyothi, Njavara* and *Chitteni*

Parameters	GABA enriched *Jyothi*	GABA enriched *Njavara*	GABA enriched *Chitteni*	CD
	GABA content (mg/kg)			
Cooked	84.34[c]	95.18[a]	92.72[b]	2.015
Pulverised	109.72[b]	125.04[a]	124.97[a]	2.040
Flaked	98.23[c]	117.85[a]	111.68[b]	2.285
Puffed	95.09[c]	112.39[a]	108.42[b]	2.132

DMRT row wise comparison

The comparison of GABA content of processed products showed that higher GABA content was retained after pulverising, followed by flaking, puffing and cooking in the descending order. In the case of cooking, the GABA enriched *Njavara* obtained the highest GABA content of 95.18 mg/kg, followed by GABA enriched *Chitteni* (92.72 mg/kg) and GABA enriched *Jyothi* (84.34 mg/kg). After pulverising, maximum GABA was retained in GABA enriched *Njavara*, followed by GABA enriched *Chitteni* and GABA enriched *Jyothi* as 125.04 mg/kg, 124.97 mg/kg and 109.72 mg/kg, respectively. After flaking, GABA enriched *Njavara,* had the maximum GABA content (117.85 mg/kg), followed by GABA enriched *Chitteni* (111.68 mg/kg) and GABA enriched *Jyothi* (98.23 mg/kg) consecutively. A gradual increase in GABA was found after puffing in GABA enriched *Jyothi* (95.09 mg/kg), GABA enriched *Chitteni* (108.42 mg/kg) and GABA enriched *Njavara* (112.3 mg/kg). The variation in GABA content of the processed products was found to be statistically significant in all three rice varieties.

4.5.3. Total antioxidant activity of GABA enriched rice products

The rice extracts of cooked, pulverised, flaked and puffed rice showed total antioxidant activity effectively by scavenging free radicals (Table 26).

The total antioxidant activity of the rice extracts of processed products was directly proportional to the concentration of extracts. The methanolic extracts of processed products from GABA enriched rice of *Jyothi, Njavara* and *Chitteni,* showed significant change in the absorbance value from 0 to 10 µg/ml.

Even though all the processed products have shown an increase in the total antioxidant activity with increasing concentration, in the case of cooking, the rice cultivar *Njavara*, showed maximum antioxidant activity with the projected EC_{50} value of 6.98 µg/ml followed by GABA enriched *Jyothi* (EC_{50}= 7.62 µg/ml) and GABA enriched *Chitteni* (EC_{50}= 9.50 µg/ml) and was found to be statistically significant. The highest antioxidant activity was found after pulverising and was 5.58 µg/ml, 5.82 µg/ml, and 8.16 µg/ml in GABA enriched *Njavara*, GABA enriched *Jyothi,* and GABA enriched *Chitteni,* respectively. After flaking, the highest antioxidant activity was obtained for GABA enriched *Njavara* with the projected EC_{50} of 6.02 µg/ml,

followed by GABA enriched *Jyothi* (EC_{50}= 6.67 µg/ml) and GABA enriched *Chitteni* (EC_{50}= 8.64 µg/ml) consecutively. In the case of puffing, maximum antioxidant activity obtained was 6.53 µg/ml, 6.91 µg/ml, and 8.98 µg/ml for GABA enriched *Njavara*, GABA enriched *Jyothi* and GABA enriched *Chitteni* and found to be statistically significant at 5 % level.

Table 26. The total antioxidant activity of processed products of *Jyothi, Njavara* and *Chitteni*

Parameters	**GABA enriched *Jyothi***	**GABA enriched *Njavara***	**GABA enriched *Chitteni***	**CD**
	Total antioxidant activity EC_{50} value (µg/ml)			
Cooked	7.62[b]	6.98[b]	9.50[a]	1.454
Pulverised	5.82[b]	5.58[b]	8.16[a]	1.231
Flaked	6.67[b]	6.02[b]	8.64[a]	1.073
Puffed	6.91[b]	6.53[b]	8.98[a]	1.735

DMRT row wise comparison

In general, it was observed that, there was a slight decrease in the organoleptic qualities, GABA content, and total antioxidant activity in the processed products of GABA enriched rice, but highest retention (more than 72%) was observed even after processing.

DISCUSSION

5. DISCUSSION

The results of the study entitled 'Medicinal properties and process optimisation for GABA enrichment in rice' are discussed in this chapter under the following major heads.

5.1. Optimisation of conditions for GABA enrichment

5.1.1. Quantification of GABA content

5.1.2. Total antioxidant activity

5.1.3. Organoleptic qualities of table rice

5.1.4. Parboiling of rice varieties

5.2. Quality evaluation of GABA enriched rice varieties

5.2.1. Chemical and nutritional qualities

5.2.2. Organoleptic qualities

5.3. Assessment of antioxidant and antiproliferative properties of GABA enriched rice

5.3.1. Antioxidant properties

5.3.2. Antiproliferatory activities

5.4. Storage qualities

5.4.1. Nutritional qualities

5.4.2. Organoleptic qualities

5.4.3. Enumeration of microbial population

5.4.4. Insect infestation

5.5. Processing GABA enriched rice for preparation of rice products

5.5.1. Organoleptic evaluation of GABA enriched rice products

5.5.2. Quantification of GABA content

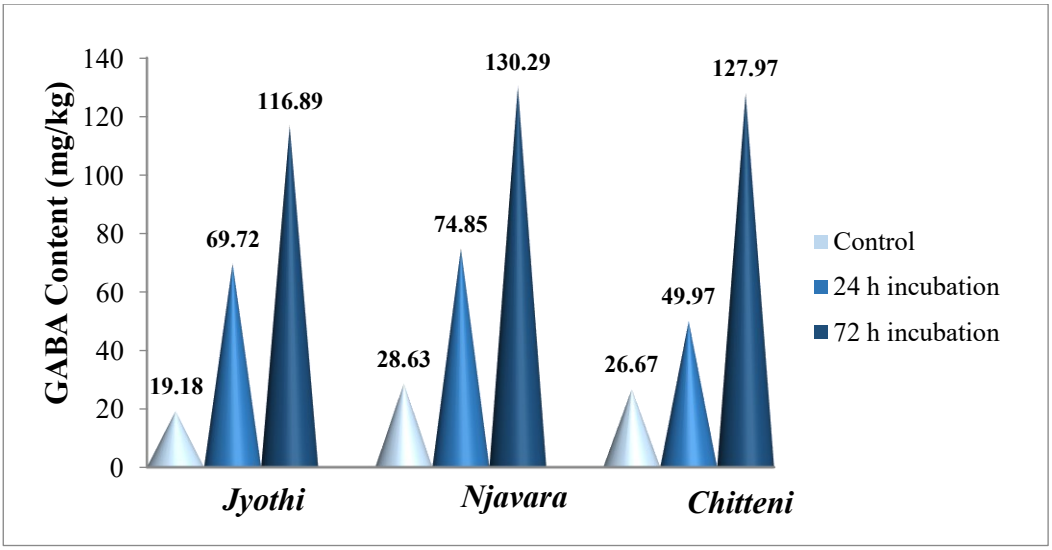
5.5.3. Total antioxidant activity of GABA enriched rice products

5.1. Optimisation of conditions for GABA enrichment

5.1.1. Quantification of GABA content

Meanwhile, Hayat *et al.* (2014) discovered that GABA derivatives showed good separation at a retention time of 7.925 minutes, whereas *Jyothi, Njavara*, and *Chitteni* obtained maximum GABA response at a retention period of 7.65 minutes in

the current study. But, Hayat *et al.* (2015) and Liu *et al.* (2015) indicated a maximal GABA response at 5.255 and 6.4 minutes. Hayat *et al.* (2015, 2014) measured the highest response of GABA at 230 nm and 254 nm wavelengths. Whereas in this study, the maximum response of GABA was noticed at 230 nm which may be due to the changes in method of analysis, the mobile and stationary phase used *etc* (Thomas *et al*., 2023).



24 h incubation – (24 h soaking and 24 h germination), 72 h incubation – (72 h soaking and 72 h germination)

Figure 11. GABA content in different soaking and germination durations

In the three rice cultivars, in the control (0h soaking and 0h germination) samples of *Jyothi, Njavara* and *Chitteni* had mean GABA levels of 19.18 mg/kg, 28.63 mg/kg, and 26.67 mg/kg consecutively (Thomas *et al*., 2023) (Figure 11). The GABA content in the Indian rice cultivars chosen for our study (*Jyothi, Njavara, Chitteni*) is better than the Taiwan rice varieties (Ng *et al.*, 2013; Thomas *et al*., 2023), and comparable with Thai rice varieties (116.9 mg/kg) (Varanyanond *et al*., 2005). The GABA content observed in the rice cultivars of the present study was lower than Korean rice varieties (2.684 mg/kg) (Kim *et al*., 2012) and much higher than Pakistan basmati rice varieties (0.524 mg/kg) (Hayat *et al*., 2014). Also found that GABA content varies with cultivar and location (Ng *et al*., 2013; Sitanggang *et al*., 2021; Thomas *et al*., 2023).

In the current study, the GABA content in the rice cultivar *Jyothi, Njavara* and *Chitteni* increased by 109.40 per cent, 74.07 per cent and 156.09 per cent after 72 hours of soaking and germination. The soaking and germination process considerably enriched the GABA content in rice cultivars of *Jyothi, Njavara* and *Chitteni* (Thomas *et al*., 2023). As per Saikusa *et al.* (1994) and Thomas *et al*. (2023), the surface of rice germ has glutamic acid in high concentration that was quickly metabolised into GABA in sprouting, which could be the reason for the elevation in GABA content subsequent to sprouting.

Based on the findings of the current study, a comparison of the cultivars revealed that the *Njavara* cultivar exhibited the highest average GABA content (98.95 mg/kg), followed by *Chitteni* (90.73 mg/kg) and *Jyothi* (81.84 mg/kg) (Thomas *et al*., 2023). The deposition of GABA in rice grain differed according to the cultivar (Saikusa *et al*., 1994; Varanyanond *et al*., 2005; Jannoey *et al*., 2010; Ng *et al*., 2013; Hayat *et al*., 2014). As per Sreejayan and Thomas (2003) and Thomas *et al*. (2023), *Njavara* exhibits significant genetic diversity and is characterised by a substantial protein content, which is positively correlated with its elevated concentration of gamma aminobutyric acid (GABA).

Nevertheless, there were changes in the response of different rice varieties to the durations of soaking and germination. Specifically, when soaked for 24 hours and germinated for 72 hours, the GABA content of *Chitteni* was found to be comparable to that of *Njavara* (Thomas *et al.*, 2023). It should be noted that the germinated rice varieties of Pakistan *Basmati* and IRRI possessed a GABA content in ranged from 115 to 935 mg/kg, as reported by Hayat *et al.* (2015) and Thomas *et al.* (2023). According to Thomas *et al.* (2023), the GABA content of *Njavara* after 72 hours of soaking and germination was found to be 130.29 mg/kg. This was seen to be greater compared to the GABA content of rice cultivars of Thailand, *La Sor Dang* (1.365 mg/kg) and *Chai Nat 1* (1.545 mg/kg) after underwent 24 hours of soaking and germination (Karladee and Suriyong, 2012). Kim *et al*. (2012) and Thomas *et al*. (2023) stated the GABA content after 72 hours of germination of the the Korean variety, *Ilpumbyeo* rough rice was 3.179 mg/kg, greater than the early reported

findings of the current study. After reviewing the GABA content in GBR, Cho and Lim (2016) and Thomas *et al.* (2023) found that, depending on the soaking time, germination time and variety, the GABA level ranged from 0.2 mg/kg to 18.1 mg/kg.

The accumulation of GABA increased significantly as the soaking time increased. As per Thomas *et al.* (2023), a higher amount of GABA was observed after 72 h of soaking time (98.63 mg/kg), followed by 48 h (88.95 mg/kg) and 24 h (83.95 mg/kg) of soaking time. This hike in GABA during soaking can be attributed to glutamate decarboxylase (GAD) activation, an enzyme that converts L- glutamic acid into carbon dioxide (CO_2) and GABA (Thomas *et al*., 2023). As a result, glutamic acid levels decrease (Komatsuzaki *et al*., 2007; Thomas *et al*., 2023). Similar findings were reported by Kaosa-Ard and Songsermpong (2012), You-Tung *et al.* (2015) and Thomas *et al.* (2023) who also stated an in elevation in GABA content with the longer soaking periods. Additionally, Maisont and Narkrugsa (2010) and Thomas *et al.* (2023) stated that soaking rice with a giant embryos in water at 30ºC for 24 hours led to an elevation in GABA content. In a study conducted by Ohtsubo *et al.* (2005) and Thomas *et al.* (2023), it was observed that immersing *Koshihikari* (Japanese) rice in water for a duration of 72 hours at a temperature of 30ºC led to a significant elevation in the concentration of gamma aminobutyric acid (GABA). The GABA content in germinated brown rice reached 6.821 mg/kg after steeping for 24 hours in distilled water. Conversely, *Haiminori* germinated brown rice soaked for 24 hours exhibited a GABA content of 1.01 mg/kg, which was considerably lower than the values reported in the current study by Komatsuzaki *et al.* (2007) and Thomas *et al.* (2023). Karladee and Suriyong (2012) and Thomas *et al.* (2023) had previously reported GABA concentrations ranging from 1.365 to 2.348 mg/kg of germinated brown rice in twentyone varieties of purple and modern white rice soaked for 24 hours at room temperature. Furthermore, soaking six Thai rice varieties for a duration of 4 hours resulted in an approximately twofold increase in GABA content in the rice grain, as reported by Varanyanond *et al.* (2005) and Thomas *et al.* (2023).

In contrast to the flow, Kavyashree *et al.* (2021) and Thomas *et al.* (2023) showed an elevation in the GABA content of variety IET-25451 after 48 h of soaking

(6.686 mg/kg), followed by a subsequent drop after 72 h of soaking (4.210 mg/kg). Furthermore, Zhang *et al.* (2014) and Thomas *et al.* (2023) stated an elevated level of GABA concentration at 36 h, followed by a subsequent fall, with the lowest concentration observed after 48 h of treatment. The accumulation of GABA can also be influenced by the medium and conditions of soaking. The immersion of substances in acidic electrolysed water led to an increased accumulation of GABA, as observed in the study assessed by Liu *et al.* (2013) and Thomas *et al.* (2023). As reported by Yu et al. (2023), the cumulative level of free amino acids demonstrated an increasing pattern with the extension of the germination period. The breakdown of polyamines was partially stimulated by heightened diamine oxidase and polyamine oxidase activity. However, the functioning of amino-aldehyde dehydrogenase, crucial for the direct synthesis of GABA in the pathway, was hindered. Numerous factors, including soaking time, germination duration, rice variety, temperature, pH, and enzymatic reactions, contribute to the modulation of GABA content in rice. These variations in GABA levels in rice could be attributed to the interplay of these factors.

During the process of germination, the nutrient stores present in grains are mobilised and utilised for various metabolic activities such as respiration and the production of newly formed cells that contribute to the development of the embryo. This utilisation of nutrient reserves leads to alterations in the nutritional and physicochemical composition of the grain (Bamforth and Barclay, 1993). The study conducted by Aurisano *et al.* (1995) and Thomas *et al.* (2023) revealed that subjecting germinating seeds to anaerobic conditions for a duration of 24 hours resulted in the synthesis of gamma aminobutyric acid (GABA) in both the root and shoot. The findings of the current study indicate that the GABA content in all rice cultivars exhibited a consistent increase throughout the duration of germination. Following a statistical analysis, it was observed that the GABA content exhibited an increase to 109.02 mg/kg after 72 hours of germination. Subsequently, the GABA content decreased to 93.60 mg/kg after 48 hours of germination; and further decreased to 68.90 mg/kg after 24 hours of germination. Additionally, Komatsuzaki *et al.* (2007), Ding *et al.* (2018), Mohammed *et al.* (2021) and Thomas *et al.* (2023) have documented an increase in GABA content following a 72 hour germination period of

various rice cultivars. As per Charoenthaikij *et al.* (2010), it was determined that the optimal duration for germination when using neutral soaking water was 36 hours. In a study conducted by Hayat *et al.* (2015) and Thomas *et al.* (2023), it was found that the highest level of GABA synthesis took place following a germination period of 96 hours. According to Maisont and Narkrugsa (2010), the GABA level in Thai waxy paddy rice increased from 8.0 to 22.0 mg/kg of fresh weight over a germination period of 12 to 60 hours.

5.1.2. Total antioxidant activity

The antioxidant capacity of pure compounds to quell and suppress the generation of a coloured radical cation generated through the process of the reagent solution is termed total antioxidant activity (Evans and Miller, 2004; García-de-la-Asunción *et al*., 2009). In the current study, the highest total antioxidant activity reported in *Jyothi, Njavara* and *Chitteni* is 4.79 µg/ml, 4.28 µg/ml, and 5.45 µg/ml, respectively. The findings were comparable with Rao *et al.* (2010) in that, the cultivar *Njavara* (yellow glumed) showed a high total antioxidant activity with 2.93 absorbance value, followed by *Jyothi* (2.52). The values obtained align with the findings of Reshmi and Nandini (2018), that among the selected rice varieties *Njavara* (yellow glumed) showed high antioxidant activity. The findings of Nayeem *et al.* (2021) stated that, the highest total antioxidant activity of *Njavara* is mainly attributable to the highest concentration of anthocyanins, proteins, phenolics and flavonoids. Pradeep *et al*. (2014) reported that, total antioxidant activity of germinated and steamed *Jyothi* rice bran was 39 mM TE/g. The results of the current study are comparable with the results of Aiswarya (2019), that both *Rakthashali* rice flour and rice bran extract showed total antioxidant activity with an absorbance of 0.95 to 9.56 and 2.13 to 10.30 respectively.

The total antioxidant activity differs with rice cultivars. The research of Finocchiaro *et al.* (2007) demonstrated that, in comparison with white rice, red rice variants had three times more antioxidant activity. As per Finocchiaro *et al.* (2010) and Walter *et al.* (2013), a large amount of the total antioxidant capacity was discovered in the soluble fraction of coloured rice types (82 % in black and 75% in

red). Meanwhile, Zaupa *et al.* (2015) stated that, black rice, followed by red, and white rice, had the highest total antioxidant activity. Furthermore, Kaur *et al*. (2018) found that, coloured rice cultivars like black, red and brown rice have higher total antioxidant activity because pigmented and coloured rice varieties have higher concentrations of bioactive components like flavonoids, anthocyanins, phenolics, and proteins than non-pigmented rice varieties.

A strong relationship between germination and total antioxidant activity was reported in germinated pigmented rice (Maisont and Narkrugsa, 2010); Sutharut and Sudarat, 2012). Meanwhile, Umnajkitikorn *et al.* (2013) found out, sprouting increased the total antioxidant activity of the Thailand rice variety *Kum Doi Saket*. Ti *et al.* (2014) analysed the role of germination on total antioxidant activity and reported that the antioxidant capacity of brown rice significantly increased during sprouting, and the highest value was found after 30 h of germination. The Sri Lankan rice varieties like *Attakkari* (EC_{50} =0.70 mg/ml), *Bg2907* (EC_{50} = 0.57 mg/ml), and *Bg406* (EC_{50} = 0.56 mg/ml) had inferior antioxidant activity with respect to the reported findings of the present study (Priyanthi and Sivakanesan, 2021). Increased antioxidant activity upon germination is attributable to the buildup of secondary metabolites like antioxidants.

5.1.3. Organoleptic evaluation of table rice

Sensory evaluation is a method for assessing the sensory qualities and acceptance of newly developed goods. Out of the major cereals, rice is the only significant cereal that is mostly consumed as cooked whole grains or table rice. In the present study, the cultivar *Jyothi* had the highest average mean score of 8.34 among all three rice varieties, followed by *Njavara* (8.16), and *Chitteni* (8). Similar results were reported by Sathyan (2012) and Chandhni (2015), that, *Jyothi* obtained the highest mean score of 7.5 out of the evaluated rice cultivars. Meanwhile, Nath (2018) noticed an average mean score of 8.56 for cooked *Jyothi* rice. From the results, it is evident that, *Jyothi* rice was widely cultivated and commonly used because of its superior organoleptic properties as a table rice. The organoleptic qualities observed in

the present study are comparable with the Thai rice variety *Aroso* (7.98) and superior to Nigerian rice variety *Ofada* (5.58) (Ebuehi and Oyewole, 2007).

According to Ong and Blanshard (1995), as rice cultivars became more diverse, the sensory qualities of rice became less predictable. The rice cultivars *Jyothi, Njavara,* and *Chitteni* had medium amylose content. According to research findings, it has been shown that a cultivar possessing a greater proportion of amylose and longer chain amylopectin molecules tends to exhibit a firmer cooked texture. Conversely, a cultivar characterized by a lower amylose content and shorter chain amylopectin molecules tends to display a softer texture (Ong and Blanshard, 1995; González *et al*., 2004).

The effect of germination was evident in the taste and texture of table rice. The current findings of Sareepuang *et al.* (2008) revealed that, increasing the soaking time enhanced the cooking characteristics of parboiled brown rice by lowering cooking time and solid loss. Furthermore, Sathyan (2012) found that, germinated and cooked *Jyothi* rice was sticky and too soft when compared to raw rice samples. Comparing to cooked brown rice, the sensory attributes of sprouted cooked rice are sweeter, softer, more swollen and more cohesive (Jiamyangyuen and Ooraikul, 2008). Meanwhile, Lu *et al.* (2010) and Patil and Khan (2012) indicated that, germination can cause the exterior of brown rice to crack, allowing moisture to enter the grain and softening the texture when cooking. These findings were reliable with those of Kyung-Ha *et al.* (2016) and Lee *et al.* (2019), who found that tissue modifications in brown rice were seen during the entire germination process, and subsequent to germination. Notably, the texture underwent a softening phenomenon, accompanied by an increase in stickiness, which contributed to the enhancement of rice flavour.

From the findings, it can be understood that, cultivar, soaking time, and germination time positively impacted organoleptic qualities, and germinated rice samples were slightly sweet. A characteristic germinated flavour was observed for the cooked grains, which improved overall acceptability.

5.1.4. Parboiling of rice varieties

Parboiling is a hydrothermal treatment primarily used to boost the head rice yield and improve nutritional, sensory, and shelflife qualities (Gunathilake *et al*., 2023).

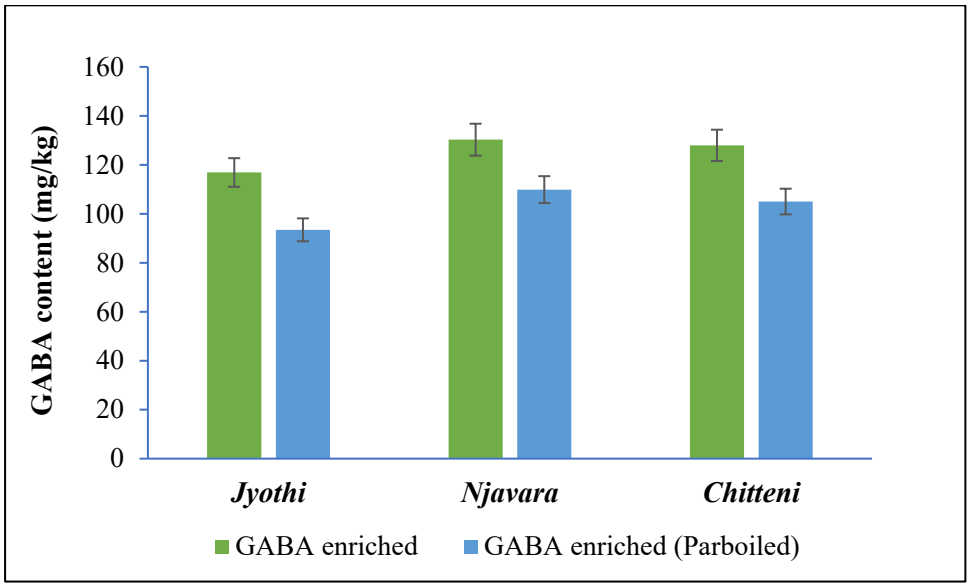


Figure 12. Effect of parboiling on GABA in germinated *Jyothi, Njavara* and *Chitteni*

As per the findings of the present study, the GABA content decreased after parboiling (Figure 12), and the *Njavara* cultivar was found to have the highest retention of GABA (84.34 %) among the three rice varieties, followed by *Chitteni* (82.05 %) and *Jyothi* (79.96 %). The results of the current study are comparable with Cheevitsopon and Noomhorm (2011) that, parboiling (15 min) considerably decreased the GABA concentration of GBR (a reduction of 62.32 %–78.39 %). Similar results were reported by Cheevitsopon and Noomhorm (2015) that, the GABA content (23.31 mg/100g) in germinated brown rice decreased slightly after parboiling to 17.91 mg /100 g. Simultaneously, Jongyingcharoen and Cheevitsopon (2016) observed that, cooked germinated brown rice had a lower GABA level (7.49 mg/100g) compared to uncooked GBR (19.71 mg/100g). In contrast to the trend, Han *et al.* (2016) found that, the GABA content significantly increased after parboiling in germinated *Jupiter* and *Wells* rice cultivars.

The results showed that the synthesis of gamma butyrolactam and structural decomposition by water loss are the causes of GABA degradation at higher temperatures (Contineanu *et al.*, 2010; Khan *et al*., 2015).

5.2. Quality evaluation of rice varieties

5.2.1. Chemical and nutritional qualities

Moisture content can be used as an index of the quality and reliability of rice grains (Sotelo *et al*., 2000). The moisture content of GABA enriched rice cultivars of *Jyothi, Njavara,* and *Chitteni* was found to be considerably higher than that of control counterparts (Figure 13). The moisture content significantly increased from 11.81 per cent, 11.63 per cent, and 11.83 per cent for control to 13.53 per cent, 13.19 per cent, and 13.58 per cent for GABA enriched rice of *Jyothi*, *Njavara* and *Chitteni* respectively. The findings of the current study were reliable to those early reported by Deepa *et al.* (2008), that a moisture content of 13 per cent and 13.1 per cent in *Jyothi* and *Njavara* rice cultivars without soaking and germination. Similar results were reported by Reshmi and Nandini (2012), with a moisture content of 13 per cent in ungerminated *Njavara*. Ayernor and Ocloo (2007) stated that for preserving the germinated rice for an extended period of time, the moisture content should be significantly lower. Instead, Sathyan (2012) stated that, a moisture content of 12.67 per cent for control and 11.27 per cent for germinated *Jyothi*. Meanwhile, Lakshmi (2011), Chandini (2015) and Nadh (2018) reported a moisture content of 12.10 per cent,10 per cent and 10.5 per cent for the *Jyothi* cultivar. Pillai *et al.* (2020) and Thomas *et al.* (2023) found out, it is considered advantageous to keep a moisture level below the range of 12-14 per cent in order to preserve the quality of rice and extend its shelf life and reported a moisture content of 11.23 per cent, 9.23 per cent, and 11.13 per cent for ungerminated *Jyothi, Njavara* and *Chitteni* consecutively. Water causes the seed coat/hull to swell, increasing the moisture content, which in turn initiates the germination cycle of the seed. The GABA enriched rice samples are soaked in water for 24 to 72 hours and are subjected to germination. The observed increase in moisture content is due to the uptake of water for the germination process.

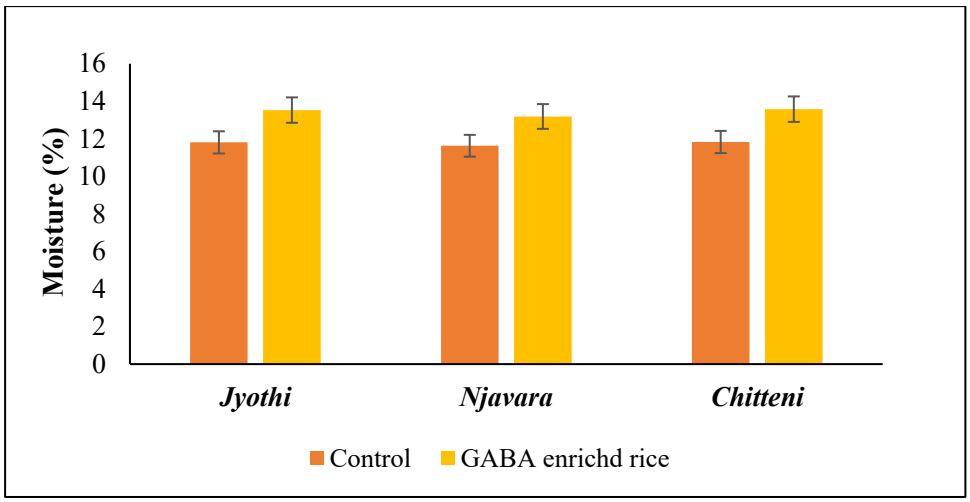


Figure 13. Moisture content of GABA enriched rice varieties

A significant number of glucose molecules condense to form the polysaccharide known as starch. In the present research, the starch content of the GABA enriched rice cultivars of *Jyothi, Njavara,* and *Chitteni* was significantly lower than control (Figure 14). In control rice samples, the starch content is ranged from 74.62 per cent (*Njavara*) to 77.8 per cent (*Jyothi*). In GABA enriched samples, it ranged from 71.52 per cent (*Njavara*) to 75.76 per cent (*Jyothi*). Instead, Chandini (2015) and Nadh (2018) found a lower starch content of 63.18 per cent and 65.83 per cent but Lakshmi (2011) and Reshmi and Nandhini (2012) observed a high amount of starch content of 75.13 per cent and 74.45 per cent in *Jyothi* cultivar. Similar results to the present study were stated by Sathyan (2012) that, the starch content of germinated *Jyothi* (71.84 per cent) was lower than the control (79.61 per cent). The reduction in starch content during germination is attributed to the activity of hydrolytic enzymes, including α and β amylases. These enzymes facilitate the hydrolysis of starch, breaking it down into smaller carbohydrates such as maltose, glucose, and dextrins (Nanogals *et al*., 2000; Traore *et al*., 2004).

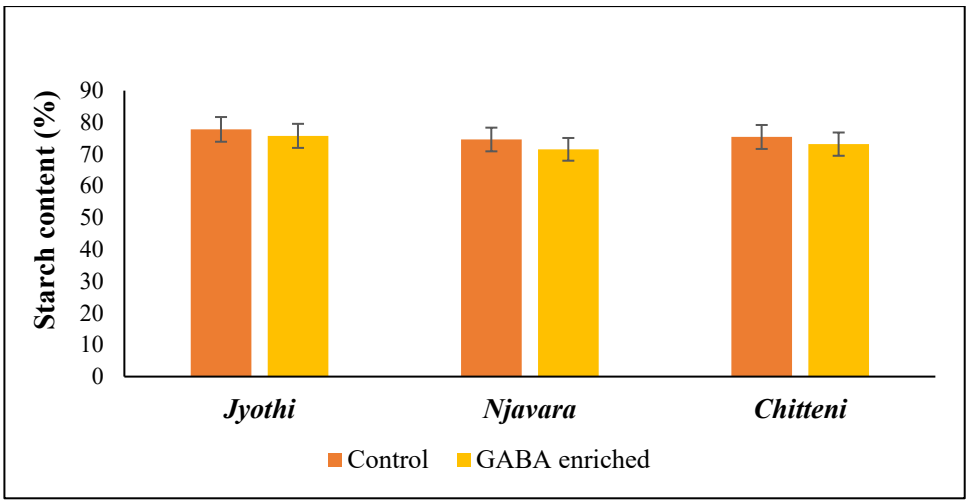


Figure 14. Starch content of GABA enriched rice varieties

Amylose is an essential part of the starch, and has a distinctive role in defining the rice's quality (Kasote *et al*., 2022). Rice with a low amount of amylose is sticky and squishy after cooking. As the amylose content rises, the rice becomes firmer. According to Resurrection *et al*. (1977), rice is classified based on amylose content as low (10–20 %), intermediate (20–24 %), or high (>25 %). In the present study, an amylose content of 25.75 per cent, 24.21 per cent and 24.66 per cent in control decreased to 23.43 per cent, 22.88 per cent and 23.87 percent in GABA enriched *Jyothi, Njavara* and *Chitteni* rice (Figure 15). The present study categorized all the rice cultivars as intermediate amylose content. The findings of the current study was comparable to the observations of Deepa *et al.* (2008), in which, the amylose content of *Njavara* was 22.7 per cent, and for *Jyothi* it was of 22.9 per cent. Similarly, Vanaja and Babu (2006), Chandhini (2015) and Nadh (2018) recorded an amylose content of 25.67 per cent, 22.17 percent and 24.20 per cent in *Jyothi* cultivar. Simultaneously, Reshmi and Nandhini (2012) reported, the amylose content of 24.27 per cent in *Njavara* (yellow). According to Sathyan (2012), the *Jyothi* rice variety had an initial amylose concentration of 24.50 per cent. However, this percentage reduced to 22.24 percent following the process of germination. Simultaneously Adujei *et al.* (1976) found out, oligosaccharides and other reserve carbohydrates undergo hydrolysis during germination. Lower amylose concentration has been reported due to increased α amylase activity and rapid starch depletion during germination (Savithri and

Desikachar, 1990). The results were found to be consistent with the low amylose concentration identified in germinated samples.

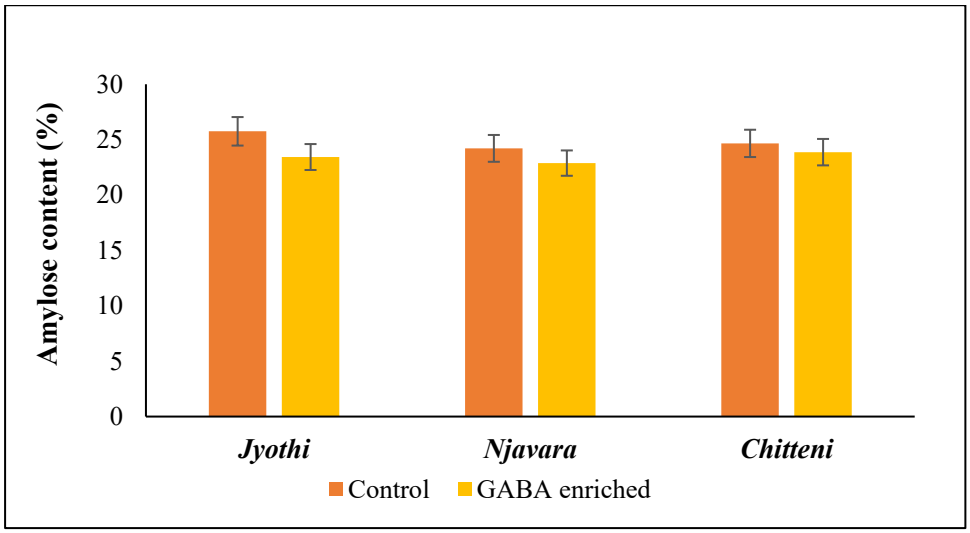


Figure 15. Amylose content of GABA enriched rice varieties

Carbohydrates represent the predominant source of dietary energy in the world, accounting for 40 to 75 per cent of total energy intake in humans. In the present study, the carbohydrate content significantly decreased from 69.14 per cent, 66.02 per cent, and 67.8 per cent to 63.23 per cent, 60.43 per cent, and 61.16 per cent in *Jyothi, Njavara,* and *Chitteni* consecutively (Figure 16). The findings of the present study were comparable with that of Pillai *et al.* (2020), who found out the *Jyothi*, *Njavara*, and *Chitteni* cultivars had carbohydrate content of 68.43 per cent, 65.20 per cent, and 67.39 per cent, respectively. Similarly, Chandini (2015) and Devraj *et al.* (2019) indicated carbohydrate content of 75.6 per cent and 76.51 per cent for *Jyothi*. Meanwhile, Deepa *et al.* (2008) stated carbohydrate content comparable to the values observed in the current study (*Jyothi* -72.8 % and *Njavara*-73.5 %). During the soaking and sprouting, the activity of α-amylose increases (Thomas *et al*., 2023). Carbohydrate content decreases significantly after germination it is used as an energy source for seed sprouting and its break down to simpler, more absorbable sugars.

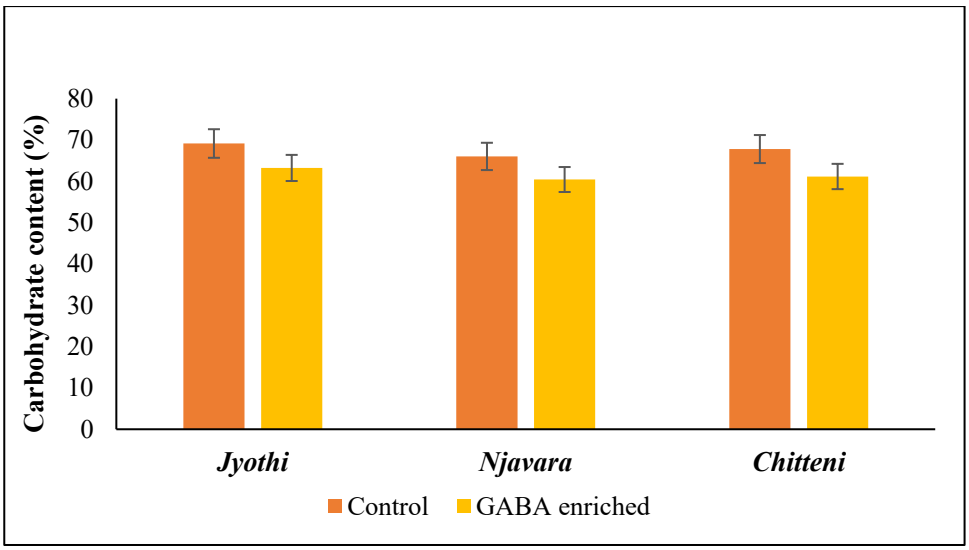


Figure 16. Carbohydrate content of GABA enriched rice varieties

Protein content in rice is used as an index for nutritional quality, and cultivars with protein content greater than 10 per cent were termed as high protein content (Ressurection *et al*., 1997). In the current study, the protein content considerably enhanced in GABA enriched rice compared to the control from 10.62 per cent to 12.43 per cent (*Jyothi*), 12.90 per cent to 14.28 percent (*Njavara*), and 11.56 per cent to 13.01 per cent in *Chitteni* cultivar (Figure 17). The findings in the present study were consistent with the protein content suggested by Pillai *et al.* (2020), which were 10.20 per cent, 11.27 per cent, and 7.77 per cent in *Jyothi, Njavara*, and *Chitteni* respectively. Simultaneously, Reshmi (2012), Chandini (2015) and Nadh (2018) noticed the protein content of 11.80 per cent, 7.5 per cent and 5.47 per cent in *Jyothi* cultivar. Compared with the protein content in the present study, Deepa *et al.* (2008) obtained a lower protein content of 9.52 per cent and 7.97 per cent in *Njavara* and *Jyothi* respectively. In contrast to the findings, Sathyan (2012) reported a decrease in protein content after germination from 8.11 per cent to 5.81 per cent in *Jyothi*. The process of germination has been found to impede trypsin activity and alleviate the inhibitory effects of phytate through hydrolysis. The protein concentration in germinated seeds is comparatively lower than that in non germinated seeds, suggesting that proteins may have undergone hydrolysis or dissociation from antinutritional components (Ohanenye *et al*., 2020). Likewise, Ongol *et al.* (2013) and

Jan *et al.* (2017) evaluated an increase in protein after sprouting due to the synthesis of some amino acids (Kim *et al*., 2012).

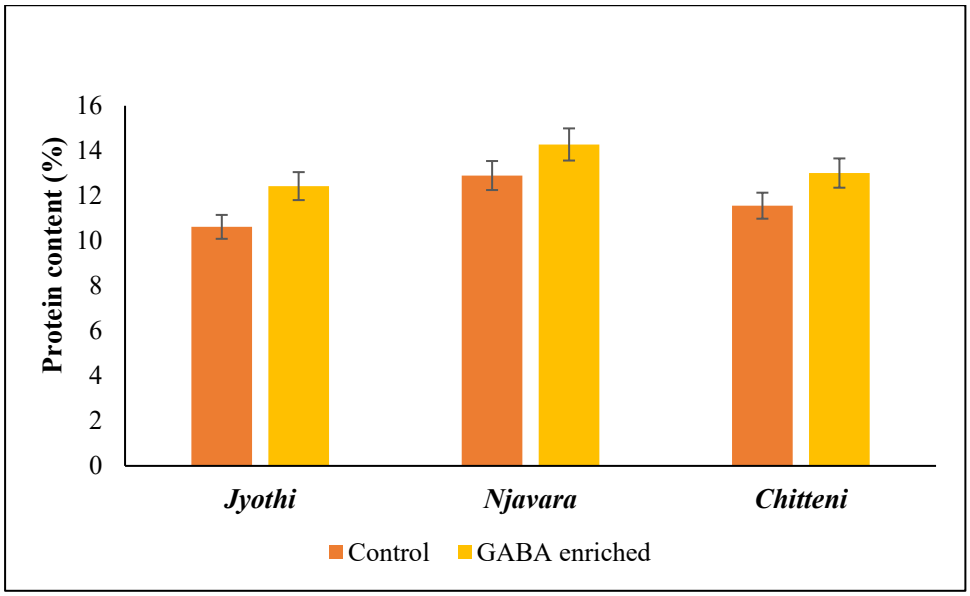


Figure 17. Protein content of GABA enriched rice varieties

Most of the fat in rice is unsaturated fatty acids, which significantly impact the appearance and eating quality of rice. In this study, the GABA enriched rice had a fat content of 2.04 per cent, 2.81 per cent and 2.59 per cent compared with the control *Jyothi* (1.59 per cent), *Njavara* (1.98 per cent) and *Chitteni* (1.75 per cent), respectively (Figure 18). Similarly, *Njavara* and *Jyothi* had a fat content of 2.48 per cent and 2.60 per cent (Deepa *et al*., 2008). Again, Devraj *et al*. (2019) determined a fat content of 2.09 per cent in the *Njavara* cultivar. Meanwhile, the fat content of *Jyothi* (3.26 per cent), *Njavara* (3.24 per cent) and *Chitteni* (3.10 per cent) reported by Pillai *et al.* (2020) were greater than the reported values of the present study. In contrast to the trend, Lakshmi (2011), Chandhini (2015), and Nadh (2018) reported a lower fat content of 1.30 per cent, 0.42 per cent, and 0.32 per cent in the *Jyothi* cultivar. According to Sathyan (2012), fat content (1.92 per cent) decreased to 1.31 per cent after germination. Similarly, the study conducted by Young *et al.* (2012) determined that, the total fat of rice grains undergoes an elevation following a 72 hour germination period, rising from around 1.6 per cent to 2.0 per cent. The primary location of lipid accumulation in rice is the bran, and the concentration of lipids can

be substantially diminished through the process of milling, which involves the removal of the bran. The current investigation employed whole grain with bran, and the elevated lipid content may be attributed to the oil content present in rice bran (Thomas *et al*., 2023).

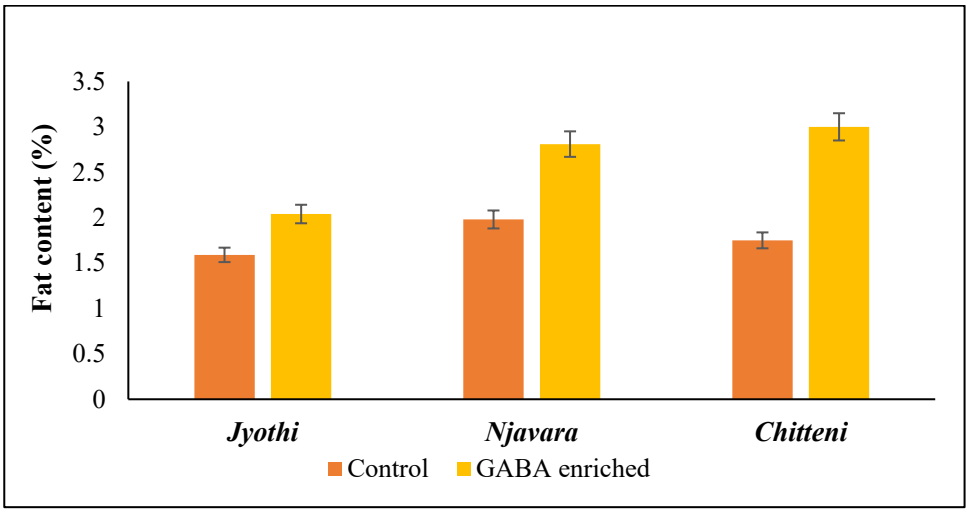


Figure 18. Fat content of GABA enriched rice varieties

Rice is the main energy source and the most popular staple food (Mujoo and Ali, 1998). In the present study, the energy content decreased from 332.68 Kcal to 324.88 Kcal (*Jyothi*), 332.84 Kcal to 326.30 Kcal (*Njavara*), and 332.54 Kcal to 325.23 Kcal in *Chitteni* rice (Figure 19). The findings of the current research were in line with Pillai *et al.* (2020), that an energy content of 1427.25 Kcal, 1393.56 Kcal and 1367.57 Kcal in *Jyothi, Njavara*, and *Chitteni* cultivars. Similarly, Reshmi and Nandhini (2012) found an energy content of 357 Kcal in *Njavara* and Chandhini (2015) observed an energy content of 335.81 Kcal in *Jyothi*. According to Deepa *et al.* (2008), the energy content in *Jyothi* (1570 kJ) and *Njavara* (1630 kJ) were superior to the determined values of the present study. After germination, the energy value of the grains falls due to the breakdown of the seedling.

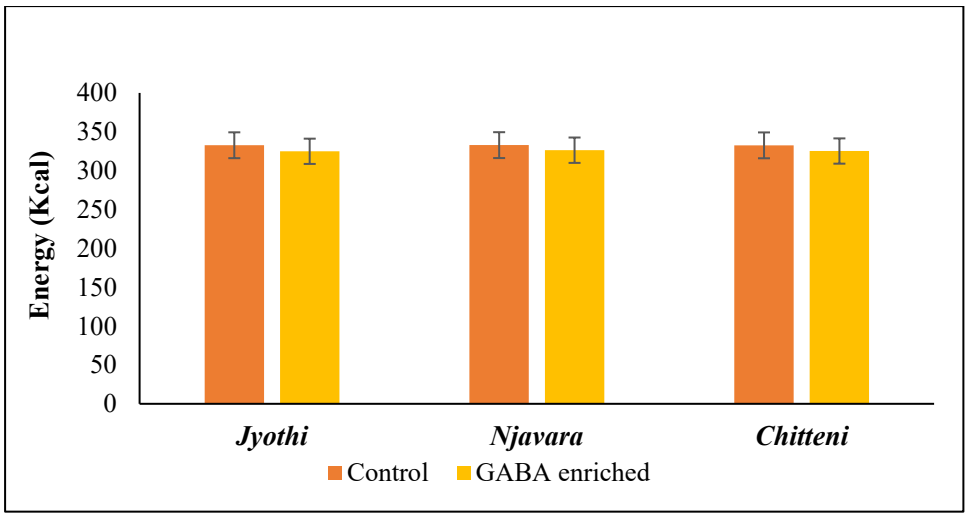


Figure 19. Energy content of GABA enriched rice varieties

Minerals are necessary for healthy metabolic activities and must be included in a balanced diet. Rice contains, minerals including calcium, phosphorus, zinc, and traces of iron (Yousaf, 1992).

According to the findings of Dutta and Barua (1982), the calcium content in rice grains ranged between 15.77 to 29.70 mg/100g. In the present study, the calcium content drastically decreased in GABA enriched rice (14.84 mg/100g, 12.11 mg/100g and 13.38 mg/100g) compared to the control (13.11 mg/100g, 11.35 mg/100g and 12.50 mg/100g) in *Jyothi, Njavara,* and *Chitteni* cultivars (Figure 20). Similar to this, Pillai *et al.* (2020) discovered a calcium content of 18.02 mg/100g, 15.81 mg/100g and 13.20 mg/100g in *Jyothi, Njavara* and *Chitteni* respectively. The calcium content of the *Jyothi* cultivar was found to be higher than the reported values of 5.94 mg/100g (Sathyan, 2012), 6.6 mg/100g (Chandhini, 2015) and 5.46 mg/100g (Nadh, 2018). According to Deepa *et al.* (2008) and Reshmi and Nandini (2012), the calcium content of the *Njavara* was 11.6 mg/100g and 12.20mg/100g which was on par with the findings of the current study. The decrease in calcium content is due to leaching during soaking.

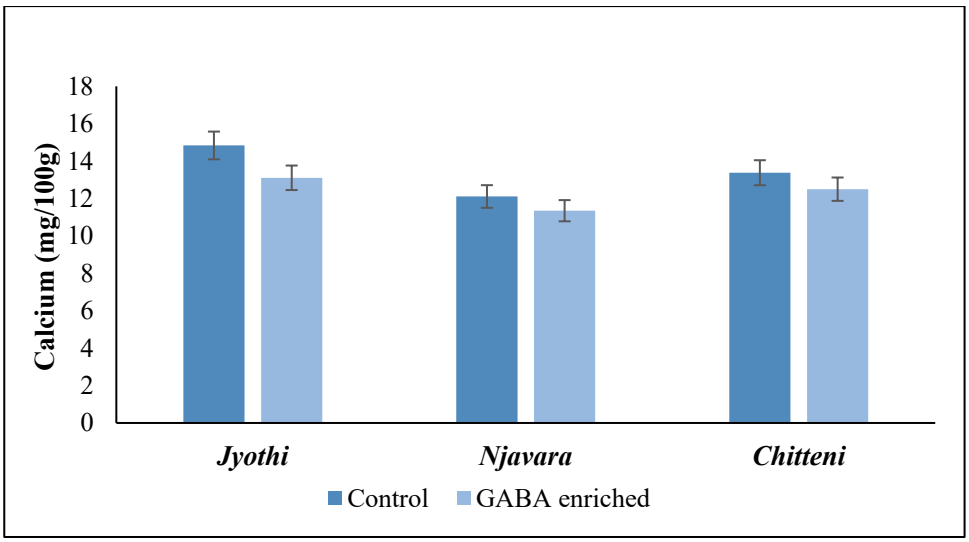


Figure 20. Calcium content of GABA enriched rice varieties

The zinc content decreased from 2.91 mg/100g, 3.28 mg/100g and 3.78 mg/100g to 2.33 mg/100g, 2.81mg/100g and 3.37 mg/100g in GABA enriched rice of *Jyothi, Njavara* and *Chitteni* consecutively (Figure 21). The findings of the current study were in line with Pillai *et al.* (2020), with a zinc content of 3.07 mg/100g, 3.31 mg/100g, and 3.31 mg/100g in *Jyothi, Njavara,* and *Chitteni*. According to Chandhini (2015) and Nadh (2018), the values of zinc in *Jyothi* (1.11 mg/100g and 1.09 mg/100g) were inferior to the determined values of the current study. According to Gopalan *et al.* (1994) and Ayernor and Ocloo (2007), the significant decline in minerals was due to leaching during soaking and utilisation of nutrients during germination.

According to Gregorio *et al.* (2000), rice was one of the cereals with low iron content. In the present study, the GABA enriched rice had an iron content of 3.12 mg/100g, 2.45 mg/100g and 2.25 mg/100g compared to the control *Jyothi* (3.60mg/100g), *Njavara* (2.62 mg/100g) and *Chitteni* (2.41 mg/100g) respectively (Figure 21). Meanwhile, the iron content of *Jyothi* (3.04 mg/100g), *Njavara* (2.76 mg/100g) and *Chitteni* (2.38 mg/100g) reported by Pillai *et al.* (2020) were on par with values reported in the present study.

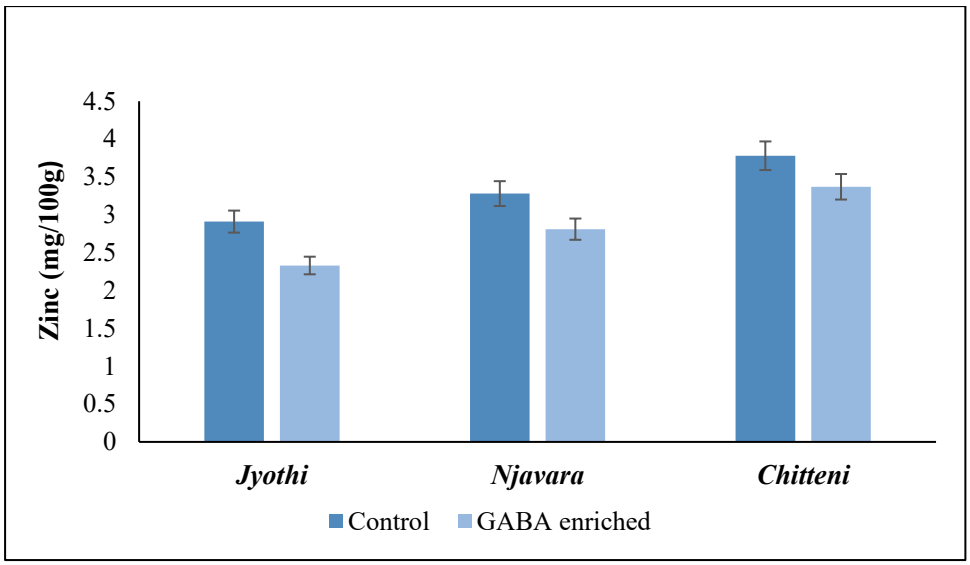


Figure 21. Zinc content of GABA enriched rice varieties

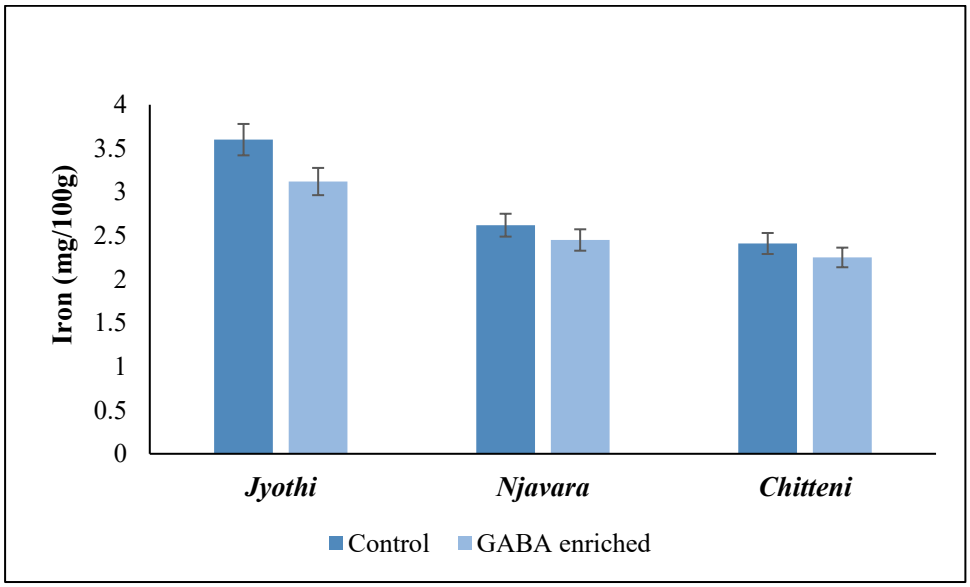


Figure 22. Iron content of GABA enriched rice varieties

Similarly, Deepa *et al.* (2008) reported an iron content of 1.93 mg/ 100g in *Njavara* and 3.95 mg/100g in *Jyothi* cultivar, which is similar to the iron content observed in *Njavara* and *Jyothi* in the present study. Instead, Chandhini (2015) and Nadh (2018) observed an iron content of 0.57 mg/100g and 0.61 mg/100g in *Jyothi* cultivar which is inferior to the results of the present research work. Reshmi and Nandini (2012) obtained a lower iron content of 0.36 mg/100g in *Njavara* cultivar.

The iron content of 1.94 mg/ 100g and 1.04 mg/100g was reported in the control and germinated *Jyothi* cultivar (Deepa *et al*., 2008).

Among the rice varieties, the phosphorus content drastically decreased in GABA enriched rice compared to the control from 328.95 mg/100g to 321.66 mg/100g (*Jyothi*), 359.15 mg/100g to 300.77 mg/100g (*Njavara*) and 338.11 mg/100g to 332.58 mg/100g in *Chitteni* (Figure 23). On par with the present study's findings, a phosphorus content of 324 mg/100g and 354 mg/100g in *Jyothi* and *Njavara* cultivars stated by Deepa *et al*. (2008). In contrast to this trend, Lakshmi (2011), Chandhini (2015) and Nadh (2018) discovered a phosphorous content of 161.83 mg/100g, 133.2mg/100g and 130.10 mg/100g in *Jyothi* cultivar. According to Reshmi (2012), the phosphorus content of *Njavara* was 351.40 mg/ 100g (Reshmi and Nandini, 2012). Meanwhile, Sathyan (2012) found a drastic decrease in phosphorous content in germinated *Jyothi* (158.60 mg/100g) compared to the control (143.30 mg/100g).

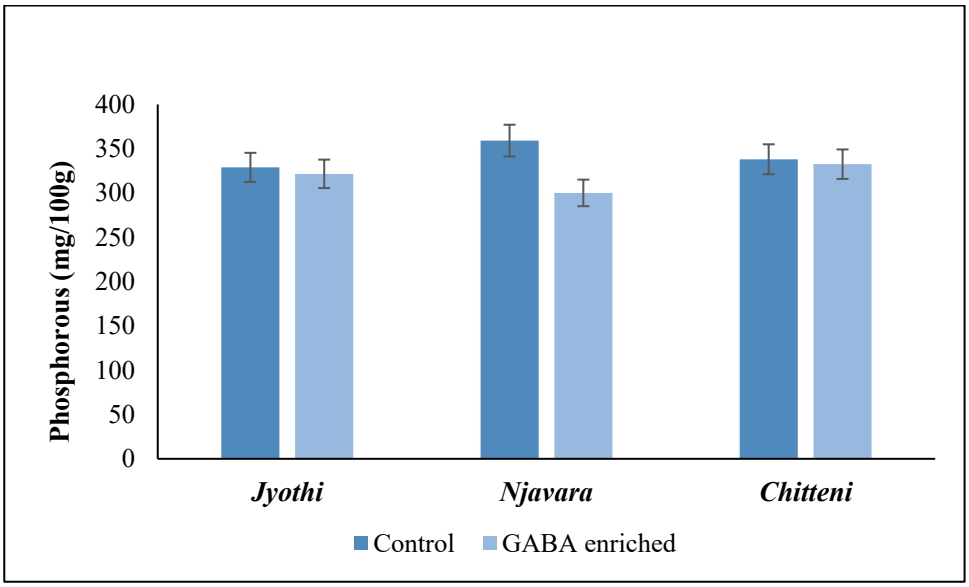


Figure 23. Phosphorous content of GABA enriched rice varieties

Nutrient content in rice, particularly thiamine, can be increased through germination (Pungwerakul, 2007). In the present study, the GABA enriched rice had a thiamine content of 0.77 mg/100g, 0.80 mg/100g, and 0.64 mg/100g compared to the control *Jyothi* (0.61 mg/100g), *Njavara* (0.68 mg/100g) and *Chitteni* (0.52

mg/100g) respectively (Figure 24). Consistent with the results of the current investigation, Deepa *et al*. (2008) stated a thiamine content of 0.35 mg/100g and 0.52 mg/100g in *Jyothi* and *Njavara* cultivars consecutively. Simultaneously, 0.058 mg/100 g of thiamine was reported in *Njavara* (Reshmi and Nandini, 2012). The high thiamine content of *Njavara* rice may help to treat neuritis, muscle weakness, and other vitamin B1 deficiency related symptoms. (Menon, 2004). In a study conducted by Sathyan (2012), the thiamine content of germinated *Jyothi* (0.22 mg/100g) increased compared to control (0.05 mg/100g). The solubilisation of rice bran and enzymatic changes will generate bioactive components during germination, making thiamine more available.

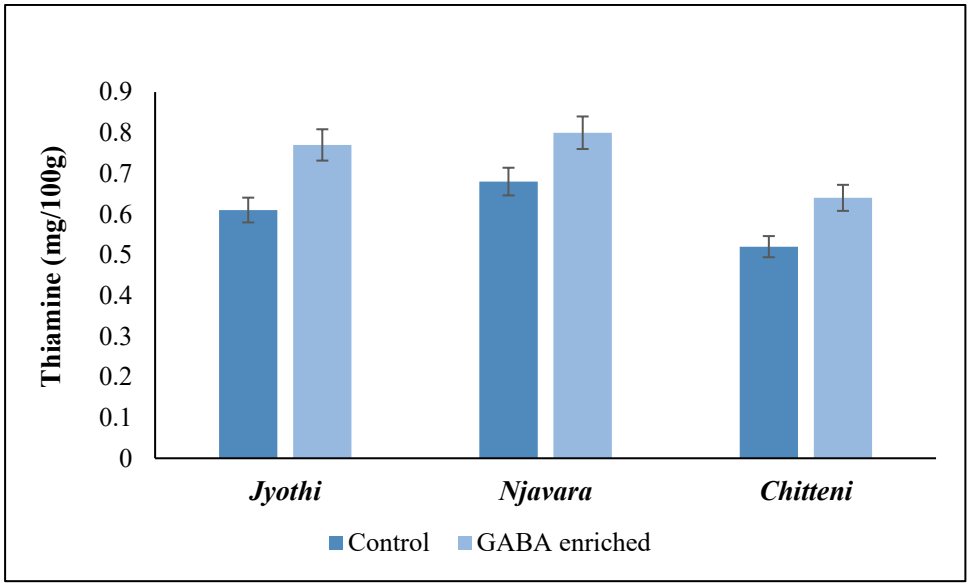


Figure 24. Thiamine content of GABA enriched rice varieties

The riboflavin content of the present study reveals that, the riboflavin content drastically increased in GABA enriched rice compared to the control from 0.14 mg/100g to 0.15 mg/100g, 0.16 mg/100g to 0.18 mg/100g, 0.11 mg/100g to 0.13 mg/100g in *Jyothi, Njavara* and *Chitteni* cultivars (Figure 25). Meanwhile, Reshmi and Nandini (2012) reported riboflavin content of 0.048 mg/100g in *Njavara*, which was seems to be lower than the reported values in the present study. Similarly, Deepa *et al.* (2008) found a riboflavin content of 0.053 mg/100g and 0.071 mg/100g in *Jyothi* and *Njavara* consecutively. According to Grewal and Sangha (1990), fertilizer

treatment, degree of processing, and variance in maturation duration can affect riboflavin levels.

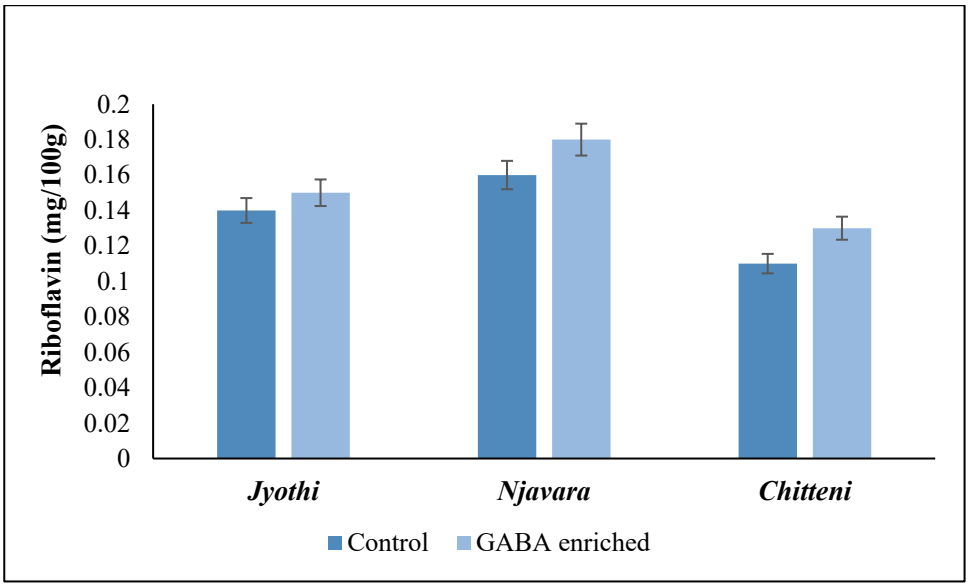


Figure 25. Riboflavin content of GABA enriched rice varieties

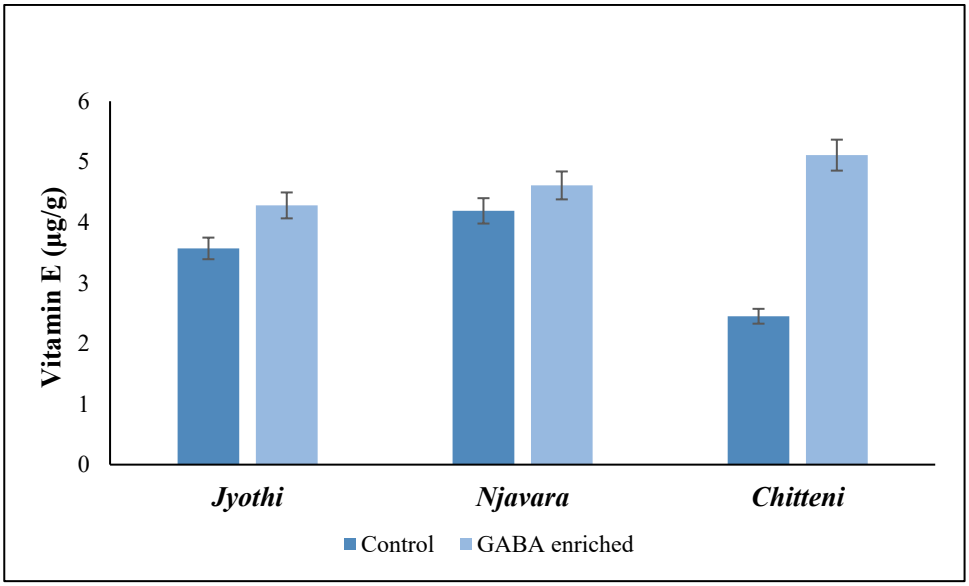


Figure 26. Vitamin E content of GABA enriched rice varieties

The fat soluble antioxidant vitamin E prevents the synthesis of reactive oxygen species (ROS), a derivative of fat oxidation (Reshmi and Nandini, 2018). In the present study, the GABA enriched rice had a vitamin E content of 4.68 µg/g, 4.61 µg/g, and 5.11 µg/g compared to the control *Jyothi* (3.57 µg/g), *Njavara* (4.19 µg/g)

and *Chitteni* (2.45 µg/g), respectively (Figure 26). In contrast to the trend, Moongngarm and Saetung (2010), reported higher vitamin E content (0.86 mg /100g) in germinated brown rice (GBR), which was similar to the results of the current study.

The *in vitro* starch digestibility and mineral absorption were found to be enhanced by the GABA enrichment in all the varieties. The *in vitro* digestibility of starch noticed in the GABA enriched rice of three rice varieties was higher than the *in vitro* starch digestibility of rice (71.2 per cent) reported by Itu *et al.* (2004). Likewise, Sathyan (2012) found the *in vitro* starch digestibility in *Jyothi* rice ranging from 77.02 to 88.61 per cent. An appreciable improvement in starch digestibility was observed with advancement in germination (Singh *et al.*, 2002). The germination process and the enhancement process of GABA in rice damages the continuous matrix of starch granules, which thus made it more bioavailable (Jabeen *et al.,* 2023). During germination, the starch is hydrolysed by α amylase and simple sugars are released, which will be utilized to synthesis cell structural polymers (Nkhata *et al.,* 2018). As per Oliveira *et al.* (2022), the enzymatic breakdown of starch by α-amylase, in conjunction with the alteration of structural polymers, leads to an enhanced availability of starch. Similar results have been reported in GABA enriched quinoa, wherein the starch digestibility was improved as the quinoa was germinated (Zhang *et al.,* 2020).

It was found that the mineral absorbability increased by the GABA enrichment. Similarly, a study on the mineral availability of cereals such as rice has reported that there were two fold increase in iron availability and one fold increase in other minerals such as calcium, zinc, and phosphorous. Phytic acid is considered an antinutritional factor that lowers mineral absorption. In rice, phytic acid is located in aleurone layers of the grains, and due to its chelating ability, it probably binds to the minerals inside the aleurone layer (Lu *et al.,* 2013). Studies have pointed out that several inherent factors in cereals inhibit mineral absorption (Luo *et al.,* 2010). Among the various inherent factors, phytic acid or phytate has an important role in mineral inhibition in rice (Ekholm *et al.,* 2003). Comparatively speaking, the GABA enriched rice had less phytic acid than the ungerminated brown rice. Thus, the

decrease of phytic acid improves the minerals' bioavailability in GABA rice (Perera *et al*., 2018). Similar findings were stated by Jabeen *et al.* (2023) in *Jhelum* brown rice, a native variety of north Himalayan regions.

5.2.2. Organoleptic qualities

The organoleptic scores of all the parameters were found to be improved in the GABA enriched rice of all three rice varieties of *Jyothi, Njavara* and *Chitteni*. The improvement in the sensory qualities is attributed to the biochemical changes that occur through the processing of GABA enriched rice. The process such as soaking and germinating can cause changes in the starch structure and in many other components. According to Ohtsubo *et al.* (2005), soaking leads to an elevated concentration of alanine, glutamic acid, and glycerin, resulting in a sweeter and more enhanced taste in comparison to normal brown rice. This change might be the reason for better scores for the sensory parameters such as flavour and taste for GABA enriched rice of all the varieties than the normal brown rice in the present study. Soaking and germination also change the starch structure by the activity of the amylase enzyme, so the gel consistency is varied from that of normal rice. This makes the texture soft and more palatable than the normal rice. The hardness of the control was reduced as it undergoes germination and texture is improved (Lee *et al.,* 2019). The harder texture of germinated brown rice (GBR) is a concern, that affects the palatability of GBR and its products. Germination can be adopted to make germinated brown rice more palatable. Prior research has demonstrated that germinated brown rice has a soft consistency as a result of both the intrinsic physiological mechanisms of the rice and the enzymatic processes at action. The outcome of this process leads to the development of rice that is characterised by its ease of cooking and its soft texture, so setting it apart from conventional brown rice (Watanabe *et al*., 2004; Kim *et al*., 2004). The rice milk from germinated brown rice (GBR) had better sensory qualities than ordinary brown rice. According to Devraj *et al.* (2019), the research findings indicated that the colour of germinated brown rice milk was perceived as more favourable compared to regular brown rice milk. The findings of the present study can be related to facts put forth by other research which further proves that GABA enrichment of brown rice varieties improves its organoleptic qualities.

5.3. Assessment of antioxidant and antiproliferative properties of GABA enriched rice

5.3.1. Antioxidant properties

Numerous modern diseases are thought to be caused by oxidative stress, which develops when pro oxidant production and neutralisation are not balanced (Doughari *et al*., 2012). Antioxidant mechanisms are developed in the cells to counteract the presence of free radicals. However, if the production of free radicals surpasses the cell's ability to eliminate them, the excess free radicals attempt to stabilize themselves by pairing with lipids, proteins and DNA in healthy human cells. This process, known as lipid peroxidation, can lead to the development of different diseases such as cancer, atherosclerosis, cardiovascular disease, ageing, and inflammatory conditions (Aswatha *et al*., 2008; Chotimarkorn *et al*., 2008; Lai *et al*., 2009). Meanwhile, rice has become known as a significant reservoir of natural antioxidants that possess the ability to function as scavengers of free radicals. (Rao *et al*., 2010).

DPPH radical scavenging activity is a reliable *in vitro* model that is frequently accustomed to evaluate antioxidant effectiveness quickly. When reduced by a radical species or an antioxidant DPPH disappears in its radical form and transforms into a stable and constant diamagnetic molecule, changing colour to yellow from purple. The observed alteration in coloration can be taken as a sign of the examined materials' ability to act as hydrogen donors (Marxen *et al*., 2007; Lee *et al*., 2007). The DPPH radical scavenging capacities of the control and GABA-enriched rice were found to be considerably inferior to that of ascorbic acid, as reported by Rao *et al.* (2010). The findings of the current investigation demonstrate that, IC_{50} values for the control group were 133.86 µg/mL, 108.38 µg/mL, and 128.92 µg/mL, while for the GABA enriched rice of *Jyothi*, *Njavara*, and *Chitteni*, the IC_{50} values were 121.01 µg/mL, 101.38 µg/mL, and 119.14 µg/mL, respectively (Thomas *et al*., 2023). In contrast to prevailing patterns, Rao *et al.* (2010) demonstrated that *Njavara* and *Jyothi* exhibited DPPH scavenging action, as evidenced by IC_{50} values of 30.85 µg/ml and 48.88 µg/ml, respectively. Reshmi (2012) and Mohanlal *et al.* (2012) indicated *Njavara* (yellow) and *Njavara* (black) had shown DPPH scavenging with an IC_{50} value of 31.52 µg/ml and 84.66 µg/ml. Deepa *et al.* (2012) demonstrated, *Jyothi* and *Njavara*

had strong DPPH radical-scavenging activity with IC_{50} values of 0.11 mg.g $^{-}$1 and 0.05 mg.g $^{-}$1 of extract, respectively. Instead, Mohanlal *et al.* (2012) stated, an IC_{50} value of 287.67 mg/ml for *Njavara* (black). Parvathy *et al.* (2014) found an IC_{50} value of 634.3 µg/mL for *Njavara* (yellow) and IC_{50} value of 206. 71 mg CAT Eq:/100g for *Jyothi* (Jayaraman *et al.,* 2019). The observed decrease in DPPH scavenging activity in this study could potentially be attributed to the use of whole grain in the DPPH assay (Thomas *et al*., 2023).

The methanolic extract from the control and GABA enriched counterpart of *Jyothi, Njavara* and *Chitteni* showed considerable reducing power activity. As per Thomas *et al.* (2023), the reducing power activity of the control and GABA enriched rice enhanced with increasing concentration. The IC_{50} values for reducing power activity ranged from 59.50 µg/ml, 51.99 µg/ml, and 55.15 µg/ml for the control group, to 53.81 µg/ml, 47.30 µg/ml, and 52.15 µg/ml for the GABA enriched counterpart of *Jyothi, Njavara*, and *Chitteni* respectively. In the selected rice cultivars, *Njavara* showed the highest reducing power. This finding was supported by Deepa *et al.* (2008) and Reshmi and Nandini (2018). The findings of the current study were in line with Rao *et al.* (2010), that the methanolic rice bran extracts at 0.5 mg/ml concentration exhibited absorbance values of 1.93 and 2.98 for *Jyothi* and *Njavara,* respectively. Meanwhile, Deepa *et al.* (2008) found ferric chloride scavenging activity with an IC_{50} value of 20.20 mg.g−1 and 27.80 mg.g-1 for *Njavara* and *Jyothi,* respectively. Similarly, Parvathy *et al.* (2014), reported an ascorbic acid equivalent of 0.38 mg/g for *Njavara* cultivar which was lower than the results of the current study. According to Laokuldilok *et al.* (2011), pigmented and coloured rice cultivars have stronger reducing power. This may be the reason for the higher reducing power activity of *Njavara,* followed by *Chitteni* and *Jyothi*.

Nitric oxide radicals are more damaging to tissues and can trigger inflammatory reactions and carcinomas. The excessive presence of nitric oxide in the acidic gastric environment leads to its interaction with oxygen, resulting in the generation of nitrite ions. These nitrite ions have been found to induce mutagenesis (Yin *et al.*, 2007). Nitric oxide scavenging activity was not detected in the current

study (Rao *et al*., 2010). According to Noh *et al.* (2002), more phenolic compounds have stronger nitric oxide scavenging efficacy. The phenol content of the control and GABA enriched *Jyothi, Njavara,* and *Chitteni* was lower in the present study, which coincided with the statement. In contrast to the trend, Rao *et al.* (2010) stated nitric oxide (NO) scavenging activity with an IC_{50} value of 55.25 µg/ml and 71.41 µg/ml for *Njavara* and *Jyothi* consecutively.

According to Parvathy *et al*. (2014), superoxide, the molecular oxygen species that has undergone one-electron reduction, serves as a precursor for various reactive oxygen species (ROS) including hydroxyl radical, singlet oxygen and hydrogen peroxide. These ROS possess the capability to interact with biological macromolecules, leading to tissue damage, redox imbalance, and other adverse physiological consequences (Aruoma,1998). The result of the current study stated that, the control and GABA enriched rice exhibited superoxide radical scavenging with IC_{50} values of 93.37 µg/ml, 65.51µg/ml, 85.10 µg/ml for control counterparts and 88.34 µg/ml, 61.19 µg/ml, 79.38 µg/ml for GABA enriched *Jyothi, Njavara,* and *Chitteni* respectively (Thomas *et al*., 2023). According to Reshmi and Nandhini (2018), *Njavara* possessed superoxide scavenging with an IC_{50} value of 55.43 µg/ml which was in line with the findings of the present study. Comparable findings were also stated by Mohanlal *et al.* (2012) and Parvathy *et al.* (2014). Superoxide scavenging activity with respect to catechin in *Jyothi* was 84.9 mg CAT Equivalent/100g and the activity of superoxide was decreased in the *Jyothi* variety due to the loss of flavonoids and phenolics (Jayaraman *et al*., 2019).

According to Jayaraman *et al.* (2019), hydrogen peroxide (-OH) is non reactive, although can occasionally be poisonous to living cells due to the creation of hydroxyl radicals (-OH), a type of free radical that interacts with biomolecules to damage tissue and break DNA, which can lead to mutagenesis, cytotoxicity, carcinogenesis and cell death (Jayaraman *et al*., 2019). In the present investigation, the control and GABA enriched rice showed hydroxyl radical scavenging activity with IC_{50} values of 101.02 µg/ml, 77.51µg/ml, 93.50 µg/ml for control counterparts, and 94.25 µg/ml, 70.42 µg/ml, 84.07 µg/ml for GABA enriched rice from *Jyothi, Njavara,*

and *Chitteni* (Thomas *et al*., 2023). Meanwhile, Reshmi and Nandini (2012) reported the hydroxyl radical scavenging (-OH) activity of *Njavara* with the IC_{50} value of 46 µg/ml which was superior to the current study's reported values (Thomas *et al*., 2023). Likewise, Parvathy *et al.* (2014) found a value of 20.97 at 100 µg/ml for hydroxyl radical (-OH) scavenging activity of *Njavara*. The findings of Jayaraman *et al.* (2019) were comparable to the result of the present study with a value of 126.1 ± 0.2 mg ascorbic acid Eq:/100g for *Jyothi* cultivar. The moderate scavenging activity of rice extracts is because of the presence of phenols and flavonoids, which may accelerate the conversion of hydrogen peroxide to water (Jayaraman *et al*., 2019).

Plant phenolics are one of the main groups of chemicals in both edible and non-edible plants, which act as principal free radical terminators or antioxidants (Parvathy *et al.,* 2014). The redox characteristics of polyphenols are crucial for quenching singlet and triplet oxygen, adsorbing free radicals, and degrading peroxides (Amakura, 2008; Petti, 2009). The total phenol content drastically increased in GABA enriched rice compared to the control from 15.34 µg/mg, 20.42 µg/mg and 17.06 µg/mg to 35.92 µg/mg, 42.50 µg/mg, and 38.87 µg/mg in *Jyothi, Njavara,* and *Chitteni* cultivars. According to Rao *et al.* (2010), the total phenol content (TPC) was in a range from 3.27 to 12.4 mg GAE/ g, and the highest total phenol content (TPC) was determined in *Njavara* (12.72mg GAE/g) followed by *Jyothi* (9.44 mg GAE/g) which was similar with the findings of the current study. Similar findings were reported by Deepa *et al.* (2012), with total phenol (GAE) of 14.75 (GAE) in *Njavara* and 14.97 (GAE) in *Jyothi* consecutively. Reshmi (2012) and Parvathy (2014) revealed a lower phenolic content of 0.29 mg and 0.70 mg/g in the *Njavara* variety. Meanwhile, Jayaraman *et al.* (2019) and Devraj *et al.* (2019) stated a total phenol content of 340 mg GA Eq:/100g in *Jyothi* and 269.04 ± 1.57 mg GAE/g in *Njavara* cultivars, which was superior than the results of the current study. Nayeem *et al.* (2021) stated superior values for phenolic contents in *Njavara* (420 ± 0. 15 mg GAE/ 100g) and *Chitteni* (380± 0.52 mg GAE/100g). The present investigation observed an elevation in the total phenol content (TPC) in germinated rice as a result of the cell wall breakdown during the germination process. This breakdown had an impact on both soluble and insoluble phenolic compounds (Vichit and Saewan, 2016). According to the study

analysed by Tian *et al.* (2011), it was shown that sinapinic acid exhibited an approximate tenfold rise, following the germination of brown rice samples. Additionally, insoluble phenolics such as p-coumaric acid and ferulic acid demonstrated an increase of around one to two times.

The antioxidant and anticancer characteristics of flavonoid molecules significantly impact human health (Havsteen, 2002; Gumul *et al.,* 2007; Petti and Scully, 2009). Catechin and myricetin are abundant flavonoid compounds in rice. The total flavonoid content drastically increased in GABA enriched rice compared to the control from 7.8 µg/mg, 9.66 µg/mg, and 8.86 µg/mg to 13.8 µg/mg, 17.5 µg/mg, and 15.97 µg/mg in *Jyothi, Njavara,* and *Chitteni* cultivars. Comparable findings were revealed by Rao *et al.* (2010), the total flavonoid content of *Jyothi* and *Njavara* varieties was 5.33 mg QTE/G, and 8.5 mg QTE/g respectively. Simultaneously, Nayeem *et al.* (2021) found a total flavonoid content of 1750 mg QE/100g for *Njavara* and 1430 ± 0. 07 mg QE /100 g for *Chitteni* consecutively. Parvathy *et al.* (2014) and Jayaraman *et al.* (2019) determined flavonoid content in *Njavara* (0.26 mg QE/g) and *Jyothi* (3.77 mg CAT/100g) which was inferior to the findings of the present study and Devraj *et al.* (2019) found higher flavonoid content in *Njavara* as 68.02 ± 0.787b mg QE/g. The flavonoids experienced favourable modifications, and hence the contents increased simultaneously after the germination of rice (Jayaraman *et al*., 2019).

These findings of the present study's antioxidant activities align with Esa *et al.* (2013) who stated that, the antioxidant activity of germinated rice are shown to be greater compared to those of ungerminated rice.

5.3.2. Antiproliferatory activities

The MTT assay, as described by Manosroi *et al.* (2006), is a simple and dependable technique employed to assess cell viability. This approach is particularly useful in the screening of antiproliferative medicines. Rice contains ferulic acid, sitosterol, gamma oryzanol, tocopherols, and phytic acid as chemo preventive agents (Barnes *et al*., 1983). The inhibition was identified in both the control and GABA enriched methanolic rice extracts of *Jyothi* as an IC_{50} value of 95.01 g/ml and 92.75

g/ml, in the present study of the cytotoxicity assay against human hepatic cancer cell line (HepG2). In *Njavara*, an inhibition was detected with the IC_{50} value of 71.51 µg/ml, and 56.53 µg/ml in control and GABA enriched rice from *Njavara.* Meanwhile, in *Chitteni*, the control and GABA enriched rice had an inhibition with the IC_{50} value of 88.68 µg/ml and 76.10 µg/ml consecutively. The ability of the chemo-preventive substances to cause apoptosis, decrease the proliferation of cells, and modify the progression of cell cycle in malignant cells mediates the antiproliferatory activity of the GABA enriched rice extracts. The results of the current investigation were on par with Tan *et al.* (2019) that, the HepG2 cancer cell lines treated with methanol extracts of *temukut* rice expressed inhibition with an IC_{50} value 56.00±8.5 µg/ml. Another study conducted by Rao *et al.* (2010) discovered cytotoxic activity in methanolic extracts of rice bran against the C6 glioma cell line and indicated that *Njavara* and *Jyothi* had antiproliferatory activity with the IC_{50} values of 17.53 g/ml and 25.52 g/ml, consecutively. The cytotoxicity of rice bran against hepatic cell lines is influenced by rice variety, growth conditions, cultivation procedure, and cancer cell line type (Cai *et al*., 2005; Norhaizan *et al*., 2011). Rice extracts having an IC_{50} of 100 g/ml are considered antiproliferative agents, according to the National Cancer Institute Guidelines (USA) (Suffness and Pezzuto, 1990). The GABA and phytochemical concentrations in germinated brown rice may be related to increased antiproliferation and apoptotic activities (Sen *et al*., 2020).

5.4. Storage qualities

5.4.1. Nutritional qualities of GABA enriched rice varieties during storage

a) Moisture

The moisture of the rice grain is an essential parameter in determining its shelflife qualities and safety in consumption. Several studies have reported that the moisture in a range from 11 to 14 per cent is ideal for long storage life (Alhendi *et al.,* 2019). If the moisture content is high, the grains tend to be frail and have higher chances of fungal invasion. Whereas, if the moisture is too low, the grains become brittle and can cause a higher rate of breakage (Shafiekhani *et al.,* 2018). The moisture content of all selected rice varieties in the current study tends to be between the range

from 11 to 15 per cent, which shows that even after six months of storage, the rice is of good quality and can be consumed without much loss of its peculiar texture.

In the present study, GABA enriched rice varieties have increased moisture content than their control. Studies have pointed out that the moisture content of rice increases in GABA enriched ones than in the control ones (Rusydi *et al.,* 2011). Even though the GABA enriched rice has higher moisture content, they are in between the safe range *i.e.* between 11 per cent to 15 per cent throughout the storage period. The gain in moisture in both control and GABA enriched varieties is also dependent on the humidity (Martinez *et al*., 2007) and the average temperature for the duration of storage (Keawpeng and Venkatachalam, 2015).

b) Energy

The energy value in the three rice varieties was much higher in the GABA enriched rice varieties (Huang and Ng, 2012). There was a decrease in the energy value of the rice varieties throughout the storage period. Meanwhile, Rusydi *et al.* (2011) have also pointed out the decrease in the energy value during storage. The results of the current investigation are similar to the results of various research on the energy value of rice varieties during storage. The changes in energy value throughout the storage period are highly dependent on its macronutrients like carbohydrate, fat, and protein content. The changes that happen to the macronutrients during germination will affect the energy value of the rice varieties during storage (Alhendi *et al.,* 2019).

c) Carbohydrate

As the rice ages, the carbohydrate content decreases and as the rice is enriched with GABA, the carbohydrate content decreases (Rusyadi *et al.,* 2011). Carbohydrate content in various rice varieties of North Sulawesi showed a decrease in six months of storage period. Among various varieties, the red rice variety showed a significant decrease in carbohydrate content. There was 72.68 % carbohydrates initially which decreased to 20.45 % by the end of 6 months of storage (Oessoe *et al.,* 2014). Whereas, by Kumar and Prasad (2017) stated, there is no specific decrease in the

carbohydrate content during a storage period of 3 months. This may be because the drastic changes in the biochemical properties are more evident only after storage of 3 to 4 months (Zhou *et al.,* 2003).

Carbohydrates is one of the major components of the grain which undergo significant changes during germination. The activation of hydrolytic enzymes occurs during the process of germination (Nascimento *et al*., 2022). The alpha amylase enzyme is primarily accountable for the hydrolysis of starch molecules present in grains, leading to the production of maltose. According to McKie and McCleary (2015), pullulanase, an amylolytic enzyme, is responsible for the breakdown of pullulan into glucose monomers, which are then utilized for cellular respiration and energy generation.

d) Protein

In the present study, as the increase in storage period to six months, the moisture increased, and the protein content decreased. This may be because of the exposure of proteins to the increased moisture content. The denaturation of protein bodies is often thought to take place during the process of aging. This denaturation is believed to be linked to the exposure of hydrophobic groups present in protein molecules, resulting in a reduction in the ability to extract proteins for each fraction (Zhou *et al*., 2003). In the present investigation, as the storage period extends, the moisture increases and the protein content decreases. This may be because of the exposure of proteins to the increased moisture content.

The decrease in protein content in rice after storage ranged from 3.93 to 7.84 per cent in germinated rice (Sathyan, 2012). The protein content of rice varieties declined after ageing, according to Kapoor *et al*. (2011), from 4.9 to 4.3 per cent in Pusa I, 7.9 to 5.3 per cent in Sugandha 4, and 6.2 to 5 per cent in Sugandha 5. According to Kalpana and Rao (1995), the decrease in soluble protein concentration caused by aging is accompanied by protein denaturation, increased superoxide dismutase activity, and a lack of ATP. Sagar *et al.* (1988) reported a drop in protein content in stored rice samples, decreasing the protein level from 7.10 to 6.70 per cent.

d) Fat

In the present study, as the storage period extends, the fat content decreases. The saturated and polyunsaturated fatty acids (PUFA) increases, and meanwhile, monounsaturated fatty acids (MUFA) decrease during brown rice sprouting (Rusyadi *et al.,* 2011). As per the study of Saikrishna *et al*. (2018) on the changes in the brown rice and after the germination during storage and it revealed, that the free fatty acids in rice varieties rose during storage for both brown rice and germinated counterparts (Parnsakhorn and Langkapin, 2013).

Rice fat content decreased during storage in germinated rice, ranging from 0.48 to 1.74 percent (Sathyan, 2012). Lipases kick off lipid hydrolysis when the lipid membrane is damaged by phospholipase during ageing (Takano, 1989). Saikrishna *et al*. (2018) found changes in the fatty acid profiles and a rise in free fatty acids in rice. Nishiba *et al.* (2000) also showed triacylglyceride breakdown during storage, and an elevation in the free fatty acid, peroxide value, and carbonyl levels during the storage of rice. Morintaka and Yasumatsu (1972) postulated that lipids derived from free fatty acids could accumulate volatile carbonyl compounds by complexing with amylose and hydroperoxides.

5.4.2. Organoleptic qualities of GABA enriched rice varieties during storage

The alterations in rice characteristics, including texture, colour, flavour, and composition, that occur over time are commonly referred to as the process of aging (Zhou *et al*., 2002). The physical qualities and chemical content of rice experience significant alterations during the storage process, which subsequently affect the rice quality in terms of cooking and consumption (Sodhi *et al*., 2003; Patindol *et al*., 2005; Singh *et al*., 2006). The process of aging leads to physiochemical alterations, which can be seen within a short duration of three months in stored rice (Perez and Juliano, 1981). The physical qualities of rice, including pasting, texture and thermal properties, are primarily influenced by the aging process. Typically, the cooked aged rice exhibits a firmer texture and lower stickiness compared to freshly harvested cooked rice (Singh *et al*., 2006; Katekhong and Charoenrein, 2012b; Katekhong and Charoenrein, 2012a). In the present investigation, the average mean scores of the sensory evaluation of all

the parameters decreased as the storage period increased. This may be because of the physicochemical changes happening in the rice varieties during storage. A considerable variation was observed in the organoleptic qualities among the three rice varieties. The lipid and protein changes in the rice may be at different rates in each variety, which in turn can cause the physical and organoleptic qualities of the rice varieties. This is further affected when the seed undergoes various processes such as soaking, and germination. The rice subjected to these processes can be further affected during storage which can change the sensory qualities (Ohtsubo *et al.* 2005). The present investigation aimed to assess and evaluate the organoleptic characteristics of brown rice (BR) and germinated brown rice (GBR). The findings indicated that, according to the overall acceptability scores, the panelists exhibited a preference for cooked GBR over cooked BR. After a storage period of eight months, both types of cooked rice exhibited reduced scores in all sensory qualities. However, it is worth noting that the cooked GBR variety obtained superior scores in each sensory attributes (Parnsakhorn and Langkapin, 2013).

5.4.2.1. Cooking time

The duration of cooking is a significant factor in determining the quality of cooked rice, and it is subject to variation based on the cooking technique employed and the processing conditions of the rice (Roy *et al*., 2008). In the present study, the cooking time taken by the rice varieties as 31.44 minutes, 32.63 minutes and 32.10 minutes for control and 24.02 minutes, 27.15 minutes and 26.89 minutes for GABA enriched rice varieties of *Jyothi, Njavara* and *Chitteni* respectively (Figure 27).

Similar decrease in cooking time after germination was reported by Sathyan (2012) that, the germinated *Jyothi* (22.33 minutes) reported less cooking time compared with the control (29.33 minutes). Raghuvanshi *et al.* (2017) reported a cooking time in the range of 30 to 40 minutes for Indian rice cultivars. Meanwhile, Nandini (1995), Chandhini (2015) and Nadh (2018) reported 37 minutes, 22.20 minutes and 23 minutes cooking time for the *Jyothi* rice variety. Water penetration occurs during soaking prior to germination may be the reason for shorter cooking time and softer texture (Ma *et al*., 2023).

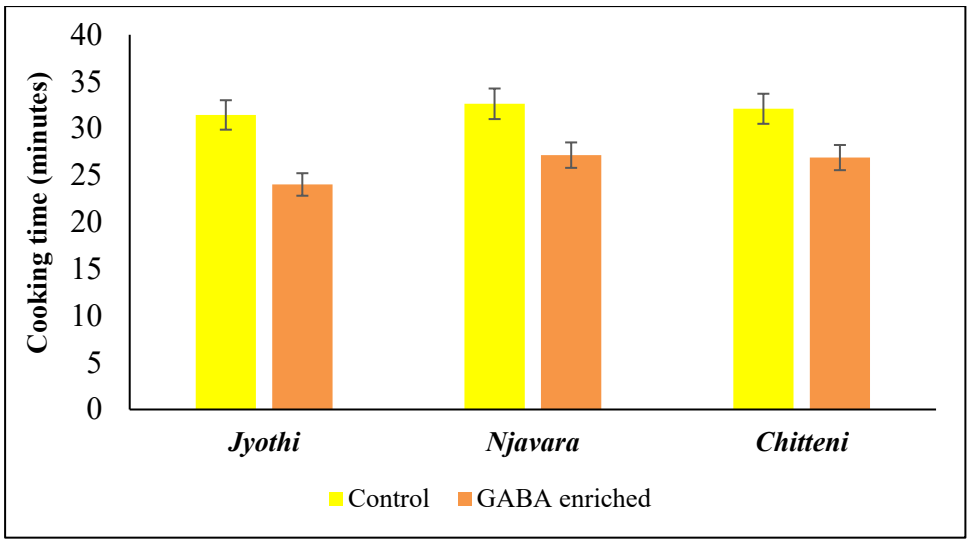


Figure 27. Cooking time of GABA enriched rice varieties

5.4.3. Microbial qualities of GABA enriched rice varieties during storage

A product's microbial growth or damage relies on certain chemical and physical variables that promote its growth (Frazier and Westhoff, 1978). The bacterial count in the GABA enriched rice was higher than in the control rice. Similarly, the findings of Kim *et al.* (2012) have also stated that the bacterial load in germinated brown rice (GBR) is much greater than in brown rice. Sathyan (2012) also found bacterial colonies in germinated rice during three months of storage period. The process of germinating rice can increase the moisture content in the rice and hence, it can increase the microbial load in the rice. Bourneow and Toontam (2019) have also pointed out that as the germination time increases, the microbial load also increases. They detected bacterial content initially and during the storage periods. The yeast and moulds were also present in that study. In the present study, fungal and yeast growth were not detected initially, but colonies appeared from the third month of storage. Sathyan (2012) also reported, fungal colonies appearing in germinated rice varieties after three months of storage and couldn't detect the presence of yeast throughout the storage period.

5.4.4. Insect infestation of GABA enriched rice varieties during storage

Insect infestation is another factor that decreases the storage stability of rice varieties. In the current study, insect infestation was not observed throughout storage. The studies conducted by Sathyan (2012), Chandhini (2015), and Nadh (2018) also reported similar results.

5.5. Processing GABA enriched rice for preparation of rice products

Puffed rice and flaked rice are popular breakfast and snack foods in India (Kumar and Prasad, 2017). Cooked, pulverised, puffed, and flaked rice was prepared with GABA enriched rice from *Jyothi, Njavara,* and *Chitteni* and the cooked rice obtained the highest average mean score, followed by puffed, flaked and pulverised, rice respectively. The average mean score of cooked rice from three GABA enriched rice varieties varied from 8.03 to 8.36. Meanwhile, the mean score was lower in puffed (7.82 to 8.07) followed by flaked (7.85 to 8.02) and pulverised (7.75 to 7.99) rice (Figure 28). The findings of the current study was comparable with Chandhini (2015), that an average mean score of 7.86 for rice flakes prepared from the *Jyothi* cultivar. Meanwhile, Gupta *et al.* (2012) and Kumar and Prasad (2017) reported an average mean score of 7.60 and 7.48 for rice flakes which were inferior to the results of the current investigation. Instead, in a study conducted by Sharon and Kareem (2013), it was discovered that rice flakes made using the *Njavara* cultivar exhibited a higher mean organoleptic scores. Similarly, Kumar and Prasad (2017) determined a range of overall acceptability values of roasted flaked rice ranging from 7.13 to 8.67 in the Gujarathy rice variety *Gurjari.* For puffed rice an average mean score of 7.53±0.68 was reported by Kumar and Prasad (2017), and another study conducted by Patel *et al*. (2021) observed, an average mean score of 7.26 to 7.67 for puffed rice of Rajasthan rice cultivars. The puffed rice prepared from Thai glutenous rice (3.18±0.01/5) have been found to be inferior to the findings of the present investigation (Wannasupchue, 2014). Based on research investigations concerning germinated brown rice, it has been observed that the germination process leads to a certain degree of softening in the texture of brown rice. This softening is attributed to the physiological activity of brown rice and the involvement of diverse enzymes. Consequently, germinated brown

rice exhibits enhanced cooking ability and a softer texture in comparison to regular brown rice (Watanabe *et al*., 2004; Sang-You *et al*., 2007). The findings of the current study proves that the processed products from GABA enriched rice varieties are superior to those from non germinated rice varieties.

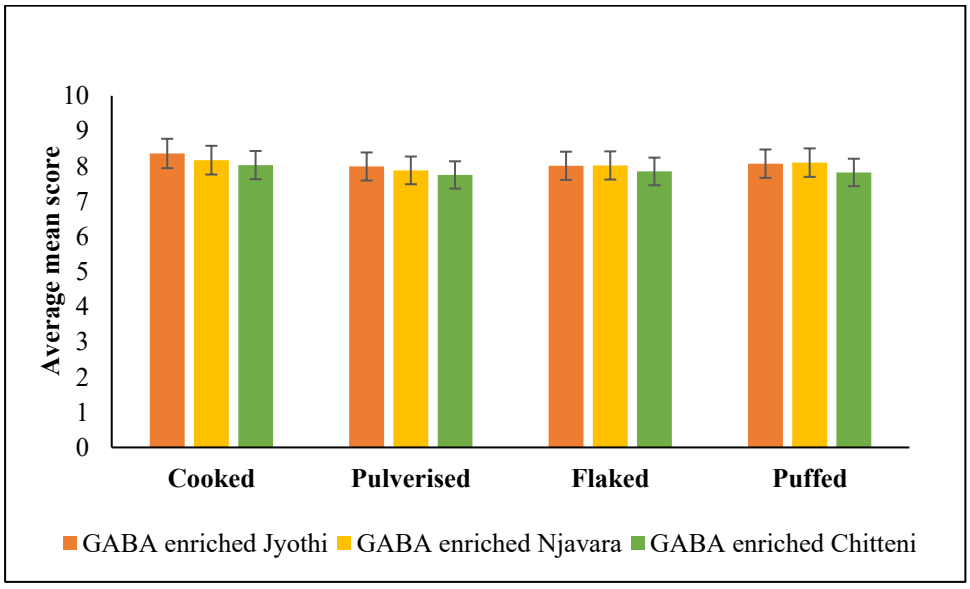


Figure 28. Average mean score of organoleptic qualities of processed products from GABA enriched *Jyothi, Njavara* and *Chitteni*

In processed products, the GABA content is slightly lower than that of GABA enriched raw rice due to short time high temperature treatments. The pulverised, flaked, puffed and cooked rice were prepared with GABA enriched rice of *Jyothi, Njavara* and *Chitteni* and pulverised rice obtained highest GABA content, followed by flaked, puffed, and cooked rice. Among the rice products, the GABA content of cooked rice from three GABA enriched rice varieties varied from 84.34 mg/kg to 95.18 mg/kg. Meanwhile, changes in GABA content in puffed rice (95.09 mg/kg to 112.39 mg/kg) followed by flaked (98.23 mg/kg to 117.85 mg/kg), and pulverised (109.72 mg/kg to 125.04 mg/kg) consecutively (Figure 29). Similarly, the research conducted by Yu *et al.* (2021) revealed that the concentration of gamma-aminobutyric acid (GABA) in uncooked brown rice was measured at 23.44 mg per 100 g of dry weight (DW). However, the GABA levels in cooked samples exhibited a range of 12.12-19.73 mg per 100 g DW. A comparison between cooked and uncooked brown

rice revealed a reduction in GABA levels ranging from 15.8 per cent to 48.3 percent. This discovery is consistent with prior studies conducted by Sirisoontaralak *et al.* (2015). Similarly, Ohtsubo *et al.* (2005) discovered that puffed germinated brown rice (GBR) have more GABA than unpuffed white rice. Hence, the observations derived from the current investigation can be rationalised by the proposition that elevated cooking temperatures have the potential to expedite the degradation of GABA. In contrast to prevailing patterns, the study conducted by Komatsuzaki *et al.* (2007) and Yu *et al.* (2021) revealed that the steaming procedure, specifically at temperatures below 100°C, did not have an impact on the GABA content of germinated brown rice (GBR).

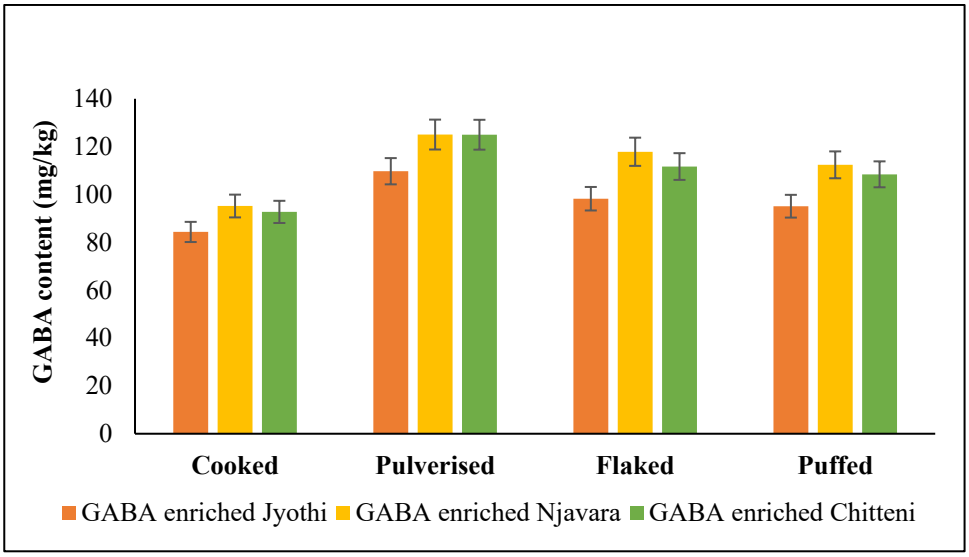


Figure 29. GABA content of processed products from GABA enriched *Jyothi, Njavara* and *Chitteni*

In germinated brown rice, the thermal treatment reduces total antioxidant activity (Konwatchara and Ahromrit, 2014). The highest total antioxidant activity (TAA) was reported in the processed products prepared from *Njavara*, followed by *Jyothi* and *Chitteni*. Among the rice products, the total antioxidant activity of cooked rice from three GABA enriched rice varieties varied from 6.98 µg/ml to 9. 50 µg/ml. Meanwhile, EC_{50} value was in the order of puffed (6.53 µg/ml to 8.98 µg/ml) followed by flaked (6.02 µg/ml to 8.64 µg/ml) and pulverised (5.58 µg/ml to 8.16 µg/ml) (Figure 30). Comparable to the findings of the current study, Mir *et al.* (2016)

observed that the antioxidant properties of puffed rice have demonstrated a notable reduction in comparison to unprocessed rice. Likewise, Liu *et al.* (2015) also stated that, as the degree of milling increased, the quantity of antioxidant molecules like ferulic, quercetin and coumaric acids found to be decreased in japonica and indica brown rice. It is worth noting that boiling coloured rice exhibits reduced levels of anthocyanin due to the removal of pigments during the process of draining excess water, thereby resulting in a decline in overall antioxidant activity (Pal *et al*., 2019). The total antioxidant activity of red rice, *Chak-hao*, *Kalanamak* and *Samba Mahsuri* was 47.73 ± 0.10 mg QE/100g, 64.38 ± 0.75 mg QEquivalent/100g, 40.34 ± 1.12 mg QEquivalent/100g and 33.32 ± 0.53 mg QEquivalent/100g respectively. In contrast to this trend, Itagi *et al.* (2023) stated that, pigmented rice cultivars retained the most bioactives even after popping. Although high temperature, short time processing like flaking and puffing affects bioactive retention. Esmaeelian *et al.* (2020) discovered that a reduction in processing time during flaking and puffing resulted in decreased temperature stress for antioxidants, leading to a higher preservation of antioxidant chemicals. The findings of the current study proves that germination increases total antioxidant activity, but puffing, flaking, pulverising, and cooking can lead to a slight decrease in antioxidant activity.

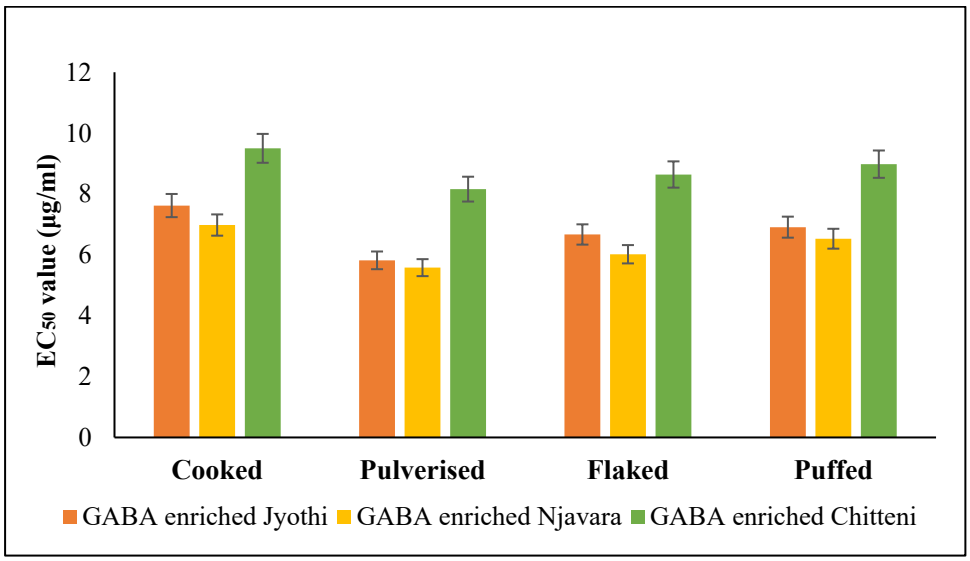


Figure 30. Total antioxidant activity of processed products from GABA enriched *Jyothi, Njavara* and *Chitteni*

SUMMARY

6. SUMMARY

The process of rice grain germination enhances the nutritional and bioactive components' bioavailability, such as gamma amino butyric acid (GABA). During the process of brown rice germination, the enzyme glutamate decarboxylase facilitates the decarboxylation of L-glutamic acid, resulting in the production of GABA, a non-protein amino acid with four carbons. Because of its ubiquity in life, gamma amino butyric acid has garnered considerable interest throughout the previous few decades. The study entitled 'Medicinal properties and process optimisation for GABA enrichment in rice' was undertaken with the objectives of optimising conditions for Gamma Amino Butyric Acid (GABA) enrichment, evaluating the ideal conditions for maximum GABA enrichment in popular and high yielding rice variety *Jyothi*, medicinal rice variety *Njavara* and indigenous rice variety *Chitteni*. The study also assessed the physicochemical, nutritional, organoleptic, antioxidant and antiproliferative activities of GABA enriched rice. The nutritional, organoleptic and microbial qualities of GABA enriched rice were evaluated, for six months of storage period. The organoleptic qualities, GABA content and total antioxidant activities of processed rice products from GABA enriched rice such as cooked, pulverised, flaked and puffed rice were also studied.

The GABA content of the selected three rice varieties was quantified after varying soaking and germination conditions (24 h, 48 h and 72 h) and the ungerminated rice grains were taken as a control. The GABA content of 19.18 mg/kg, 28.63 mg/kg, and 26.67 mg/kg was reported in control samples of *Jyothi, Njavara* and *Chitteni* respectively. The GABA content of the rice cultivar *Jyothi* enhanced from 19.18 mg/kg to 116.89 mg/kg (6.09 fold) after a soaking and germination period of 72 h. The *Njavara* cultivar had the highest GABA content (28.63 mg/kg) that gradually increased from 24 h of soaking and germination (74.85 mg/kg) to 72 h of soaking and germination (130.29 mg/kg). In the traditional cultivar *Chitteni*, the GABA content was found to be the lowest among the selected cultivars, and it enhanced to 127.97 mg/kg (4.79 fold) after a soaking and germination period of 72 hours. The findings revealed that, the GABA content gradually enhanced with 24 h, 48 h and 72 h of incubation and the highest content was recorded after a soaking and

germination period of 72 hours. It was discovered that *Njavara* has the highest average GABA concentration (98.95 mg/kg), followed by *Chitteni* (90.73 mg/kg) and *Jyothi* (81.84 mg/kg). After soaking for 72 hours (98.63 mg/kg), the maximum GABA level was found, followed by 48 hours (88.95 mg/kg) and 24 hours (83.95 mg/kg) respectively. The concentration of gamma aminobutyric acid (GABA) exhibited an increase to 109.02 mg/kg after 72 hours of germination, 93.60 mg/kg after 48 hours of germination, and 68.90 mg/kg after 24 hours of germination.

The total antioxidant activity was evaluated using standard procedure and the antioxidant activity of the control samples was quantified, yielding EC_{50} values of 7.61 µg/ml in *Jyothi*, 7.24 µg/ml in *Njavara* and 8.83 µg/ml in *Chitteni* respectively. In rice cultivar *Jyothi*, 48 h soaked and 24 h germinated rice grains showed the highest total antioxidant activity (EC_{50} = 4.79 µg/ml). The total antioxidant activity was the highest in *Njavara* with an EC_{50} value of 4.28 µg/ml after a soaking time of 72 h and germination time of 48 h. The traditional rice cultivar *Chitteni* reported maximum antioxidant activity (EC_{50} = 5.45 µg/ml) after a soaking time of 24 h and a germination period of 48 h. The average total antioxidant activity was the highest in *Njavara* (4.94 µg/ml) followed by *Jyothi* (5.58 µg/ml) and *Chitteni* (7.46 µg/ml). The maximum total antioxidant activity was observed at 24 h (5.72 µg/ml), followed by 72 h (6.03 µg/ml) and 48 h (6.24 µg/ml) of soaking time. The highest total antioxidant activity (TAA) was reported with an average EC_{50} value of 5.79 µg/ml, 5.79 µg/ml, and 6.41 µg/ml after 48 h, 24 h and 72 h of germination respectively.

Assessment of organoleptic qualities reported that, the average mean score of 7.5, 7.36 and 7.42 were found in control samples of *Jyothi, Njavara* and *Chitteni.* The highest average mean score of 8.34, 8.16 and 8 was recorded for *Jyothi, Njavara* and *Chitteni* after a soaking and germination period of 72 h. The highest organoleptic qualities were registered in *Jyothi*, *Chitteni* and *Njavara*, with an average mean score of 7.74, 7.67 and 7.65, respectively. The organoleptic qualities increased considerably with soaking time, with the maximum mean score at 72 h (7.96), followed by 48 h (7.58) and 24 h (7.50) in selected cultivars. The highest average mean score was

reported after 72 h of germination (7.80), followed by 48 h of germination (7.69), and after 24 h of germination (7.57), respectively.

Based on GABA content, total antioxidant activity and organoleptic qualities, the most suitable conditions for GABA enrichment in each variety were identified by calculating the total index score. Among the cultivars, the highest total index score of 1.5, 1.29 and 1.07 was reported after 72 h of soaking and 72 h of germination and was selected as the best suitable condition for maximum GABA enrichment.

The parboiling effect on GABA enriched rice was evaluated and the highest GABA retention was reported in *Njavara* (84.34 %), followed by *Chitteni* (82.05 %) and *Jyothi* (79.96 %) cultivars. The study revealed that, an average more than 79 per cent of GABA was retained in all three rice cultivars after parboiling.

The nutritional composition of GABA enriched rice was estimated. The parameters such as moisture, starch, amylose, carbohydrate, protein, total fat, energy, calcium, zinc, iron, phosphorus, thiamine, riboflavin, vitamin E were analysed. The GABA enriched rice from *Jyothi, Njavara* and *Chitteni* had a moisture content in a range from 13.19 per cent to 13.58 per cent respectively. The starch content in GABA enriched rice significantly decreased in a range from 71.5 per cent to 75.76 per cent. Amylose content of 22.88 per cent to 23.87 per cent was noticed in GABA enriched rice from *Jyothi, Njavara* and *Chitteni*. A significant decrease in carbohydrate content (60.43 % to 63.23 %) was found in *Jyothi, Njavara* and *Chitteni*. The protein was observed to be in a range from 12.43 per cent to 14.28 per cent in GABA enriched rice. The fat content was noticed in GABA enriched rice from *Jyothi, Njavara* and *Chitteni* ranges from 2.54 per cent to 3.24 per cent respectively. The energy content was significantly decreased after GABA enrichment to the range of 324.88 Kcal/100g to 326.30 Kcal/100g.

GABA enriched rice from *Jyothi, Njavara* and *Chitteni* have a calcium content ranges from 11.35 mg/100g to 13.38 mg/100g respectively. Whereas, the corresponding values of zinc content were in the range of 2.33 mg/100g to 3.37 mg/100g. Similarly, the content of iron in GABA enriched rice was recorded in a

range of 2.25 mg/100g to 3.12 mg/100g. The phosphorous content was in a range of 300.77 mg/100g to 332.58 mg/100g in GABA enriched rice from *Jyothi, Njavara* and *Chitteni*.

The vitamin content of the GABA enriched rice were estimated and a thiamine content of 0.64 mg/100g to 0.80 mg/100g was noticed in GABA enriched rice of *Jyothi, Njavara* and *Chitteni*. Similarly, the riboflavin content of 0.13 mg/100g to 0.18 mg/100g was observed in GABA enriched rice. Meanwhile, the vitamin E of the GABA enriched rice varieties was ranges from 4.61 µg/g to 5.11 µg/g respectively.

The starch *in vitro* digestibility was observed in GABA enriched rice and found as 75.13 per cent to 78.18 per cent. The *in vitro* availability of calcium, zinc, iron and phosphorus was assessed and the availability of calcium was reported in a range from 44.09 per cent to 49.55 per cent in GABA enriched rice. Whereas, the corresponding values of zinc availability were ranges from 34.54 per cent to 38.14 per cent. The iron content in GABA enriched rice was comparatively lower with the range of 30.09 per cent to 33.24 per cent respectively. The availability of phosphorous was the highest, among other minerals and ranges from 55.89 per cent to 59.95 per cent in GABA enriched *Jyothi, Njavara* and *Chitteni.*

The organoleptic qualities were determined using standard procedure and the GABA enriched *Jyothi, Njavara* and *Chitteni* had the maximum mean score for all the sensory parameters. The maximum mean score for organoleptic qualities was 8.40, 8.16 and 7.95 for GABA enriched *Jyothi, Njavara* and *Chitteni* respectively.

Antioxidant activities including DPPH (1,1-diphenyl 1,2- picrylhydrazyl) radical scavenging activity, ferric reducing power (FRAP) assay, nitric oxide (NO) scavenging activity, superoxide and hydroxyl scavenging activity was assessed and the findings revealed that, the methanolic rice extract of GABA enriched cultivars showed the DPPH radical scavenging activity within a range from 101.38 µg/ml to 121.01 µg/ml respectively. The reducing power activity of the GABA enriched rice extracts (in methanol) enhanced with the increase in concentration and was found with the IC_{50} values ranges from 47.30 µg/ml to 53.81 µg/ml consecutively. The GABA

enriched *Jyothi, Njavara* and *Chitteni* prevented the superoxide radicals with the projected IC_{50} values of 61.19 µg/ml to 88.34 µg/ml. The GABA enriched rice of *Jyothi, Njavara* and *Chitteni* also scavenged hydroxyl groups with a projected IC_{50} value of 70.42 µg/ml to 94.25 µg/ml.

The total phenolic content (TPC) of methanolic extract of GABA enriched rice was estimated. The phenol content of GABA enriched rice ranged from 35.92 to 42.50 GAE µg/mg and the flavonoid content varied from 13.8 to 17.5 QE µg/mg.

Anti proliferative activity in hepatic cancer cell line (HepG2) using MTT [3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide] assay was carried out in methanolic extract of GABA enriched *Jyothi, Njavara* and *Chitteni*. Against the hepatic cancer cell lines, the methanolic rice extracts were found to have moderate antiproliferative activity. The GABA enriched *Njavara* recorded the highest antiproliferative activity (IC_{50} = 56.53 µg/ml), followed by *Chitteni* (76.10 µg/ml) and *Jyothi* (92.75 µg/ml).

The GABA enriched rice was subjected to storage for a period of six months and the nutritional, organoleptic and microbial qualities were analysed initially, and at three and six month intervals. The moisture content was 13.53 per cent, 13.19 per cent and 13.58 per cent initially which increased with increase in six months of storage period. Meanwhile, energy decreased from 321.01 Kcal/100g, 324.15 Kcal/100g, 323.69 Kcal/100g to 296.90 Kcal/100g, 298.09 Kcal/100g and 298.16 Kcal/100g after a storage period of six months. Likewise, a considerable decrease was also found in carbohydrate, protein and fat with an increase in the storage period. Organoleptic evaluation of GABA enriched rice was carried out in third and sixth month and it could be inferred that, the mean scores decreased as the storage period increased. Even then the GABA enriched *Jyothi, Njavara* and *Chitteni* were acceptable till the end of six months with an average mean score of more than 7. Microbial population was observed and the GABA enriched *Jyothi, Njavara* and *Chitteni* were safe to consume till six months of storage. No yeast and fungi were detected initially, but bacteria were presented and the count slightly increased by six months of storage.

The rice varieties with higher GABA content and medicinal properties were subjected to the processing conditions like cooking, pulverising, flaking, puffing and the organoleptic qualities, GABA content and total antioxidant activity were analysed. The cooked rice obtained the highest average mean score, followed by puffed, flaked and pulverised rice respectively. The average mean score of cooked GABA enriched rice varieties varied from 8.03 to 8.36. Meanwhile, a variation in the average mean scores was observed in puffed (7.82 to 8.07) followed by flaked (7.85 to 8.02) and pulverised (7.75 to 7.99) rice grains of all the varieties consecutively.

The GABA content of the processed products from *Jyothi, Njavara* and *Chitteni* showed a slight decline in values than that of GABA enriched raw rice probably due to the application of short time high temperature treatments. The pulverised rice obtained the higher GABA content, followed by that flaked, puffed and cooked rice. Among the rice products, the GABA content of cooked rice from three GABA enriched rice varieties varied from 84.34 mg/kg to 95.18 mg/kg. Meanwhile, changes in GABA content was found in puffed (95.09 mg/kg to 112.39 mg/kg) followed by flaked (98.23 mg/kg to 117.85 mg/kg) and pulverised (109.72 mg/kg to 125.04 mg/kg).

Processed food products from GABA enriched *Jyothi, Njavara* and *Chitteni* have shown total antioxidant activity (TAA) effectively by eliminating the free radical and among the processed rice products, the total antioxidant activity of cooked rice from three GABA enriched rice varieties varied from 6.98 µg/ml to 9. 50 µg/ml. Meanwhile, a variation of EC_{50} value was observed in puffed (6.53 µg/ml to 8.98 µg/ml) followed by flaked (6.02 µg/ml to 8.64 µg/ml) and pulverised (5.58 µg/ml to 8.16 µg/ml) consecutively.

The current study evaluated the possibilities for enriching GABA content in rice cultivars like *Jyothi, Njavara* and *Chitteni*. The processing methods like soaking and sprouting considerably improved the GABA content accumulation in rice varieties studied. The GABA enriched rice of all the three varieties were found to be nutritionally superior over raw rice and is a promising source of potential antioxidants. GABA enriched rice showed moderate antiradical and antiproliferative activities in

reducing hepatic cancer cell proliferation. Processed products made from GABA enriched rice also showed excellent sensory scores, and retained GABA and total antioxidant activity and could be inferred that, GABA enriched rice can be utilised for product diversification and value addition.

Future line of the study:

- The GABA enriched rice can be utilised as important ingredient for the preparation of several functional products
- Profiling of other bioactive compounds and antiproliferatory activity in different cancer cell lines can be done
- Applications of the neuroprotective effect of GABA enriched rice and the possibility for the development of nutraceuticals can be done
- Studies on the stability, photosensitivity and depletion of GABA during storage are yet to be done
- Effect of different packaging materials to increase the storage period of GABA enriched rice can be done

REFERENCES

Adamu, H. A., Imam, M. U., Ooi, D. J., Esa, N. M., Rosli, R., and Ismail, M. 2016. Perinatal exposure to germinated brown rice and its gamma amino-butyric acid-rich extract prevents high fat diet-induced insulin resistance in first generation rat offspring. *Food Nutr. Res.* 60: 30209.

Adijei, T., Splittstoesser, W., and Vandemark, J. 1976. Use of soybeans as sprouts. *Hort. Sci.* 11: 235-241.

Agarwal, G. P. and Hasija, S. K. 1986. *Micro Organism in the Laboratory*. Print House India Ltd., Lucknow, 155p.

Ainsworth, E. A. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nat. Protoc*. 2(4): 861-875.

Aiswarya, G. 2019. Nutritional and antioxidant potential of medicinal rice variety rakthashali. PhD thesis, Kerala Agricultural University, Thrissur, 180p.

Akama, K., Kanetou, J., Shimosaki, S., Kawakami, K., Tsuchikura, S., and Takaiwa, F. 2009. Seed-specific expression of truncated OsGAD2 produces GABA-enriched rice grains that influence a decrease in blood pressure in spontaneously hypertensive rats. *Transgenic Res.* 18: 865-876.

Alhendi, A. S., Al-Rawi, S. H., and Jasim, A. M. 2019. Effect of moisture content of two paddy varieties on the physical and cooked properties of produced rice. *Braz. J. Food Technol.* 22: 1-12.

Ali, B. H., Al-Salam, S., Al Za'abi, M., Al Balushi, K. A., AlMahruqi, A. S., Beegam, S., Al-Lawatia, I., Waly, M. I., and Nemmar, A. 2015. Renoprotective effects of gamma-aminobutyric acid on cisplatin-induced acute renal injury in rats. *Basic Clin. Pharmacol. Toxicol.* 116: 62-68.

Amakura, Y. 2008. Influence of jam processing on the radical scavenging activity and phenolic content in berries. *J. Agric. Food Chem*. 48(12): 6292-6297.

Anawachkul, M. and Jiamyangyuen, S. 2009. The study of GABA content and development of GABA-enriched yogurt from germinated red rice (Munpu rice). *Agric. Nat. Resour.* 43(5): 224-231.

AOAC [Association of Official Analytical Chemists]. 2012. *Official Methods of Analysis* (19th Ed.). Association of Official Analytical Chemist. Washington D.C, 130p.

Aquino, R., Morelli, S., Lauro, M. R., Abdo, S., Saija, A., and Tomaino, A. 2001. Phenolic constituents and antioxidant activity of an extract of anthurium versicolor leaves. *J. Nat. Prod*. 64(8): 1019-1023.

Aruoma, O. I. 1998. Free radicals, oxidative stress, and antioxidants in human health and disease. *J. Am. Oil Chem. Soc.* 75(2): 199-212.

Aswatha, R. H. N., Shreedhara, C. S., Falguni, G. P., and Sachin, Z. B. 2008. Antioxidant studies of aqueous extract of *Phyllanthus reticulates* Poir. *Pharmacol. Online* 1: 351-364.

Aurisano, N., Bertani, A., and Reggiani, R. 1995. Anaerobic accumulation of 4-aminobutyrate in rice seedlings–causes and significance. *Phytochemi.* 38(5): 1147-1150.

Ayemor, G. S. and Ocjoo, F. C. K. 2007. Physico-chemical changes and diastatic activity associated with germinating paddy rice (PSB.Rc 34). *Afr. J. Food Sci*. 1: 37-41.

Azizah, A. H. and Zainon, H. 1997. Effect of processing on dietary fiber contents of selected le gumes and cereals. *Malays. J. Nutr*. 3(2):131-136.

Azmi, N. H., Ismail, M., Ismail, N., Imam, M. U., Alitheen, N. B. M., and Abdullah, M. A. 2015. Germinated brown rice alters Aβ (1-42) aggregation and modulates Alzheimer's disease-related genes in differentiated human SH-SY5Y cells. *Evid-Based Complement. Altern. Med.* 2015*:* 1-12*.*

Bagli, E., Goussia, A., Moschos, M. M., Agnantis, N., and Kitsos, G. 2016. Natural compounds and neuroprotection: Mechanisms of action and novel delivery systems. *In Vivo*. 30: 535-547.

Bahadur, H. K. C. 2003. Useful germinated brown rice. *The Kathmandu*, 15 April 2009, p.13.

Bahnwar, S., Bamnia, M., Ghosh, M., and Ganguli, A. 2013. Use of Lactococcus lactis to enrich sourdough bread with γ-aminobutyric acid. *Int. J. Food Sci. Nutr.* 64: 77-81.

Bamforth, C.W. and Barclay, A.H.P. 1993. Malting technology and the uses of malt. In: Mac-Gregor, A.W. and Bhatty, R.S. (eds), *Barley: Chemistry and Technology*. AACC, St Paul, MN. pp. 297-354.

Bansal, P., Wang, S., Liu, S., Xiang, Y. Y., Lu, W. Y., and Wang, Q. 2011. GABA coordinates with insulin in regulating secretory function in pancreatic INS-1 β-cells. *PLoS ONE* 6(10): e26225.

Bao, G., Ashraf, U., Wang, C., He, L., Wei, X., Zheng, A., Mo, Z., and Tang, X. 2018. Molecular basis for increased 2-acetyl-1-pyrroline contents under alternate wetting and drying (AWD) conditions in fragrant rice. *Plant Physiol. Biochem.*133:149-157.

Barnes, D. S., Clapp, N. K., Scott, D. A., Oberst, D. L., and Berry, S. G. 1983. Effects of wheat, rice, corn, and soybean bran on 1, 2-dimethylhydrazine-induced large bowel tumorigenesis in F344 rats. *Nutr. Cancer* 5(1): 1-9.

Bau, H. M., Villaume, C, Nicolas, J. P., and Mejean, L. 1997. Effect of germination on chemical composition, biochemical constituents and antinutritional factors of soybean (Glycine max) seeds. *J. Sci. Food Agric*. 73(1): 1-9.

Beal, A. D. and Mottram, D. S. 1993. An evaluation of the aroma characteristics of malted barley by free-choice profiling. *J. Sci. Food Agric.* 61(1):17-22.

Bean, M. M. and Nishita, K. D. 2000. Rice flour for baking. In: Julino, (ed.), *Rice chemistry and technology* (2nd ed.). American Association of Cereal Chemists, St. Paul, U. S. A, pp. 539-556.

Bello, M., Marcela, P. T., and S. Constantino. 2004. Factors affecting water uptake of rice grain during soaking. *LWT – Food Sci. Technol.* 37: 811-816.

Bhat, F. M. and Riar, C. S. 2015. Health benefits of traditional rice varieties of temperate regions. *Med. Aromat. Plants 4*(3): 1-3.

Bishwajith, G., Sarker, S., Amara, K. M., Gao, H., Jun, L., Yin, D., and Ghosh, S. 2013. Self sufficiency in rice and food security a South Asian perspective. *Agric. Food Secur.* 2:10- 18.

Borden, L., Murali Dhar, T., and Smith, K. 1994. Tiagabine, S. F, 89976-A, CI-996 and NNC- 711 are selective for the cloned GABA transporter GAT-1. *Eur. J. Pharmacol.* 269(2): 219-224.

Bourneow, C. and Toontam, N. 2019. Microbiological quality and some bioactive compounds in relation to sensory profiles during germination of brown-purple-pigmented rice. *Int. Food Res. J.* 26(3): 1011-1021.

Bowery, N. G., Bettler, B., Froestl, W., Gallagher, J. P., Marshall, F., Raiteri, M., Bonner, T. I. and Enna, S. J. 2002. International Union of Pharmacology. XXXIII. Mammalian γ-aminobutyric acidB receptors: structure and function. *Pharmacol. Rev.* 54(2): 247-264.

Bown, A. W. and Shelp, B. J. 1997. The cloning of GABAb receptors. *Nat.* 386: 223-224.

Brar, B., Jain, S., Singh, R. and Jain, R. K. 2011. Genetic diversity for iron and zinc contents in a collection of 220 rice (*Oryza sativa* L.) genotypes. *Indian J. Genet. Plant Breed.* 71(1): 67-73.

Brousse, G., Arnaud, B., Vorspan, F., Richard, D., Dissard, A., Dubois, M., Pic, D., Geneste, J., Xavier, L., Authier, N., and Sapin, V. 2012. Alteration of

glutamate/GABA balance during acute alcohol withdrawal in emergency department: a prospective analysis. *Alcohol Alcohol.* 47(5): 501-508.

Caceres, P. J., Martinez-Villaluenga, C., Amigo, L., and Frias, J. 2014. Assessment on proximate composition, dietary fibre, phytic acid and protein hydrolysis of germinated Ecuatorian brown rice. *Plant Foods Hum. Nutr.* 69: 261-267.

Caceres, P. J., Penas, E., Martinez-Villaluenga, C., Amigo, L., and Frias, J. 2017. Enhancement of biologically active compounds in germinated brown rice and the effect of sun- drying. *J. Cereal Sci.* 73:1-9.

Cagno, R. D., Mazzacane, F., Rizzello, C. G., De Angelis, M., Giuliani, G., Meloni, M., De Servi, B., and Gobbetti, M. 2010. Synthesis of γ-aminobutyric acid (GABA) by Lactobacillus plantarum DSM19463: functional grape must beverage and dermatological applications. *Appl. Microbiol. Biotechnol.* 86: 731-741.

Cai, H., Al-Fayez, M., Tunstall, R. G., Platton, S., Greaves, P., Steward, W. P., and Gescher, A. J. 2005. The rice bran constituent tricin potently inhibits cyclooxygenase enzymes and interferes with intestinal carcinogenesis in Apc Min mice. *Mol. Cancer Ther.* 4(9): 1287-1292.

Cataldo, P. G., Villena, J., Elean, M., Savoy de Giori, G., Saavedra, L., and Hebert, E. M. 2020. Immunomodulatory Properties of a γ-Aminobutyric Acid-Enriched Strawberry Juice Produced by Levilactobacillus brevis CRL 2013. *Front Microbiol*. 11: 1-14.

CFTRI [Central Food Technological Research Institute]. 1969. *Parboiling of Paddy*. Central Food Technological Research Institute, Mysore, 92p.

Chaiyasut, C., Sivamaruthi, B. S., Pengkumsri, N., Keapai, W., Kesika, P., Saelee, M., and Tojing, P. 2016. Germinated Thai black rice extract protects experimental diabetic rats from oxidative stress and other diabetes-related consequences. *Pharma.*10(1): 1-6.

Chamba, J. F. and Irlinger, F. 2004. Secondary and adjunct cultures. *Cheese: Chem. Phy. Microbiol.* 1(C): 191-206.

Chandhni, A. A. 2015. Quality evaluation of newly released KAU rice (*Oryza sativa* L.) varieties and their suitability for traditional food products. MSc. (Home Science) thesis, Kerala Agricultural University, Thrissur, 250p.

Chang, C. C., Yang, M. H., Wen, H. M., and Chern, J. C. 2002. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J. Food. Drug Anal*. 10(3): 121-126.

Charoenthaikij, P., Jangchud, K., Jangchud, A., Piyachomkwan, K., Tungtrakul, P., and Prinyawiwatkul, W. 2009. Germination conditions affect physicochemical properties of germinated brown rice flour. *J. Food Sci.* 74(9): C658-C665.

Charoenthaikij, P., Jangchud, K., Jangchud, A., Prinyawiwatkul, W., and Tungtrakul, P. 2010. Germination conditions affect selected quality of composite wheat-germinated brown rice flour and bread formulations. *J. Food Sci*. 75(6): 312-318.

Chatchavanthatri, N., Junyusen, T., Arjharn, W., Treeamnuk, T., Junyusen, P., and Pakawanit, P. 2021. Effects of parboiling and infrared radiation drying on the quality of germinated brown rice. *J. Food Process. Preserv.* 45(11): 1-13.

Cheevitsopon, E. and Noomhorm, A. 2011. Effects of parboiling and fluidized bed drying on the physicochemical properties of germinated brown rice. *Int. J. Food Sci. Technol.* 46(12): 2498-2504.

Cheevitsopon, E. and Noomhorm, A. 2015. Effects of superheated steam fluidized bed drying on the quality of parboiled germinated brown rice. *J. Food Process. Preserv.* 39(4): 349-356.

Chen, J., Xie, W., Huang, Z., Ashraf, U., Pan, S., Tian, H., Duan, M., Wang, S., Tan, Z., and Mo, Z. 2021. Light quality during booting stage modulates fragrance, grain yield and quality in fragrant rice. *J. Plant Interact*. 16(1): 42-52.

Chen, L., Zhao, H., Zhang, C., Lu, Y., Zhu, X., and Lu, Z. 2016. γ-Aminobutyric acid-rich yogurt fermented by Streptococcus salivarius subsp. thermophiles fmb5 apprars to have anti-diabetic effect on streptozotocin-induced diabetic mice. *J. Funct. Foods* 20: 267-275.

Chen, X., Zhao, Z., Li, Z., Li, D., Xu, L., and Zhou, Z. 2017. Changes in the physicochemical properties, flavor components, and microbial community during the germination of rough rice. *J. Cereal Sci*. 74: 1-8.

Chen, Y., Jiang, W., Jiang, Z., Chen, X., Cao, J., Dong, W., and Dai, B. 2015. Changes in physicochemical, structural, and sensory properties of irradiated brown japonica rice during storage. *J. Agric. Food Chem*. 63(17): 4361-4369.

Cho, D. H. and Lim, S. T. 2016. Germinated brown rice and its bio-functional compounds. *Food Chem.* 196: 259-271.

Choe, H., Sung, J., Lee, J., and Kim, Y. 2021. Effects of calcium chloride treatment on bioactive compound accumulation and antioxidant capacity in germinated brown rice. *J. Cereal Sci.* 101: 1-6.

Choi, H., Kim, Y., Kim, W., Park, J., and Kim, K. 2014. Optimization of germination conditions for enhancing γ-aminobutyric acid content and antioxidant activities in brown rice. *Food Chem.* 156: 130-135.

Choi, I., Suh, S. J., Kim, J. H., and Kim, S. L. 2009. Effects of germination on fatty acid and free amino acid profiles of brown rice 'Keunnun'. *Food Sci. Biotechnol.* 18: 799-802.

Chompoopong, S., Jarungjitaree, S., Punbanlaem, T., Rungruang, T., Chongthammakun, S., Kettawan, A., and Taechowisan, T. 2016. Neuroprotective effects of germinated brown rice in rotenone-induced Parkinson's-like disease rats. *Neuromolecular Med.* 18: 334-346.

Chotimarkorn, C., Benjakul, S., and Silalai, N. 2008. Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand. *Food Chem*. 111(3): 636-641.

Chua, J. Y., Koh, M. K. P., and Liu, S. Q. 2019. Gamma-aminobutyric acid: a bioactive compound in foods. In: Feng, H., Nemzer, B., and DeVries, J. W. (eds), *Sprouted Grains: Nutritional Value*, *Production and Applications,* Woodhead Publishing and AACC International Press, Sawston, Cambridge, pp. 25-54.

Chung, H. J., Jang, S. H., Cho, H. Y., and Lim, S. T. 2009. Effects of steeping and anaerobic treatment on GABA (γ-aminobutyric acid) content in germinated waxy hull-less barley. *LWT-Food Sci. Technol.* 42(10): 1712-1716.

Chung, S. I., Lee, S. C., Yi, S. J., Kang, M. Y. 2018. Antioxidative and antiproliferative activities of ethanol extracts from pigmented giant embryo rice (*Oryza sativa* L. cv. Keunnunjami) before and after germination. *Nutr. Res. and Pract*. 12(5): 365-370.

Chungcharoen, T., Prachayawarakorn, S., Tungtrakul, P., and Soponronnarit, S. 2015. Quality attributes of germinated high-amylose and waxy rice in superheated steam and hot air drying. *Dry. Technol.* 33(7): 876-885.

Contineanu, I., Contineanu, M., Neacsu, A., and Perisanu, S. 2010. A DSC study of γ aminobutyric acid gamma irradiated. *Analele Universităţii Din Bucureşti – Chimie* 19(1): 79-86.

Cunha, D. S., Coelho, M. C., Ribeiro, S. C., Silva, C. C. G. 2022. Application of Enterococcus malodoratus SJC25 for the Manufacture of Whey-Based Beverage Naturally Enriched with GABA. *Foods* 11: 447.

Dagorn, A., Hillion, M., Chapalain, A., Lesouhaitier, O., Duclairoir Poc, C., Vieillard, J., Chevalier, S., Taupin, L., Le Derf, F., and Feuilloley, M. G. 2013. Gamma-aminobutyric acid acts as a specific virulence regulator in Pseudomonas aeruginosa. *Microbiol.* 159(2): 339-351.

Dai, W., Xie, D., Lin, Z., Yang, C., Peng, Q., Tan, J., and Lin, Z. 2020. A nontargeted and targeted metabolomics study on the dynamic changes in metabolite levels during the anaerobic treatment of γ-aminobutyric acid (GABA) tea. *LWT- Food Sci. Technol.* 126: 109313.

Deepa, G., Singh, V., and Naidu, K. A. 2008. Nutrient composition and physicochemical properties of Indian medicinal rice–Njavara. *Food Chem. 106*(1): 165-171.

Deepa, G., Singh, V., and Naidu, K. A. 2012. Characterization of antioxidant compounds and antioxidant activity of Indian rice varieties. *J. Herbs, Spices Medicinal Plants* 18(1): 18-33.

Devraj, L., Panoth, A., Kashampur, K., Kumar, A., and Natarajan, V. 2019. Study on physicochemical, phytochemical, and antioxidant properties of selected traditional and white rice varieties. *J. Food Process. Eng.* 43(4): 1-13.

Dhakal, R., Bajpai, V. K., and Baek, K. H. 2012. Production of GABA (γ-aminobutyric acid) by microorganisms: a review. *Brazilian J. Microbiol*. 43(4): 1230-1241.

Dhaliwal, Y. S. and Aggarwal, R. A. K. 1999. Composition of fat in soybeans as affected by duration of germination and drying temperature. *J. Food Sci. Technol.* 36(3): 266-267.

Diana, M., Rafecas, M., and Quilez, J. 2014a. Sourdough bread enriched with γ-aminobutyric acid (GABA). A comparison of free amino acid, biogenic amine and acrylamide content in GABA enriched sourdough and commercial breads. *J. Cereal Sci.* 60(3): 639-644.

Diez-Gutierrez, L., San Vicente, L., R. Barron, L. J., Villaran, M. del C., and Chavarri, M. 2020. Gamma-aminobutyric acid and probiotics: Multiple health benefits and their future in the global functional food and nutraceuticals market. *J. Funct. Foods* 64: 1-14.

Ding, J., Ulanov, A. V., Dong, M., Yang, T., Nemzer, B. V., Xiong, S., Zhao, S., and Feng, H. 2018. Enhancement of gama-aminobutyric acid (GABA) and other health-related metabolites in germinated red rice (*Oryza sativa* L.) by ultrasonication. *Ultrasonics Sonochem*. 40(Pt A): 791-797.

Divyashri, G., Gokul, K., and Prapulla, S.G. 2017. Oral supplementation of gaba containing rice flour alleviate acrylamide induced oxidative impairments and neurotoxicity in mice. *EC Nutr.* 8(6): 191-203.

Dodge, Y. 2003. *The Oxford Dictionary of Statistical Terms*. Oxford University Press, Oxford, 506p.

Doughari, J. H., Ndakidemi, P. A., Human, I. S., and Benade, S. 2012. Antioxidant, antimicrobial and antiverotoxic potentials of extracts of *Curtisia dentata*. *J. Ethnopharmacol.* 141(3): 1041-1050.

Dutta, K. K. and Barua, K. D. 1982. Physiological analysis of growth, yield development and grain quality of some deep-water rice (*Oryza sativa* L.) cultivars. *J. Agron. Crop Sci*. 192(3): 228-232.

Ebizuka, H., Ihara, M., and Arita, M. 2009. Antihypertensive effect of pre-germinated brown rice in spontaneously hypertensive rats. *Food Sci. Technol. Res.* 15(6): 625-630.

Ebuehi, O. A. T. and Oyewole, A. C. 2007. Effect of cooking and soaking on physical characteristics, nutrient composition and sensory evaluation of indigenous and foreign rice varieties in Nigeria. *Afr. J. Biotechnol.* 6(8): 1016-1020.

Ekholm, P., Virkki, L., Ylinen, M., and Johansson, L. 2003. The effect of phytic acid and some natural chelating agents on the solubility of mineral elements in oat bran. *Food Chem.* 80(2): 165-170.

El-Adawy, T. A., Rahma, E. H., El-Bedawey, A. A., and El-Beltagy, A. E. 2004. Nutritional potential and functional properties of germinated mung bean, pea and lentil seeds. *Plant Foods Hum. Nutr*. 58(3): 1-13.

Elizabeth, K., Yang, M. H., and Rao, M. N. A. 1990. Oxygen radical scavenging of curcumin. *Int. J. Pharm*. 58: 237-240.

Esa, N. M., Abdul-Kadir, K. K., Amomd, Z., and Azlan, A. 2013. Antioxidant activity of white rice, brown rice and germinated brown rice (*in vivo* and *in vitro*) and the

effects on lipid peroxidation and liver enzymes in hyperlipidaemic rabbits. *Food Chem*. 141(2): 1306-1312.

Esmaeelian, M., Jahani, M., Einafshar, S., and Feizy, J. 2020. Optimization of experimental parameters in subcritical water extraction of bioactive constituents from the saffron (*Crocus sativus* L.) corm based on response surface methodology. *J. Food Meas. Charact.* 14(25): 1822-1832.

Evans, R. and Miller, C.A. 2004. The relative antioxidant activities of plant derived polyphenolic flavonoids. *Free Radic. Res*. 22(4): 375-383.

FAO [Food and Agriculture Organization]. 2020. Production, trade and prices of commodities. Fao statistical year book 2020. World food and agriculture, Rome, Italy, 307p.

Finocchiaro, F., Ferrari, B., and Gianinetti, A. 2010. A study of biodiversity of flavonoid content in the rice caryopsis evidencing simultaneous accumulation of anthocyanins and proanthocyanidins in a black-grained genotype. *J. Cereal Sci.* 51(1): 28-34.

Finocchiaro, F., Ferrari, B., Gianinetti, A., Dall'Asta, C., Galaverna, G., Scazzina, F., and Pellegrini, N. 2007. Characterization of antioxidant compounds of red and white rice and changes in total antioxidant capacity during processing. *Mol. Nutr. Food Res.* 51(8): 1006-1019.

Frazier W. C. and Westhoff, D. C. 1978. *Food Microbiology* (3rd Ed.). MacGraw-Hill Publishing Company, New York, 540p.

Gan, R. Y., Li, H. B., Gunaratne, A., Sui, Z. Q., and Corke, H. 2017. Effects of fermented edible seeds and their products on human health: bioactive components and bioactivities. *Compr. Rev. Food Sci. Food Saf.* 16: 489-531.

García-de-la-Asunción, J., de Toro, P., Planelles, V., Belda, J., Perez-Griera, J., Gambini, J., and Domenech, E. 2009. Bemiparin improves the total antioxidant status in plasma. *Eur. J. Pharmacol.* 602(2-3): 380-382.

Geethakutty, P. S., Nair, S., and Pratheesh, V. S. 2004. *Medicinal Rice and Traditional Knowledge of Women: Science – Society Interface on Medicinal and Aromatic Rices*. M. S. Swaminathan Research Foundation, Chennai and Kerala Agricultural University, Thrissur. 123p.

German, J. B. and Dillard, C. J. 2006. Composition, structure and absorption of milk lipids: a source of energy, fat-soluble nutrients and bioactive molecules. *Crit. Rev. Food Sci. Nutr.* 46(1): 57-92.

Ghavidel, R. A. and Prakash, J. 2007. The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. *LWT-Food Sci. Technol.* 40(7): 1292-1299.

Gong, J., Wang, J., Jin, Y., Xiao, G., You, Y., Yuan, H., Li, L., Huang, J., Liu, S., Mao, J., and Li, B. 2019. Effect of γ-aminobutyric acid supplementation on the composition of Chinese rice wine. *J. Inst. Brewi. 125*(1): 110-117.

González, R. J., Livore, A., and Pons, B. 2004. Physico-chemical and cooking characteristics of some rice varieties. *Braz. Arch. Biol. Technol.* 47(1): 71-76.

Gopalan, C., Sastri, B. V. R. and Balasubramanian, S. C. 2012. *Nutritive Value of Indian Foods.* National Institute of Nutrition, Hyderabad, 137p.

Gopalan, C., Sastri, B. V. R. and Balasubramanian, S.C. 1994. *Nutritive Value of Indian Foods*. National Institute of Nutrition, ICMR, Hyderabad, 137p.

Gramazio, P., Takayama, M., and Ezura, H. 2020. Challenges and Prospects of New Plant Breeding Techniques for GABA Improvement in Crops: Tomato as an Example. *Front. Plant Sci.* 11: 1-16.

Gregorio, G. B., Senadhira, D., Htut, H., and Graham, R. D. 2000. Breeding for trace mineral density in rice. *Food and Nutr. Bull*. 21(4): 382-386.

Grewal, P. and Sangha, J.K. 1990. Effect of processing on thiamin and riboflavin contents of some high yielding rice varieties of Punjab. *J. Sci. Food Agric.* 52(3): 387-391.

Guiyun, C., Yushan, W., Mingyue, Z., Wanxing, M., Xixian, X., and Ye, C. 2022. Cold atmospheric plasma treatment improves the γ-aminobutyric acid content of buckwheat seeds providing a new anti-hypertensive functional ingredient. *Food Chem.* 388: 133064.

Gumul, D., Korus, J., and Achremowicz, B. 2007. The influence of extrusion on the content of polyphenols and antioxidant/antiradical activity of rye grains (*Secale cereale* L.). *Acta Sci. Pol. Technol Aliment*. 6(4): 103-111.

Gunathilake, D. C. and Senadeera, W. 2023. Mild hydrothermal treatment for improving outturn of basmati rice. *AgriEng*. 5(2): 992-1004.

Gupta, E., Sinha, J., and Dubey, R. P. 2012. Utilization of dehydrated herbs in the formulation of value added snack "rice flakes mix". *J. Food Process. Technol.* S1-002: 1-4.

Hagiwara, H., Seki, T., and Ariga, T. 2004. The effect of pre-germinated brown rice intake on blood glucose and PAI-1 levels in streptozotocin-induced diabetic rats. *Biosci. Biotechnol. Biochem.* 68: 444 - 447.

Hahm, T., Park, S., and Lo, Y. M. 2008. Effects of germination on chemical composition and functional properties of sesame (Sesamum indicum L.) seeds. *Bioresourc. Technol.* 100(4): 1643-1647.

Han, A., Jinn, J. R., Mauromoustakos, A., and Wang, Y. J. 2016. Effect of parboiling on milling, physicochemical, and textural properties of medium- and long-grain germinated brown rice. *Cereal Chem*. 93(1): 47-53.

Han, D., Kim, H. Y., Lee, H. J., Shim, I., and Hahm, D. H. 2007. Wound healing activity of gamma- aminobutyric acid (GABA) in rats. *J. Microbiol. Biotechnol.* 17:1661-1669.

Han, M., Liao, W. Y., Wu, S. M., Gong, X., and Bai, C. 2020. Use of Streptococcus thermophilus for the in situ production of γ-aminobutyric acid-enriched fermented milk. *J. Dairy Sci.* 103(1): 98-105.

Havsteen, B. H. 2002. The biochemistry and medical significance of the flavonoids. *Pharmacol Ther*. 96(2-3): 67-202.

Hayakawa, T., Suzuki, S., Kobayashi, S., Fukutomi, T., Ide, M., Ohno, T., Ohkouchi, M., Taki, M., Miyamoto, T., and Nimura, T. 2009. Effect of pre-germinated brown rice on metabolism of glucose and lipid in patients with diabetes mellitus type 2. *J. Jpn. Assoc. Rural Med.* 58: 438 - 446.

Hayat, A., Jahangir, T. M., Khuhawar, M. Y., Alamgir, M., Hussain, Z., Haq, F. U., and Musharraf, S. G. 2015. HPLC determination of gamma amino butyric acid (GABA) and some biogenic amines (BAs) in controlled, germinated, and fermented brown rice by pre-column derivatization. *J. Cereal Sci*. 64: 56 -62.

Hayat, A., Jahangir, T. M., Khuhawar, M. Y., Alamgir, M., Siddiqui, A. J., and Musharraf, S. G. 2014. Simultaneous HPLC determination of gamma amino butyric acid (GABA) and lysine in selected pakistani rice varieties by pre-column derivatization with 2- hydroxynaphthaldehyde. *J. Cereal Sci*. 60: 356 -360.

Hepsomali, P., Groeger, J. A., Nishihira, J., and Scholey, A. 2020. Effects of oral gamma-aminobutyric acid (GABA) administration on stress and sleep in humans: A systematic review. *Front. Neurosci.* 14(923): 1-13.

Hinton, T. and Johnston, G. A. R. 2020. GABA-enriched teas as neuronutraceuticals. *Neurochem. Int*. 141: 104895.

Horie, T. 2019. Global warming and rice production in Asia: Modeling, impact prediction and adaptation. *Proc. Jpn. Acad, Ser.* 95(6): 211-245.

Hsu, T. F., Kise, M., Wang, M. F., Ito, Y., Yang, M. D., Aoto, H., Yoshihara, R., Yokoyama, J., Kunii, D., and Yamamoto, S. 2008. Effects of pre-germinated brown rice on blood glucose and lipid levels in free-living patients with impaired fasting glucose or type 2 diabetes. *J. Nutr. Sci. Vitaminol.* 54: 163-168.

Huang, C. Y., Kuo, W. W., Wang, H. F., Lin, C. J., Lin, Y. M., Chen, J. L., Kuo, C. H., Chen, P. K., and Lin, J. Y. 2014. GABA tea ameliorates cerebral cortex apoptosis and autophagy in streptozotocin-induced diabetic rats. *J. Funct. Foods* 6: 534-544.

Huang, S. H. and Ng, L. T. 2012. Quantification of polyphenolic content and bioactive constituents of some commercial rice varieties in Taiwan. *J. Food Compos. Anal.* 26(1-2): 122-127.

Hwang, S. S., Cheng, Y. C., Chang, C., Lur, H. S., and Lin, T. T. 2009. Magnetic resonance imaging and analyses of tempering processes in rice kernels. *J. Cereal Sci*. 50: 36-42.

Imam, M. U., Azmi, N. H., Bhanger, M. I., Ismail, N., and Ismail, M. 2012. Antidiabetic properties of germinated brown rice: a systematic review. *Evid. Complement. Altern. Med.* 2012:1-13.

Imam, M. U., Ishaka, A., Ooi, D. J., Zamri, N. D. M., Sarega, N., Ismail, M., and Esa, N. M. 2014. Germinated brown rice regulates hepatic cholesterol metabolism and cardiovascular disease risk in hypercholesterolaemic rats. *J. Funct. Foods* 8: 193-203.

IRRI [International Rice Research Institute]. 2002. Rice Almanac (3rd Ed.). International Rice Research Institute, Metro Manila, Philippines, 254p.

Ismail, N., Ismail, M., Fathy, S. F., Musa, S. N. A., Imam, M. U., Foo, J. B., and Iqbal, S. 2012. Neuroprotective effects of germinated brown rice against hydrogen peroxide induced cell death in human SH-SY5Y cells. *Int. J. Mol. Sci.* 13(8): 9692-9708.

Itagi, H., Sartagoda, K. J. D., Pratap, V., Roy, P., Tiozon, R. N., Regina, A., and Sreenivasulu, N. 2023. Popped rice with distinct nutraceutical properties. *LWT-Food Sci. Technol.* 173(7): 1-10.

Ito, Y., Mizukuchi, A., Kise, M., Aoto, H., Yamamoto, S., Yoshihara, R., and Yokoyama, J. 2015. Postprandial blood glucose and insulin responses to pre-germinated brown rice in healthy subjects. *J. Med. Investig.* 52: 159-164.

Itu, N., Hibui, H., Li, T., Horibata, T., Fuwa, H., and Itani, T. 2004. Structure and properties of endosperm starches from cultivated rice of Asia and other countries. *J. Appl. Glycosci*. 52(3): 239-246.

Jabeen, R., Hussain, S. Z., Jan, N., Fatima, T., Naik, H. R., and Jabeen, A. 2023. Comparative study of brown rice and germinated brown rice for nutritional composition, in vitro starch digestibility, bioactive compounds, antioxidant activity and microstructural properties. *Cereal Chem.* 100(2): 434-444.

Jackson, M. L. 1973. *Soil Chemical Analysis*. Prentice Hall of India Private Ltd., New Delhi, 299p.

Jan, R., Saxena, D. C., and Singh, S. 2017. Physico-chemical, textural, sensory and antioxidant characteristics of gluten e Free cookies made from raw and germinated Chenopodium (*Chenopodium album*) flour. *LWT – Food Sci. Technol.* 71: 281-287 (in press).

Jannoey, P., Niamsup, H., Lumyong, S., Suzuki, T., Katayama, T., and Chairote, G. 2010. Comparison of gamma-aminobutyric acid production in Thai rice grains. *World J. Microbiol. Biotechnol.* 26(2): 257-263.

Jayaraman, R., Yadavalli, C., Singh, V., and Khanum, F. 2019. Phenolic compounds and antioxidant activities in dehusked and polished pigmented rice varieties. *ORYZA-An Int. J. Rice* 56(3): 263-284.

Jellenick, G. 1985. *Sensory Evaluation of Food-Theory and Practice*. Ellis Horwood Ltd., Chichtester, England and VCH, Weinheim, Federal Republic of Germany, 204p.

Jiamyangyuen, S. and Ooraikul, B. 2008. The physico-chemical, eating and sensorial properties of germinated brown rice. *J. Food Agric. Environ.* 6(2): 119-124.

Jiang, T., Yue, Y., and Li, F. 2018. Improvement of gamma-aminobutyric acid on intestinal mucosalbarrier injury of colitis induced by 2,4,6-trinitrobenzene sulfonic acid and alcohol. *Her. Med.* 37: 931-938.

Jongyingcharoen, J. S. and Cheevitsopon, E. 2016. Development of UV-treated cooked germinated brown rice and effect of UV-C treatment on its storability, GABA content, and quality. *LWT-Food Sci. Technol.* 71: 243-248 (in press).

Juliano, B. O. 1994. Utilization of rice functional properties to produce rice food products with modern processing technologies. *Int. Rice Comm. Newsl*. 39: 163-179.

Juliano, B. O. and Betchtel, D. B. 1985. The rice grain and its gross composition. In: Juliano, B. O. (ed.). Rice: Chemistry and technology. Am. Assoc. Cereal Chem. St. Paul, MN, USA, pp. 17-57.

Kakee, A., Takanaga, H., Terasaki, T., Naito, M., Tsuruo, T., and Sugiyama, Y. 2001. Efflux of a suppressive neurotransmitter, GABA, across the blood–brain barrier. *J. Neurochem.* 79(1): 110-118.

Kalpana, R. and Rao, M. 1995. On the ageing mechanism in pigeonpea (*Cajanus cajan* (L.) Millsp) seeds. *Seed Sci. Technol.* 23(1): 1-9.

Kaminsky, N., Bihari, O., Kanner, S., and Barzilai, A. 2016. Connecting malfunctioning glial cells and brain degenerative disorders. *Genom. Proteom. Bioinform.* 14:155-165.

Kaosa-Ard, T. and Songsermpong, S. 2012. Influence of germination time on the GABA content and physical properties of germinated brown rice. *Asian J. Food Agro-Ind.* 5(4): 270-283.

Kapoor, N., Arya, A., Siddiqui, M.A., Kumar, H., and Amir, A. 2011. Physiological and biochemical changes during seed deterioration in aged seeds of rice (*Oryza sativa* L.). *Am. J. Plant Physiol.* 6(1): 28-35.

Karladee, D. and Suriyong, S. 2012. γ-Aminobutyric acid (GABA) content in different varieties of brown rice during germination. *Sci. Asia* 38(1): 13-17.

Kasote, D., Sreenivasulu, N., Acuin, C., and Regina, A. 2022. Enhancing health benefits of milled rice: Current status and future perspectives. *Crit. Rev. Food Sci. Nutr. 62*(29): 8099-8119.

Katekhong, W. and Charoenrein, S. 2012a. The effect of rice aging on the freeze–thaw stability of rice flour gels. *Carbohydr. Polym*. 89(3): 777-782.

Katekhong, W. and Charoenrein, S. 2012b. Effect of rice storage on pasting properties, swelling and granular morphology of rice flour. *Asian Journal of Food and Agro-Ind*. 5(4): 315-321.

Kaur, P., Singh, N., Pal, P., and Kaur, A. 2018. Variation in composition, protein and pasting characteristics of different pigmented and non-pigmented rice (*Oryza sativa* L.) grown in Indian Himalayan region. *J. Food Sci. Technol.* 55(9): 3809-3820.

Kaushik, G., Satya, S., and Naik, S. N. 2010. Effect of domestic processing techniques on the nutritional quality of the soybean. *Mediterr. J. Nutr. Metabol*. 3: 39-46.

Kavyashree, N. M., Diwan, J. R., Mahantashivayogayya, K., Lokesha, R., Naik, N. M., and Shakuntala, N. M. 2021. Germinated brown rice as a potential source of Gaba: An inhibitory neurotransmitter. *Pharma Innov.* 10(11): 2993-2996.

Kawakami, K., Yamada, K., Yamada, T., Nabika, T., and Nomura, M. (2018). Antihypertensive effect of γ-aminobutyric acid-enriched brown rice on spontaneously hypertensive rats. *J. Nutr. Sci. Vitaminol.* 64(1): 56-62.

Kawasaki, A., Hara, T., and Joh, T. 2014. Inhibitory effect of γ-aminobutyric acid (GABA) on histamine release from rat basophilic leukemia RBL-2H3 cells and rat peritoneal exudate cells. *Japn. Soc. Food Sci. Technol*. 61: 362-366.

Kayahara, H., Tsukahara, K., and Tatai, T. 2001. Flavor, health and nutritional quality of pre-germinated brown rice. In: Spanier, A. M., Shahidi, F., Parliament, T. H.,

Mussinan, C., Ho, C. T., and Contis, E. T. (eds), *Food flavors and chemistry: advances of the new millennium: Proceedings of the 10th International Flavor Conference* 4-7 July 2000. Royal Society of Chemistry, Paros, Greece, pp. 546-551.

Keawpeng, I. and Venkatachalam, K. 2015. Effect of aging on changes in rice physical qualities. *Int. Food Res. J.* 22(6): 2180-2187.

Khan, W., Bhatt, P. C., and Panda, B. P. 2015. Degradation Kinetics of Gamma Amino Butyric Acid in M onascus-Fermented Rice. *J. Food Qual.* 38(2): 123-129.

Kharroubi, A. T. and Darwish, H. M. 2015. Diabetes mellitus: The epidemic of the century. *World J. Diabetes* 6(6): 850-867.

Khatoon, N. and Prakash, J. 2006. Nutrient retention in microwave cooked germinated legumes. *Food Chem.* 97(1):115-121.

Khwanchai, P., Chinprahast, N., Pichyangkura, R., and Chaiwanichsiri, S. 2014. Gamma- aminobutyric acid and glutamic acid contents, and the GAD activity in germinated brown rice (Oryza sativa L.): Effect of rice cultivars. Food Sci. Biotechnol. 23: 373-379.

Kim, H. Y., Hwang, I. G., Kim, T. M., Woo, K. S., Park, D. S., and Kim, J. H. 2012. Chemical and functional components in different parts of rough rice (*Oryza sativa* L.) before and after germination. *Food Chem*. 134(1): 288-293.

Kim, H. Y., Yokozawa, T., Nakagawa, T., and Sasaki, S. 2004. Protective effect of gamma-aminobutyric acid against glycerol-induced acute renal failure in rats. *Food Chem. Toxicol.* 42: 2009-2014.

Kim, H., Kim, O. W., Ha, A. W., and Park, S. 2016. Determination of optimal harvest time of chuchung variety green rice (Oryza sativa L.) with high contents of GABA, γ-oryzanol, and α-tocopherol. *Prev. Nutr. Food Sci.* 21(2): 97-103.

Kim, J. K., Kim, Y. S., Lee, H. M., Jin, H. S., Neupane, C., Kim, S., Lee, S. H., Min, J. J., Sasai, M., Jeong, J. H., and Choe, S. K. 2018. GABAergic signaling linked to autophagy enhances host protection against intracellular bacterial infections. *Natur. Commun.* 9(1): 4184.

Kim, K. S., Kim, B. H., Kim, M. J., Han, J. K., Kum, J. S., and Lee, H. Y. 2012. Quantitative microbiological profiles of brown rice and germinated brown rice. *Food Sci. Biotechnol.* 21(6): 1785-1788.

Kim, H. 2013. Functional foods and the biomedicalisation of everyday life: A case of germinated brown rice. *Sociol. of Health Ill.* 35(6): 842–857.

Kittibunchakul, S., Yuthaworawit, N., Whanmek, K., Suttisansanee, U., and Santivarangkna, C. 2021. Health beneficial properties of a novel plant-based probiotic drink produced by fermentation of brown rice milk with GABA-producing Lactobacillus pentosus isolated from Thai pickled weed. *J. Funct. Foods*. 86: 104710.

Klausberger, T. and Somogyi, P. 2008. Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. *Sci.* 321(5885): 53-57.

Ko, C. Y., Lin, H. T. V., and Tsai, G. J. 2013. Gamma-aminobutyric acid production in black soybean milk by Lactobacillus brevis FPA 3709 and the antidepressant effect of the fermented product on a forced swimming rat model. *Process Biochem.* 48(4): 559-568.

Komatsuzaki, N., Shima, J., Kawamoto, S., Momose, H., and Kimura, T. 2005. Production of γ-aminobutyric acid (GABA) by *Lactobacillus paracasei* isolated from traditional fermented foods. *Food microbiol.* 22(6): 497-504.

Komatsuzaki, N., Tsukahara, K., Toyoshima, H., Suzuki, T., Shimzu, N., and Kimura, T. 2007. Effect of soaking and gaseous treatment on GABA content in germinated brown rice. *J. Food Eng*. 78(2): 556-560.

Konwatchara, T. and Ahromrit, A. 2014. Effect of cooking on functional properties of germinated black glutinous rice (KKU-ULR012). *Songklanakarin J. Sci. Technol.* 36(3): 283-290.

Kubota, A., Kobayashi, M., Sarashina, S., Takeno, R., Yasuda, G., Narumi, K. Furugen, A., Takahashi-Suzuki, N., and Iseki, K. 2018. Gamma-aminobutyric acid (GABA) attenuates ischemia reperfusion-induced alterations in intestinal immunity. *Biol. Pharm. Bull.* 41: 1874-1878.

Kumar, S. H. I. V. and Prasad, K. 2017. Optimization of flaked rice dry roasting in common salt and studies on associated changes in chemical, nutritional, optical, physical, rheological and textural attributes. *Asian J. Chem.* 29(6):1380-1392.

Kunze, O. R. 2001. Effect of drying on grain quality: Moisture read sorption causes fissured grains. *Cereal Food World* 46(1): 16-19.

Kyung-Ha, L., Soon-Duck, Y., Jeong-Heui, L., Yong-Jae, W., Induck, C., Hye-Young, P., Koan-Sik, W., and Sea-Kwan, O. 2016. Studies on the degree of polymerization of amylopectin and texture analysis test of brown rice after germination. *Korean J Crop Sci*. 61(1):1-8.

Lai, P., Li, K.Y., Lu, S., and Chen, H. H. 2009. Phtochemicals and antioxidant properties of solvent extracts from Japonica rice bran. *Food Chem*. 117(3): 538-544.

Lai, S. C., Ho, Y. L., Huang, S. C., Huang, T. H., Lai, Z. R., Wu, C. R., Lian, K. Y., and Chang,Y. S. 2010. Antioxidant and antiproliferative activities of Desmodium triflorum (L.) DC. *Am. J. Chin. Med.* 38: 329-342.

Lakshmi, A. S. 2011. Quality evaluation of parboiled rice and its products from germinated rice. M. Sc. (Home Science) thesis, Kerala Agricultural University, Thrissur, 84p.

Lamberts, L., Joye, I., Belien, T., and Delcour, J. 2012. Dynamics of γ-aminobutyric acid in wheat flour bread making. *Food Chem.* 130: 896-901.

Laokuldilok, T., Shoemaker, C. F., Jongkaewwattana, S., and Tulyathan, V. 2011. Antioxidants and antioxidant activity of several pigmented rice brans. *J. Agric. Food Chem.* 59(1): 193-199.

Latifah, S. Y., Armania, N., Tze, T. H., Azhar, Y., Nordiana, A. H., Norazalina, S., Hairuszah, I., Saidi, M., and Maznah, I. 2010. Germinated brown rice (GBR) reduces the incidence of aberrant crypt foci with the involvement of β-catenin and COX-2 in azoxymethane-induced colon cancer in rats. *Nutr J.* 9(1): 1-8.

Lee, G., Piao, R., Lee, Y., Kim, B., Seo, J., Lee, D., Jang, D. S., Jin, Z., Lee, C., Chin, J. H., and Koh, H. J. 2019b. Identification and characterization of large embryo, a new gene controlling embryo size in rice (*Oryza sativa* L.). *Rice* 12(1): 1-12.

Lee, J. S., Sreenivasulu, N., Hamilton, R. S., and Kohli, A. 2019a. Brown rice, a diet rich in health promoting properties. *J. Nutr. Sci. Vitaminol.* 65(Supplement): S26-S28.

Lee, J., Kim, K., Kim, K., Kim, Y., and Lee, H. 2009. Effects of germination conditions on γ- aminobutyric acid content and α-amylase activity in brown rice. *J. Korean Soc. Food Sci. Nutr.* 38(6): 797-802.

Lee, S. Y., Park, S. L., Nam, Y. D., and Lee, S. H. 2013. Anti-diabetic effects of fermented green tea in KK-Ay diabetic mice. *Korean J. Food Sci. Technol. 45*: 488-494.

Lee, Y. J., Kim, M. Y., Kim, H. Y., Woo, K. S., Lee, M. J., Lee, J., and Jeong, H. S. 2019. Cooking characteristics and sensory evaluation of rice cooked with germinated barley. *Int. J. Food Eng.*15(11-12): 1-11.

Lee, Y. R., Woo, K. S., Kim, K. J., Son, J. R., and Jeong, H. S. 2007. Antioxidant activities of ethanol extracts from germinated specialty rough rice. *Food Sci. Biotechnol*. 16(5): 765-770.

Leena Kumari S. 2012. *Status Paper on Rice in Kerala-Rice Knowledge Management Portal.* Directorate of Rice Research, Rajendranagar, Hyderabad, India. 32 pp.

Li, H., Gao, D., Cao, Y., and Xu, H. 2008. A high γ-aminobutyric acid-producing *Lactobacillus brevis* isolated from Chinese traditional paocai. *Ann. Microbiol.* 58(4): 649-653.

Li, S. C., Lin, H. P., Chang, J. S., and Shih, C. K. 2019. Lactobacillus acidophilus-fermented germinated brown rice suppresses preneoplastic lesions of the colon in rats. *Nutr.* 11(11): 2718-2734.

Li, Y., Liu, K .L., Chen, F. .S, and ,Cheng Y. Q. 2018.Comparative proteomics analysis reveals the effect of germination and selenium enrichment on the quality of brown rice during storage. *Food Chem.* 269: 220-227.

Liang, J., Han, B. Z., Nout, M. J. R., and Hamer, R. J. 2008. Effect of soaking, germination and fermentation on phytic acid, total and in vitro soluble zine in brown rice. *Food Chem.* 110: 821-828.

Lin, S. D., Mau, J. L., and Hsu, C.A. 2012. Bioactive components and antioxidant properties of gamma-aminobutyric acid (GABA) tea leaves. *Lwt-Food Sci. Technol.* 46(1): 64-70.

Liu, J., Xu, Y., Nie, Y., and Zhao, G. A. 2012. Optimization production of acid urease by *Enterobacter* sp in an approach to reduce urea in Chinese rice wine. *Bioproc. Biosyst. Eng.* 35:651-657

Liu, L., Guo, J., Zhang, R., Wei, Z., Deng, Y., Guo, J., and Zhang, M. 2015. Effect of degree of milling on phenolic profiles and cellular antioxidant activity of whole brown rice. *Food Chem.* 185: 318-325.

Liu, R., He, X., Shi, J., Nirasawa, S., Tatsumi, E., Li, L., and Liu, H. 2013. The effect of electrolyzed water on decontamination, germination and γ-aminobutyric acid accumulation of brown rice. *Food Control*. 33(1): 1-5.

Liu, W., Son, D. O., Lau, H. K., Zhou, Y., Prud'homme, G. J., Jin, T., and Wang, Q. 2017. Combined oral administration of GABA and DPP-4 inhibitor prevents beta cell damage and promotes beta cell regeneration in mice. *Front. Pharmacol.* 8: 362.

Liu, X. P., Lai, T., Huang, S. P., Tang, J., Zhang, J. H., Wang, K. K., and Yao, E. P. 2018. Effects of different harvesting periods on japonica rice yield. *Bull. Agric. Sci. Technol.* 7(7):105-107.

Lobo, V., Patil, A., Phatak, A., and Chandra, N. 2010. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn. Rev.* 4(8): 118.

Lu, L., Tian, S., Liao, H., Zhang, J., Yang, X., Labavitch, J.M., and Chen, W. 2013. Analysis of metal element distributions in rice (*Oryza sativa* L.) seeds and relocation during germination based on X-ray fluorescence imaging of Zn, Fe, K, Ca, and Mn. *PLoS One* 8(2): 1-9.

Lu, Z.H., Li, L.T., Min, W.H., Feng, W., and Tatsumi, E. 2010.The effects of natural fermentation on the physical properties of rice flour and the rheological characteristics of rice noodles. *Int. J. Food Sci. Technol.* 40(9): 985-992.

Luo, Y., Xie, W., and Cui, Q. 2010. Effects of phytases and dehulling treatments on in vitro iron and zinc bioavailability in faba bean (*Vicia faba* L.) flour and legume fractions. *J. Food Sci.* 75(2): 191-198.

Ma, Z., Zhai, X., Zhang, N., and Tan, B. 2023. Effects of germination, fermentation and extrusion on the nutritional, cooking and sensory properties of brown rice products: a comparative study. *Foods 12*(7): 1542.

Mabunga, D. F. N., Gonzales, E. L. T., Kim, H. J., and Choung, S. Y. 2015. Treatment of GABA from fermented rice germ ameliorates caffeine-induced sleep disturbance in mice. *Biomol. Ther.* 23(3): 268.

Maisont, S. and Narkrugsa, W. 2010. The effect of germination on GABA content, chemical composition, total phenolics content and antioxidant capacity of Thai waxy paddy rice. *Agric. Nat. Resour*. 44(5): 912-923.

Maksup, S., Pongpakpian, S., Roytrakul, S., Cha-Um, S., and Supaibulwatana, K. 2018. Comparative proteomics and protein profile related to phenolic compounds and antioxidant activity in germinated Oryza sativa 'KDML105'and Thai brown rice 'Mali Daeng'for better nutritional value. *J. Sci. Food Agric.* 98(2): 566-573.

Manna, K. M., Naing, K. M., and PE, H. 1995. Amylase activity of some roots and sprouted cereals and beans. *Food Nutr. Bull.*16(2): 1-4.

Manosroi, J., Dhumtanom, P., and Manosroi, A. 2006. Anti-proliferative activity of essential oil extracted from Thai medicinal plants on KB and P388 cell lines. *Cancer Lett.* 235(1): 114-120.

Martinez, A.W., Phillips, S.T., Butte, M.J., and Whitesides, G. M. 2007. Patterned paper as a platform for inexpensive, low-volume, portable bioassays. *Angewandte. Chemie*.*119*(8): pp.1340-1342.

Marxen, K., Vanselow, K.H., Lippemeier, S., Hintze, R., Ruser, A., and Hansen, U.P. 2007. Determination of DPPH radical oxidation caused by methanolic extracts of some microalgal species by linear regression analysis of spectrophotometric measurements. *Sens*. 7(10): 2080-2095.

Matsubara, F., Ueno, H., Tadano, K., Suyama, T., Imaizumi, K., Suzuki, T., and Saruta, T. 2002. Effects of GABA supplementation on blood pressure and safety in adults with mild hypertension. *Jpn. Pharmacol. Ther.* 30: 963-972

McCord, J.M. and Fridovich, I. 1969. The utility of superoxide dismutase in studying free radical reactions generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. *J. Biol. Chem.* 244(22): 6056-6063.

McKie, V. A. and McCleary, B. V. 2015. A rapid, automated method for measuring α-amylase in pre-harvest sprouted (sprout damaged) wheat. *J. Cereal Sci.* 64: 70-75.

Mei, X., Chen, Y., Zhang, L., Fu, X., Wei, Q., Grierson, D., Zhou, Y., Huang, Y., Dong, F., and Yang, Z. 2016. Dual mechanisms regulating glutamate decarboxylases and accumulation of gamma-aminobutyric acid in tea (Camellia sinensis) leaves exposed to multiple stresses. *Sci. Rep.* 6(1): 23685.

Mei, X., Xu, X., and Yang, Z. 2020. Characterization of two tea glutamate decarboxylase isoforms involved in GABA production. *Food Chem.* 305: 125440.

Meldrum, B. S. and Chapman, A. G. 1999. Basic mechanisms of Gabitril (tiagabine) and future potential developments. *Epilepsia* 40:2-6.

Menon, M.V. 2004. Njavara: the healing touch. *Sci. Report*. 41(2): 28-30.

Mir, S.A., Bosco, S.J.D., Shah, M.A., and Mir, M.M. 2016. Effect of puffing on physical and antioxidant properties of brown rice. *Food Chem.* 19: 139-146.

Mody, I. and Pearce, R. A. 2004. Diversity of inhibitory neurotransmission through GABAA receptors. *Trends Neurosci.* 27(9): 569-575.

Mohan, V., Spiegelman, D., Sudha, V., Gayathri, R., Hong, B., Praseena, K., Anjana, R. M., Wedick, N. M., Arumugam, K., Malik, V., and Ramachandran, S., 2014. Effect of brown rice, white rice, and brown rice with legumes on blood glucose and insulin responses in overweight Asian Indians: a randomized controlled trial. *Diabetes Technol. Ther.*16(5): 317-325.

Mohanlal, S., Parvathy, R., Shalini, V., Helen, A., and Jayalekshmy, A. 2011. Isolation, characterization and quantification of tricin and flavonolignans in the medicinal rice Njavara (Oryza sativa L.), as compared to staple varieties. *Plant Foods Hum. Nutr.* 66: 91-96.

Mohd Esa, N., Abdul Kadi,r K. K., Amom, Z., and Azlan, A. 2013. Antioxidant activity of white rice, brown rice and germinated brown rice (in vivo and in vitro) and the effects on lipid peroxidation and liver enzymes in hyperlipidaemic rabbits. *Food Chem.* 141(2):1306-1312.

Mohmmed, N., Kuna, A., Sarkar, S., Azam, M.M., Prasanna, K.L., and Kiran, V.K. 2021. Effect of germination on yield, physic-chemical properties, nutritional composition and GABA content in germinated brown rice. *ORYZA-An Int. J. Rice* 58(4): 496-505.

Moongngarm, A. and Saectung, N. 2010. Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. *Food Chem*. 122(3): 782-788.

Morintaka, S. and Yasumatsu, K. 1972. Storage deterioration of defatted rice. *Eiyo To Shokuryo* 25: 16-20.

Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65(1-2): 55-63.

Mujoo, R. and Ali, S.Z. 1998. Susceptibility of starch to *in vitro* enzyme hydrolysis in rice, rice flakes and intermediary products. *LWT-Food Sci. Technol.* 31(2): 114-121.

Mukesh, M., Vidyabhushan, J., Anand, K., Mankesh, K., and Shweta, K. 2018. Genetic divergence in mid early rice genotypes. *J Pharmacogn. Phytochem*. 7(4S): 274-276.

Murray, B. A. and FitzGerald, R. J. 2007. Angiotensin converting enzyme inhibitory peptides derived from food proteins: biochemistry, bioactivity and production. *Curr. Pharma. Des.* 13(8): 773-791.

Murthy, K. S., Balasubramanyam, P., Talpaga, K. K., and Singh, S. 2023. Impact of soil minerals and other factors on the paddy production with a specific focus on the seven blocks of tiruvarur district, Tamilnadu. *J. Data Acquis Process*. 38(1): 2399.

Nadh, R. G. 2018. Quality evaluation of KAU red rice (*Oryza sativa* L.) varieties. M.Sc. (Home Science) thesis, Kerala Agricultural University, Thrissur, 168p.

Nandini, P. V. 1995. A multivariate approach to define the quality of rice. Ph. D (Home Science) thesis, Kerala Agricultural University, Thrissur, 199p.

Nanogals, R., Harakari, T., and Ahro, N. 2000. Phosphorus, potassium and magnesium contents and their balance in cereal grain. *Japan J. Crop Sci*. 71: 461-467.

Nascimento, L. Á., Abhilasha, A., Singh, J., Elias, M. C., and Colussi, R. 2022. Rice germination and its impact on technological and nutritional properties: a review. *Rice Sci.* 29(3): 201-215.

Nayeem, S., Sundararajan, S., Ashok, A. K., Abusaliya, A., and Ramalingam, S. 2021. Effects of cooking on phytochemical and antioxidant properties of pigmented and non-pigmented rare Indian rice landraces. *Biocatal. Agric. Biotechnol.* 32: 1-7.

Ng, L. T., Huang, S. H., Chen, Y. T., and Su, C. H. 2013. Changes of tocopherols, tocotrienols, γ-oryzanol, and γ-aminobutyric acid levels in the germinated brown rice of pigmented and nonpigmented cultivars. *J. Agric. Food Chem*. 61(51): 12604-12611.

Ngo, D. H. and Vo, T. S. 2019. An updated review on pharmaceutical properties of gamma-aminobutyric acid. *Molecules* 24(15): 1-23.

Ngo, D. H., Tran, Q. T., Kim, Y. S., Hang, N. T. N., Ngo, D. N., and Vo, T. S. 2022. GABA-enriched rice bran inhibits inflammation in LPS-stimulated macrophages via suppression of TLR4-MAPK/NF-κB signaling cascades. *J. Food Biochem.* 46(12), e14421.

Nikmaram, N., Dar, B. N., Roohinejad, S., Koubaa, M., Barba, F. J., Greiner, R., and Johnson, S. K. 2017. Recent advances in γ-aminobutyric acid (GABA) properties in pulses: An overview. J. Sci. Food Agric. 97(9): 2681-2689.

Nishiba, Y., Sato, T., and Suda, I. 2000. Convenient method to determine free fatty acid of rice using thin-layer chromatography and flame-ionization detection system. *Cereal Chem.* 77(2): 223-229.

Nishimura, M., Yoshida, S. I., Haramoto, M., Mizuno, H., Fukuda, T., Kagami-Katsuyama, H., Tanaka, A., Ohkawara, T., Sato, Y., and Nishihira, J., 2016. Effects of white rice containing enriched gamma-aminobutyric acid on blood pressure. *J. Tradit. Complement. Med.* 6(1): 66-71.

Nkhata, S. G., Ayua, E., Kamau, E. H., and Shingiro, J. B. 2018. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food Sci. Nutr.* 6(8): 2446-2458.

Noh, K. S., Yang, M. O., and Cho, E. J. 2002. Nitrite scavenging effect of Umbelligeraeceae. *Korean J. Food Cook. Sci*. 18: 8-12.

Nomura, M., Kimoto, H., Someya, Y., Furukawa, S., and Suzuki, I. 1998. Production of γ- aminobutyric acid by cheese starters during cheese ripening. *J. Dairy Sci*. 81(6): 1486-1491.

Nonogaki, H., Bassel, G. W., and Bewley, J. D. 2010. Germination-still a mystery. *Plant Sci.* 179(6): 574-581.

Noomhorm, A., Ahmad, I., and Anal, A. K. 2014. *Functional Foods and Dietary Supplements*. John Wiley and Sons Ltd, The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK. 512p.

Noonari, S., Irfana, N. M., Raiz, A. B., Muhammed, I. K., and Ali, S. 2015. Price flexibility and seasonal varations of major vegetables in Sindh Pakistan. *J. Food Process Technol*. 6(12): 421-430

Norhaizan, M., Ng, S., Norashareena, M., and Abdah, M. 2011. Antioxidant and cytotoxicity effect of rice bran phytic acid as an anticancer agent on ovarian, breast and liver cancer cell lines. *Malaysian J. Nutr.* 17(3): 367-375.

Nuss, P. 2015. Anxiety disorders and GABA neurotransmission: a disturbance of modulation. *Neuropsychiatric Dis Treat.* 11: 165-175.

Oessoe, Y. Y. E., Maramis, R., Warouw, O. O. J., and Mandey, L. C. 2014. Changes on carbohydrates and protein content in North Sulawesi local rice during storage. *IOSR J. Environ. Sci. Toxicol. Food Technol*. 8(2): 16-21.

Oh, C. H. and Oh, S. H. 2004. Effects of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. *J. Med. Food* 7: 19-23.

Oh, I., Saitoh, K., and Kuroda, T. 2007. Effects of high temperature on growth, yield and dry-matter production of rice grown in the paddy field. *Plant Prod. Sci.* 10(4): 412-422.

Oh, S. H., Soh, J. R., and Cha, Y. S. 2003. Germinated brown rice extract shows a nutraceutical effect in the recovery of chronic alcohol-related symptoms. *J. Med. Food* 6: 115-121.

Oh, S. J., Kim, H. S., Lim, S. T., and Reddy, C. K. 2019. Enhanced accumulation of gamma-aminobutyric acid in rice bran using anaerobic incubation with various additives. *Food chem.* 271:187-192.

Ohanenye, I. C., Tsopmo, A., Ejike, C. E., and Udenigwe, C. C. 2020. Germination as a bioprocess for enhancing the quality and nutritional prospects of legume proteins. *Trends Food Sci. Technol.* 101: 213-222.

Ohmori, T., Tahara, M., and Ohshima, T. 2018. Mechanism of gamma-aminobutyric acid (GABA) production by a lactic acid bacterium in yogurt-sake. *Process Biochem.* 74: 21-27.

Ohtsubo, K. I., Suzuki, K., Yasui, Y., and Kasumi, T. 2005. Bio-functional components in the processed pre-germinated brown rice by a twin-screw extruder. *J. Food Compos. Anal.* 18(4): 303-316.

Ohtsubo, T. 2004. Studies on textural and chemical changes in aged rice grains. *Food Sci. Technol. Res.* 11: 385-389.

Okada, T., Sugishita, T., Murakami, T., Murai, H., Saikusa, T., Horino, T., Onoda, A., Kajimoto, O., Takahashi, R., and Takahashi, T., 2000. Effect of the defatted rice germ enriched with GABA for sleeplessness, depression, autonomic disorder by oral administration. *J. Japn. Soc. Food Sci.Technol.* 47(8): 596-603.

Oki, T., Masuda, M., Nagai, S., Take'ichi, M., Kobayashi, M., Nishiba, Y., Sugawara, T., Suda, I., and Sato, T. 2005. Radical-scavenging activity of red and black rice. In: Toriyama, K., Heong, K. L., and Hardy, B. (eds), *Rice is life: scientific perspectives for the 21st century.* Proceedings of the World Rice Research

Conference, International Rice Research Institute (IRRI), Tsukuba, Japan, 4-7 November 2004. pp. 256-259.

Oliveira, M. E. A. S., Coimbra, P. P. S., Galdeano, M. C., Carvalho, C. W. P., and Takeiti, C. Y. 2022. How does germinated rice impact starch structure, products and nutrional evidences–a review. *Trends Food Sci. Technol.* 122: 13-23.

Ong, M. H. and Blanshard, J. M. V. 1995. Texture determinants in cooked, parboiled rice. I: rice starch amylose and the fine structure of amylopectin. *J. Cereal Sci.* 21(3): 251-260.

Ongol, M. P., Nyozima, E., Gisanura, I., and Vasanthakaalam, H. 2013. Effect of germination and fermentation on nutrients in maize flour. *Pak. J. Food Sci*. 23(4): 183-188.

Oo, E. M., Ruamyod, K., Khowawisetsut, L., Turbpaiboon, C., Chaisuksunt, V., Uawithya, P., Pholphana, N., Rangkadilok, N., and Chompoopong, S. 2020. Germinated brown rice attenuates cell death in vascular cognitive impaired mice and glutamate-induced toxicity in HT22 cells. *J. Agric. Food Chem.* 68(18): 5093-5106.

Pal, S., Bagchi, T. B., Dhali, K., Kar, A., Sanghamitra, P., Sarkar, S., Samaddar, M., and Majumder, J. 2019. Evaluation of sensory, physicochemical properties and Consumer preference of black rice and their products. *J. Food Sci. Technol.* 56(4): 1484-1494.

Park, K. B. and Oh, S. H. 2005. Production and characterization of GABA rice yogurt. Korean soc. *Food Sci. Technol*. 14(4): 518-522.

Park, K. B. and Oh, S. H. 2007. Production of yogurt with enhanced levels of gammaaminobutyric acid and valuable nutrients using lactic acid bacteria and germinated soybean extract. *Bioresour. Technol*. 98(8): 1675-1679.

Parnsahkorn, S. and Langkapin, J. 2013. Changes in physicochemical characteristics of germinated brown rice and brown rice during storage at various temperatures. *Agric. Eng. Int.: CIGR J.* 15(2): 293-303.

Parvathy, R., Mohanlal, S., Pushpan, C., Helen, A., and Jayalekshmy, A. 2014. Antioxidant properties, anti-inflammatory effects, and bioactive constituents of the Indian medicinal rice Njavara yellow compared with staple varieties. *Food Sci. Biotechnol.* 23(5): 1379-1388.

Parvez, M. K. 2018. Natural or plant products for the treatment of neurological disorders: Current knowledge. *Curr. Drug. Metab.* 19: 424-428.

Patel, H., Rajput, L. P. S., Ansari, F., Rana, G. K., and Soni, R. 2021. Effects of physicochemical and processing characteristics on nutritional and sensorial profile of puffed rice. *J. Pharmacogn. Phytochem.* 10(1S): 635-642.

Patil, S. B. and Khan, M. K. 2011. Germinated brown rice as a value added rice product: A review. *J. Food Sci. Technol.* 48: 661-667.

Patil, S. B. and Khan, M. K. 2012. Some cooking properties of germinated brown rice. *Agric. Eng. Int.: CIGR J.* 14(4): 156-162.

Patindol, J., Wang, Y. J., and Jane, J. I. 2005. Structure functionality changes in starch following rough rice storage. *Starch/Stärke* 57(5): 197-207.

Perera, I., Seneweera, S., and Hirotsu, N. 2018. Manipulating the phytic acid content of rice grain toward improving micronutrient bioavailability. *Rice* 11(1): 1-13.

Perez, C.M. and Juliano, B.O. 1981. Texture changes and storage of rice. *J. Texture Stud.* 12(3): 321-333.

Petti, M. S. 2009. Antioxidant determinations by the use of stable free radicals. *Nat*. 81(4617): 1199-2000.

Petti, S. and Scully, C. 2009. Polyphenols, oral health and disease: a review. *J. Dent*. 37(6): 413-423.

Phuapaiboon, P. 2017. Gamma-aminobutyric acid, total anthocyanin content and antioxidant activity of vinegar brewed from germinated pigmented rice. *Pak. J. Nutr*. 16(3): 109-118.

Pillai, C., Faseela, K.V., and Thampi, H. 2020. Nutritional composition of selected traditional rice varieties of Kerala. *J. Trop. Agric.* 58(1): 33-43.

Pino, A., Nicosia, F.D., Agolino, G., Timpanaro, N., Barbagallo, I., Ronsisvalle, S., Caggia, C., and Randazzo, C. L. 2022. Formulation of germinated brown rice fermented products functionalized by probiotics. *Innov. Food Sci. Emerg. Technol.* 80(24): 103-176.

Poojary, M. M., Dellarosa, N., Roohinejad, S., Koubaa, M., Tylewicz, U., Gomez-Galindo., Saraiva, J. A., Rosa M. D., and Barba, F. J. 2017. Influence of innovative processing on γ-aminobutyric acid (GABA) contents in plant food materials. *Compr. Rev. Food Sci. Food Saf.* 16(5): 895-905.

Powers, M. E., Borst, S. E., McCoy, S. C., Conway, R., and Yarrow, J. F. 2003. The effects of gamma aminobutyric acid on growth hormone secretion at rest and following exercise. *Med. Sci. Sports Exerc.* 35: S271.

Pradeep, P.M., Jayadeep, A., Guha, M., and Singh, V. 2014. Hydrothermal and biotechnological treatments on nutraceutical content and antioxidant activity of rice bran. *J. Cereal Sci.* 60(1): 187-192.

Prieto, P., Pineda, M., and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analyt. Biochem.* 269(2): 337-341.

Priyanthi, C. and Sivakanesan, R. 2021. The total antioxidant capacity and the total phenolic content of rice using water as a solvent. *Int. J. Food Sci.* 2021: 1-6.

Promtang, S., Turbpaiboon, C., Oo, E. M., Khowawisetsut, L., Uawithya, P., and Chompoopong, S. 2023. Germinated brown rice protects against glutamate toxicity in HT22 hippocampal neurons through the jnk-mediated apoptotic pathway via the GABAA receptor. *IBRO Neurosci. Rep.*14: 38-49.

Prud'homme G., Glinka Y., and Wang Q. 2013. GABA exerts anti-inflammatory and immunosuppressive effects (P5175). *J. Immunol*. 190(1):68.

Puangwerakul, Y. 2007. Change of GABA and vitamine B1 during parboiled rice processing of brown germinated rice and germinated KDML105 brown rice. *J. Biochem*. 62: 46-49.

Pulido, R. 2000. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food Chem*. 48(8): 3396-3402.

Quilez, J. and Diana, M. 2017. Gamma-Aminobutyric acid-enriched fermented foods. in: Frias, J., C. MartinezVillaluenga, C., and Penas, E. (eds), *Fermented Foods in Health and Disease Prevention.* Academic Press, Boston, pp. 85-103.

Radulovic, N., Stankov-Jovanović, V., Stojanović, G., Smelcerović, A., Spiteller, M., and Asakawa, Y. 2007. Screening of in vitro antimicrobial and antioxidant activity of nine Hypericum species from the Balkans. *Food Chem.* 103(1): 15-21.

Raghuvanshi, R. S., Dutta, A., Tewari, G., and Suri, S. 2017. Qualitative characteristics of red rice and white rice procured from local market of Uttarakhand: a comparative study. *J. Rice Res.* 10(1): 49-53.

Rai, P. 2009. Study on physiochemical and nutritional quality of Kavuni rice flour. *Biosci. Trends* 7(11): 1142-1146.

Ramakrishna, A. and Roshchina, V. V. 2018. *Neurotransmitters in Plants: Perspectives and Applications*. CRC Press, Boca Raton, 448p.

Rao, A. S., Reddy, S. G., Babu, P. P., and Reddy, A. R. 2010. The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran. *BMC Complement. Altern. Med.* 10(1):1-9.

Rao, R. S. P. and Muralikrishna, G. 2004. Non-starch polysaccharide–phenolic acid complexes from native and germinated cereals and millet. *Food Chem.* 84(4): 527-531.

Rashmi, D., Zanan, R., John, S., Khandagale, K., and Nadaf, A. 2018. γ-aminobutyric acid (GABA): Biosynthesis, role, commercial production, and application. In:

Rahman, A. (ed.), *Study in Natural Products Chemistry.* Elsevier, 1000 AE Amsterdam, The Netherlands, pp. 413-452.

Rasolt, D. H. 2008. Chemical in germinated brown rice could benefit diabetics. Latest News and Information, Defeat Diabetes Foundation, Inc.

Reddy, C. M., Kimi, L., Haripriya, S., and Kang, N. 2017. Effects of polishing on proximate composition, physico-chemical characteristics, mineral composition and antioxidant properties of pigmented rice. *Rice Sci.* 24(5): 241- 252.

Reshmi, R. 2012. Quality Evaluation of medicinal rice (*Oryza sativa* L.). *Njavara* for product development and therapeutic value. Ph.D. (H. Sc.) thesis, Kerala Agricultural University, Thrissur, 273p.

Reshmi, R. and Nandini, P. V. 2012. Nutrient composition and physico-chemical properties of medicinal rice-Njavara. *Asian J. Home Sci.* 1: 211-215.

Reshmi, R. and Nandini, P.V. 2018. Antioxidant activity of Indian medicinal rice (*Oryza sativa* L.) Njavara. *Int. J. Adv. Sci. Eng. Inf. Technol*. 4(3): 236-245.

Resurrection, A., Hara, T., Juliano, B., and Yoshida, S. 1997. Effect of temperature during the ripening on grain quality of rice. *Soil Sci. Plant Nutr*. 23(1): 109-112.

Ribas, V., Garcia-Ruiz, C., and Fernandez-Checa, J. C. 2016. Mitochondria, cholesterol and cancer cell metabolism. *Clin. Transl. Med.* 5: 22.

Rizzello, C. G., Cassone, A., Di Cagno, R., and Gobbetti, M., 2008. Synthesis of angiotensin-I converting enzyme (ACE)-inhibitory peptides and γ-aminobutyric acid (GABA). *J. Agric. Food Chem*. 56 (16): 6936-6943.

Roberts, E. and Frankel, S. 1950. γ-Aminobutyric acid in brain: its formation from glutamic acid. *J. Biol. Chem.* 187(1): 55-63.

Roy, P., Ijiri, T., Okadome, H., Nei, D., Orikasa, T., Nakamura, N., and Shina, T. 2008. Effect of processing condition on overall energy consumption and quality of rice (*Oryza sativa* L.). *J. Food Eng*. 89 (3): 343-348.

Rungtip, S. A., Sarawut, J., Surin, K., Sompong, L., Wanphen, K., and Supranee, U. 2012. Antioxidative and neuroprotective activities of the pre-germinated brown rice extract. *Food Nutr. Sci.* 3(1): 136-140.

Rusydi, M. M. R., Noraliza, C. W., Azrina, A., and Zulkhairi, A. 2011. Nutritional changes in germinated legumes and rice varieties. *Int. Food Res. J.* 18(2): 705-713.

Sadasivam, S. and Manickam, A. 2017. *Biochemical Methods* (4th Ed.). New Age International Pvt. Ltd., New Delhi, 254p.

Sadasivam, S. and Manikkam, A. 1992. *Biochemical Methods for Agricultural Sciences*. Wiley Eastern Ltd., New Delhi, 73p.

Sagar, M. A., Ahsraf, M., and Akmal, M. A. 1988. Grain quality characteristics of Pakistani commercial rice varieties. *Pak. J. Agric. Res*. 9: 431-6.

Sahab, N. R., Subroto, E., Balia, R. L., and Utama, G. L. 2020. γ-Aminobutyric acid found in fermented foods and beverages: current trends. *Heliyon* 6(11): e05526.

Saidi, V., Sheikh-Zeinoddin, M., Kobarfard, F., and Soleimanian-Zad, S. 2020. Bioactive characteristics of a semi-hard non-starter culture cheese made from raw or pasteurized sheep's milk. *3 Biotech*. 10: 1-8.

Saikrishna, A., Dutta, S., Subramanian, V., Moses, J. A., and Anandharamakrishnan, C. 2018. Ageing of rice: A review. *J. Cereal Sci.* 81: 161-170.

Saikusa, T., Horino, T., and Mori, Y. 1994. Distribution of free amino acids in the rice kernel and kernel fractions and the effect of water soaking on the distribution. *J. Agric. Food Chem.* 42(5): 1122-1125.

Sakashita, M., Nakamura, U., Horie, N., Yokoyama, Y., Kim, M., and Fujita, S. 2019. Oral supplementation using gamma-aminobutyric acid and whey protein improves whole body fat-free mass in men after resistance training. *J. Clin. Med. Res.* 11: 428-434.

Saleh, A. S., Zhang, Q., Chen, J., and Shen, Q. 2013. Millet grains: nutritional quality, processing, and potential health benefits. *Compr. Rev. Food Sci. Food Saf.* 12(3): 281-295.

Saleh, M. I. and Meullener, J. F. 2007. The effect of moisture content at harvest and degree of milling (based on surface lipid content) on the texture properties of cooked long-grain rice. *Cereal Chem*. 84(2): 119-124.

Sang-You, K., Hyun-Jun, P., and Sang-Jin, B. 2007. Method for preparing germinated brown rice having improved texture and cookability without microbial contamination and a germinated brown rice obtained therefrom. United States Patent No. US, 7,217,436 B2. Available: https://patents.google.com/patent/US7217436B2/en [10-7-2023]

Sarasa, S. B., Mahendran, R., Muthusamy, G., Thankappan, B., Selta, D. R. F., and Angayarkanni, J. 2020. A brief review on the non-protein amino acid, gamma-amino butyric acid (GABA): its production and role in microbes. *Curr. Microbiol*. 77: 534-544.

Sareepuang, K., Siriamornpun, S., Wiset, L., and Meeso, N. 2008. Effect of soaking temperature on physical, chemical and cooking properties of parboiled fragrant rice. *World J. Agric. Sci.* 4(4): 409-415.

Sasaki, S., Yokozawa, T., Cho, E. J., Oowada, S., and Kim, M. 2006. Protective role of gamma-aminobutyric acid against chronic renal failure in rats. *J. Pharm. Pharmacol.* 58: 1515-1525.

Sasimar, W., Narissara, L., Bhagavathi, S. S., Wiwat, W., Sophon, S., Sartjin, P., and Chaiyavat, C. 2016. Evaluation of factors that influence the L-glutamic and γ-aminobutyric acid production during Hericium erinaceus fermentation by lactic acid bacteria. *CyTA- J Food* 14: 47-54.

Sathyan, N. T. 2012. Quality evaluation of germinated rice and rice products. M. Sc. (Home Science) thesis, Kerala Agricultural University, Thrissur, 97p.

Satterlee, L.D., Marshall, H.F., and Tennyson, J.M. 1979. Estimation of starch digestibility. *J. Am. Oil Chem. Soc.* 56: 103-104. Adijei, T., Splittstoesser, W., and Vandemark, J. 1976. Use of soybeans as sprouts. *Hort Sci.* 11(3): 235-241.

Savithri, A. and Desikachar, H. S. R. 1990. Effect of germination of Kalatur (black variety soyabean and tur (pigeon pea) on carbohydrate profile and its relation to flatus formation. *Legume Res*. 13: 1-4.

Schellack, N. and Naicker, P. 2015. Hypertension: A review of antihypertensive medication, past and present. *S. Afr. Pharm. J.* 82:17-25.

Schmit, J. C. and Brody, S. T. U. A. R. T. 1975. Neurospora crassa conidial germination: role of endogenous amino acid pools. *J. Bacteriol.* 124(1): 232-242.

Scimemi, A. 2014. Structure, function, and plasticity of GABA transporters. *Front. Cell. Neurosci.* 8: 161.

Sen, S., Chakraborty, R., and Kalita, P. 2020. Rice-not just a staple food: A comprehensive review on its phytochemicals and therapeutic potential. *Trends Food Sci. Technol.* 97(8): 265-285.

Seo, W. D., Ryu, Y. B., Curtis-Long, M. J., Lee, C. W., Ryu, H. W., Jang, K. C., and Park, K. H. 2010. Evaluation of anti-pigmentary effect of synthetic sulfonylamino chalcone. *European J Medici Chem.* 45(5): 1-2.

Shafiekhani, S., Wilson, S. A., and Atungulu, G. G. 2018. Impacts of storage temperature and rice moisture content on color characteristics of rice from fields with different disease management practices. *J. Stored Prod. Res.* 78: 89-97.

Sharon, C. L. and Kareem, K. A. 2013. *Njavara* rice flakes a novel value added medicinal snacks [abstract]. In: *3rd International Conference on Food Technology* (3rd intoftech); 4-5, January, 2013, Thanjavoor, Indian Institute of Crop Processing Technology, Thanjavoor, p.31. Abstract No. 054.

Shawky, B. T., Nagah, M., Ghareeb, M. A., El-Sherbiny, G. M., Moghannem, S. A., and Abdel-Aziz, M. S. 2019. Evaluation of antioxidants, total phenolics and

antimicrobial activities of ethyl acetate extracts from Fungi grown on rice straw. *J. Renew. Materi.* 7(7): 667.

Shelp, B. J., Bown, A. W., and McLean, M. D. 1999. Metabolism and functions of gamma-aminobutyric acid. *Trends Plant Sci.* 4(11): 446-452.

Shelp, B. J., Bozzo, G. G., Trobacher, C. P., Zarei, A., Deyman, K. L., and Brikis, C. J. 2012. Hypothesis/review: contribution of putrescine to 4-aminobutyrate (GABA) production in response to abiotic stress. *Plant Sci.* 193: 130-135.

Shen, L., Gao, M., Zhu, Y., Liu, C., Wang, L., Kamruzzaman, M., Liu, C., and Zheng, X. 2021. Microwave drying of germinated brown rice: Correlation of drying characteristics with the final quality. *Innovative Food Sci. Emerg. Technol.* 70(10): 102-173.

Shen, S., Wang, Y., Li, M., Xu, F., Chai, L., and Bao, J. 2015. The effect of anaerobic treatment on polyphenols, antioxidant properties, tocols and free amino acids in white, red, and black germinated rice (*Oryza sativa* L.). *J. Funct. Foods* 19: 641-648.

Shi, F., Ni, Y., and Wang, N. 2016. Metabolism and Biotechnological Production of Gamma-Aminobutyric Acid (GABA). In: Vandamme, E. J. and Revuelta, J. L. (eds), *Industrial Biotechnology Vitamins, Biopigments, and Antioxidants*, John Wiley and Sons Ltd., The Atrium, Southern Gate, Chichester, West Sussex, PO19 8SQ, UK. pp.445-468.

Shi, F., Xie, Y. L., Jiang, J. J., Wang, N., Li, Y., and Wang, Z. 2014. Directed evolution and mutagenesis of glutamate decarboxylase from Lactobacillus brevis Lb85 to broaden the range of its activity toward a near-neutral pH. *Enzyme Microb. Tech*. 61-62: 35-43.

Shijagurumayum, S., Shantibala Devi, G. A., and Singh, C. B. 2018. Grain quality evaluation of some aromatic rice varieties of Manipur, India. *Res. Crop*. 19(2): 169-181.

Si, X., Shang, W., Zhou, Z., Shui, G., Lam, S. M., Blanchard, C., and Strappe, P. 2018. Gamma-aminobutyric acid enriched rice bran diet attenuates insulin resistance and balances energy expenditure via modification of gut microbiota and short-chain fatty acids. *J. Agric. Food Chem.* 66: 881-890.

Singh, A. and Sharma, S. 2017. Bioactive components and functional properties of biologically activated cereal grains: a bibliographic review. *Crit. Rev. Food Sci. Nutr.* 57(14): 3051-3071.

Singh, G. H., Kumar, V., and Kaur, A. 2002. Effect of the enzymatic removal of endosperm cell wall on the gelatinisation properties of aged and unaged rice flours. *J. Food Eng*. 59: 117- 121.

Singh, N., Kaur, L., Sandhu, K.S., Kaur, J., and Nishinari, K. 2006. Relationships between physicochemical, morphological, thermal, rheological properties of rice starches. *Food Hydrocoll.* 20(4): 532-542.

Singh, T and Pun, K. B. 2020. Response of Nitrogen Levels on Productivity and Profitability of Rice Cultivars in Shallow Lowlands of Assam. *Int. J. Curr. Microbiol. App. Sci*. 9(6): 1597-1604.

Siragusa, S., Angelis, M. De., Cagno, R. Di., Rizzello, C. G., Coda, R., and Gobbetti, M. 2007. Synthesis of γ -aminobutyric acid by lactic acid bacteria isolated from a variety of Italian cheeses. *Appl. Environ. Microbiol*. 73(22): 7283-7290.

Sirisoontaralak, P., Nakornpanom, N.N., Koakietdumrongkul, K., and Panumaswiwath, C. 2015. Development of quick cooking germinated brown rice with convenient preparation and containing health benefits. *LWT-Food Sci. Technol.* 61(1): 138-144.

Sitanggang, A. B., Joshua, M., Munarko, H., Kusnandar, F., and Budijanto, S. 2021. Increased γ-aminobutyric acid content of germinated brown rice produced in membrane reactor. *Food Technol. Biotechnol*. 59(3): 295-305.

Sivakumar, R., Saravanan, R., Perumal, A. E., and Iniyan, S. 2016. Fluidized bed drying of some agro products-A review. *Renew. Sustain. Energy Rev.* 61: 280-301.

Sivamaruthi, B. S., Kesika, P., Prasanth, M. I., and Chaiyasut, C. A. 2018. Mini review on antidiabetic properties of fermented foods. *Nutr.* 10: 1973.

Sodhi, N. S., Singh, N., Arora, M., and Singh, J. 2003. Changes in physico-chemical, thermal, cooking and textural properties of rice during aging. *J. Food Process. Preserv*. 27(5): 387-400.

Sotelo, A., Sousa, V., Montalvo, I., Hernandez, M., and Hernandez-Aragon, L. 2000. Chemical composition of different fractions of 12 Mexican varieties of rice obtained during milling. *Cereal Chem*. 67(2): 209-212.

Sreejayan, V. R. K. and Thomas, G. 2003. Collection and morphological evaluation of Njavara, a traditional medicinal rice (*Oryza sativa* L.), in Kerala, India. Plant Genetic *Resour. Newslett*. 135: 12-17.

Srilakshmi, B. 2003. *Food Science*. New age international Pvt. Ltd., New Delhi. 401p.

Streeter, J. G. and Thompson, J. F. 1972. *In vivo* and *in vitro* studies on 4-aminobutyric acid metabolism with the radish plant (*Raphanus sativus* L.). *Plant Physiol*. 49: 579-584.

Suda, M., Watanabe, T., Kobayashi, M., and Matsuda, K. 1986. Changes in starch content and related enzyme activities during the growth of germinating soybeans. *Agric. Biol. Chem.* 50(12): 3195-3196.

Suffness, M. and Pezzuto, J. M. 1990. Assays related to cancer drug discovery. In: Hostettmann, K. (ed.), *Methods in Plant Biochemistry: Assays for Bioactivity*. Academic Press, London, pp. 71-133.

Sugimoto, T., Tanaka, K., and Kasai, Z. 1998. Improved extraction of rice prolamin. *Agric Biol. Chem*. 50: 2409-2411.

Sutharut, J. and Sudarat, J. 2012. Total anthocyanin content and antioxidant activity of germinated colored rice. *Int. Food Res. J.* 19(1): 215-221.

Takano, K. 1989. Studies on the mechanism of lipid-hydrolysing in rice bran. *Nippon Shokuhin Kogyo Gakkaishi* 36(6): 519-524.

Talebi, N., Nematbakhsh, M., Monajemi, R., Mazaheri, S., Talebi, A., and Vafapour, M. 2016. The protective effect of γ-aminobutyric acid on kidney injury induced by renal ischemia-reperfusion in ovariectomized estradiol-treated rats. *Int. J. Prev. Med.* 7: 1-6.

Tan, B. L. and Norhaizan, M. E. 2019. *Manilkara zapota* (L.) P. Royen leaf water extract triggered apoptosis and activated caspase-dependent pathway in HT-29 human colorectal cancer cell line. *Biomed. Pharmacother.* 110: 748-757.

Taylor, J. R. N. and Njintang, Y. N. 2012. Germinated brown rice: a novel functional ingredient. In Germinated Cereals for Sustainable Food and Nutrition Security (pp. 59-70). Springer, Dordrecht.)

Techo, J., Soponronnarit, S., Devahastin, S., Wattanasiritham, L. S., Thuwapanichayanan, R., and Prachayawarakorn, S. 2019. Effects of heating method and temperature in combination with hypoxic treatment on γ-aminobutyric acid, phenolics content and antioxidant activity of germinated rice. *Int. J. Food Sci. Technol*. 54(4): 1330-1341.

Thakur, A. K. and Gupta. A. K. 2006. Water absorption characteristics of paddy, brown rice and husk during soaking. *J. Food Eng*. 75: 252-257.

Thitinunsomboon, S., Keeratipibul, S., and Boonsiriwit, A. 2013. Enhancing gamma-aminobutyric acid content in germinated brown rice by repeated treatment of soaking and incubation. *Food Sci. Technol. Int.* 19(1): 25-33.

Thomas, S., Aneena, E. R., Pathrose, B., Panjikkaran, S. T., Sharon, C. L., Prameela, P., Ajisha, K. H., and Athira, K. A. 2023. Augmentation of gamma amino butyric acid (GABA), antioxidant potential and nutrient qualities in germinated brown rice. *Food Humanit*. 1: 349-357.

Ti, H., Zhang, R., Zhang, M., Li, Q., Wei, Z., Zhang, Y., Tang, Z., De3ng, Y., Liu, L., and Ma, Y. 2014. Dynamic changes in the free and bound phenolic compounds and antioxidant activity of brown rice at different germination stages. *Food Chem.* 161: 337-344.

Tian, J., Dang, H. N., Yong, J., Chui, W. S., Dizon, M. P., Yaw, C. K., and Kaufman, D. L. 2011. Oral treatment with γ-aminobutyric acid improves glucose tolerance and insulin sensitivity by inhibiting inflammation in high fat diet-fed mice. *PLoS ONE* 6: e25338.

Tian, S., Nakamura, K., and Kayahara, H. 2004. Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. *J. Agric. Food Chem.* 52(15): 4808-4813.

Tiansawang, K., Luangpituksa, P., Varanyanond, W., and Hansawasdi, C. 2016. GABA (γ-aminobutyric acid) production, antioxidant activity in some germinated dietary seeds and the effect of cooking on their GABA content. *Food Sci. Technol.* 36: 313-321.

Tipkanon, S., and Abdallah, A. M. 2014. Factor affecting on accumulation of gamma-aminobutyric acid (GABA) in rice germ (Khao Dawk Mali 105). *Appl. Sci. Eng. Prog*. 7(3): 43-48.

Torimitsu, M., Nagase, R., Yanagi, M., Homma, M., Sasai, Y., Ito, Y., Hayamizu, K., Nonaka, S., Hosono, T., and Kise, M. 2010. Replacing white rice with pre-germinated brown rice mildly ameliorates hyperglycemia and imbalance of adipocytokine levels in type 2 diabetes model rats. *J. Nutr. Sci. Vitaminol. (Tokyo)* 56: 287-292.

Toyoizumi, T., Kosugi, T., Toyama, Y., and Nakajima, T. 2021. Effects of high-temperature cooking on the gamma-aminobutyric acid content and antioxidant capacity of germinated brown rice (*Oryza sativa* L.). *CyTA-J. Food* 19(1): 360-369.

Trachoo, N., Boudreaux, C., Moongngarm, A., Samappito, S., and Gaensakoo, R. 2006. Effect of germinated rough rice media on growth of selected probiotic bacteria. *Pak. J. Biol. Sci*. 9: 2657-2661.

Traore, G., Juliano, B. O., and Ganales, A. L. 2004. Physico-chemical and economic aspects of rice grain quality. *Rice J*. 69: 7-12.

Tsushida, T. and Murai, T. 1987. Conversion of glutamic acid to γ-aminobutyric acid in tea leaves under anaerobic conditions. *Agric. Biol. Chem*. 51: 2865-2871.

Tu, J., Liu, G., Jin, Y., Tang, C., Yao, T., Zhuo, J., and Wang, J. 2022. Enrichment of γ-aminobutyric acid in mulberry leaves and the inhibitory effects of the water extract on ACE and α-glucosidase activity. *Ind. Crops Prod.* 177: 114485.

Tujioka, K., Ohsumi, M., Horie, K., Kim, M., Hayase, K., and Yokogoshi, H. 2009. Dietary gamma-aminobutyric acid affects the brain protein synthesis rate in ovariectomized female rats. *J. Nutr. Sci. Vitaminol. (Tokyo)* 55: 75-80.

Tujioka, K., Okuyama, S., Yokogoshi, H., Fukaya, Y., Hayase, K., Horie, K., and Kim, M. 2007. Dietary gamma-aminobutyric acid affects the brain protein synthesis rate in young rats. *Amino Acids* 32: 255-260.

Tumpanuvatr, T., Jittanit, W., and Surojanametakul, V. 2018. Effects of drying conditions in hybrid dryer on the GABA rice properties. *J. Stored Prod. Res*. 77: 177-188.

Tung, Y. T., Lee, B. H., Liu, C. F., and Pan, T. M. 2011. Optimization of culture condition for ACEI and GABA production by lactic acid bacteria. *J. Food Sci.* 76(9): M585-M591.

Tuntipopipat S., Muangnoi C., Thiyajai P., Srichamnong W., Charoenkiatkul S., and Praengam K. A. 2015. bioaccessible fraction of parboiled germinated brown rice exhibits a higher anti- inflammatory activity than that of brown rice. *Food Funct*. 6: 1480-1488.

Umnajkitikorn, K., Faiyue, B., and Saengnil, K. 2013. Enhancing antioxidant properties of germinated Thai rice (*Oryza sativa* L.) cv. Kum Doi Saket with salinity. *J. Rice Res*. 1: 103-110.

Urbano, G., Aranda, P., Vılchez, A., Aranda, C., Cabrera, L., Porres, J. M., and Lopez-Jurado, M. 2005. Effects of germination on the composition and nutritive value of proteins in Pisum sativum, L. *Food Chem.* 93(4): 671-679.

Usuki, S., Ito, Y., Morikawa, K., Kise, M., Ariga, T., Rivner, M., and Yu, R. K. 2007. Effect of pre-germinated brown rice intake on diabetic neuropathy in streptozotocin-induced diabetic rats. *Nutr. Metab.* 4: 1-11.

Vanaja, T. and Babu, L. C. 2006. Heterosis for yield and yield components in rice (*Oryza sativa* L.). *J. Trop. Agric.* 42(1-2): 43-44.

Varanyanond, W., Tungtrakul, P., Surojanametakul, V., Watanasiritham, L., and Luxiang, W. 2005. Effects of water soaking on gamma-aminobutyric acid (GABA) in germ of different Thai rice varieties. *Kasetsart J. Nat. Sci*. 39: 411-441.

Vellupillai, S., Nithyanantharajah, K., Vasantharuba, S., Balakumar, S., and Arasaratnam, V. 2009. Biochemical Changes Associated with Germinating Rice Grains and Germination Improvement. *Rice Sci*. 16(3): 240-242.

Venturi, M., Galli, V., Pini, N., Guerrini, S., and Granchi, L. 2019. Use of Selected Lactobacilli to Increase γ-Aminobutyric Acid (GABA) Content in Sourdough Bread Enriched with Amaranth Flour. *Foods* 8 (6): 218.

Vichit, W. and Saewan, N. 2016. Effect of germination on antioxidant, anti-inflammatory and keratinocyte proliferation of rice. *Int. Food Res. J.* 23(5): 2006–2015.

Vijayan, F. P., Rani, V. J., Vineesh, V. R., Sudha, K. S., Michael, M. M., and Padikkala, J. 2007. Protective effect of Cyclea peltata Lam on cisplatin-induced nephrotoxicity and oxidative damage. *J. Basic Clin. Physiol. Pharmacol*. 18(2): 101-114.

Walter, M., Marchesan, E., Massoni, P. F. S., da Silva, L. P., Sartori, G. M. S., and Ferreira, R. B. 2013. Antioxidant properties of rice grains with light brown, red and black pericarp colors and the effect of processing. *Food Res. Int.* 50(2): 698-703.

Wang, D., Wang, Y., Lan, H., Wang, K., Zhao, L., and Hu, Z. 2021. Enhanced production of γ-aminobutyric acid in litchi juice fermented by Lactobacillus plantarum HU-C2W. *Food Biosci.* 42: 101155.

Wang, H. K., Dong, C., Chen, Y. F., Cui, L. M., and Zhang, H. P. 2010. A new probiotic Cheddar cheese with high ACE-inhibitory activity and gamma-aminobutyric acid content produced with Koumiss-derived Lactobacillus casei Zhang. *Food Technol. Biotechnol*. 48: 62-70.

Wangpradit, N., Prom-in, S., Kumar, J., Chua, K. H., and Ugusman, A. 2020. Modulation of spatial learning and memory of obese mice by germinated Sang-Yod rice. *J Nutri Med. Diet. Care* 6: 042.

Wannasupchue, W2014. Chemical properties and sensory characteristics of tomyum puffed rice. *Khon Kaen Agric. J.* 42(4): 218-225.

Wassanai, W. and Royintarat, T. 2012. Implementation of quality function deployment and kansei engineering for GABA rice snack development. *Environ. Nat. Resour. J.* 10(2):1-10.

Watanabe, M., Maeda, T., Tsukahara, K., Kayahara, H., and Morita, N. 2004. Application of pregerminated brown rice for breadmaking. *Cereal Chem.* 81(4): 450-455.

Watanabe, N., Fujimoto, K., and Aoki, H. 2007. Antioxidant activities of the water-soluble fraction in tempeh-like fermented soybean (GABA-tempeh). *Int. J. Food Sci. Nutr,* 58(8): 577-587.

Watcharatparpaiboon, W., Laohakunjit, N., and Kerdchoechuen, O. 2010. An improved process for high quality and nutrition of brown rice production. *Food Sci. Technol. Int.*16(2): 147-158.

Wunjuntuk, K., Kettawan, A., Charoenkiatkul, S., and Rungruang, T. 2016. Parboiled germinated brown rice protects against CCl4-induced oxidative stress and liver injury in rats. *J. Med. Food* 19(1):15-23.

Xie, H., Sugimoto, T., Tanaka, K., and Kasai, Z. 2008. Biosynthesis of storage proteins in developing rice seeds. *Plant Physiol.* 108: 1094-1100.

Xie, M., Chen, H. H., Nie, S. P., Yin, J. Y., and Xie, M. Y. 2017. Gamma-aminobutyric acid increases the production of short-chain fatty acids and decreases pH values in mouse colon. *Molecules* 22: 653.

Xie, W., Li, Y., Li, Y., Ma, L., Ashraf, U., Tang, X., and Mo, Z. 2021. Application of γ-aminobutyric acid under low light conditions: effects on yield, aroma, element status, and physiological attributes of fragrant rice. *Ecotoxicol. Environ. Saf.* 213: 111941.

Xu, J. G., Hu, Q. P., Duan, J. L., and Tian, C. R. 2010. Dynamic changes in γ-aminobutyric acid and glutamate decarboxylase activity in oats (Avena nuda L.) during steeping and germination. J. Agric. Food Chem. 58(17): 9759-9763.

Yadav, B. K. and Jindal, V. K. 2007. Water uptake and solid loss during cooking of milled rice (*Oryza sativa* L.) in relation to its physicochemical properties. *J. Food Eng*. 80: 46-54.

Yang, H., Xing, R., Liu, S., Yu, H., and Li, P. 2019. Analysis of the protective effects of γ-aminobutyric acid during fluoride-induced hypothyroidism in male Kunming mice. *Pharm. Biol.* 57: 29-37

Yang, Y., Lian, Y. T., Huang, S. Y., Yang, Y., Cheng, L. X., and Liu, K. 2014. GABA and topiramate inhibit the formation of human macrophage-derived foam cells by modulating cholesterol-metabolism-associated molecules. *Cell. Physiol. Biochem.* 33: 1117-1129.

Yao, F., Sumners, C., O'Rourke, S. T., and Sun, C. 2008. Angiotensin II increases $GABA_B$ receptor expression in nucleus tractus solitarii of rats. *Am. J. Physiol. Heart Circulatory Physiol.* 294(6): H2712-H2720.

Yasuhiko, A., Satoshi, U., Koh-ichi, S., NobuhitoHirawa., Yoshio, K., Nobuyuki, Y., Tomoko, Y., Junichi, I., and Masao, I. 1995. Effect of green tea rich in γ-aminobutyric acid on blood pressure of Dahl salt-sensitive rats. *Am. J. Hypertens.* 8(1): 74-79.

Yavarzadeh, M., Anwar, F., Saadi, S., and Saari, N. 2023. Production of glycerolamines based conjugated γ-aminobutyric acids using microbial COX and LOX as successor enzymes to GAD. *Enzym. Microbiol. Technol.* 169: 110-282.

Yin, J. Y., Chen, H. Y., and Yen, G. C. 2007. Evaluation of antioxidant activity and inhibitory effect on nitric oxide production of some common vegetables. *J. Agric. Food Chem*. 54(5): 1680-1686.

Yogeeswari, P., Ragavendran, J. V., and Sriram, D. 2006. An update on GABA analogs for CNS drug discovery. *Recent Patents CNS Drug Discover*. 1(1): 113-118.

Yoshida, S., Haramoto, M., Fukuda, T., Mizuno, H., Tanaka, A., and Nishimura, M. 2015. Optimization of a γ-aminobutyric acid (GABA) enrichment process for hokkaido white rice and the effects of GABA-enriched white rice on stress relief in humans. *Japn. Soc. Food Sci. Technol.* 62: 95-103.

Young, H., Guk, I., Myoung, T., Woo, K.S., Park, D.S., Kim, J. H., Kim, D. J., Lee, J., Lee, Y. R., and Jeong, H. S. 2012. Chemical and functional components in different parts of rough rice (*Oryza sativa* L.) before and after germination. *Food Chem*. 134(1): 288-293.

Yousaf, M. 1992. Study on some physicochemical characteristics affecting the cooking and eating qualities of some Pakistani rice varieties. M.Sc (Food Technology) thesis, University of Agriculture Faisalabad, Pakistan, 46p.

Yousaf, M. 2000. Study on some physio-chemical characteristics affecting the cooking and eating qualities of some Pakistani rice varieties. M. Sc. (Food Technology) thesis. Department of food Technology, University of Agriculture, Faisalabad, Pakistan, 46p

You-Tung, L., Cheng-Cheng, P., Shwu-Tzy, W., and Chi-Yeu, C. 2015. Effect of different germination conditions on antioxidative properties and bioactive compounds of germinated brown rice. *BioMed Res. Int*. 1: 1-11.

Yu, C., Zhu, L., Zhang, H., Bi, S., Wu, G., Qi, X., Zhang, H., Wang, L., Qian, H., and Zhou, L. 2021. Effect of cooking pressure on phenolic compounds, gamma-aminobutyric acid, antioxidant activity and volatile compounds of brown rice. *J. Cereal Sci.* 97: 103-127.

Yu, Y., Li, M., Li, C., Niu, M., Dong, H., Zhao, S., Jia, C. and Xu, Y. 2023. Accelerated accumulation of γ-aminobutyric acid and modifications on its metabolic pathways in black rice grains by germination under cold stress. *Foods*. *12*(6):1290.

Zamri Md, N. D., Imam, M. U., Abd Ghafar, S. A., and Ismail, M. 2014. Antioxidative effects of germinated brown rice-derived extracts on H 2 O 2-induced oxidative stress in HepG2 cells. *Evid-based Complement. Alter. Med.* 2014: 1-12.

Zaupa, M., Calani, L., Del Rio, D., Brighenti, F., and Pellegrini, N. 2015. Characterization of total antioxidant capacity and (poly) phenolic compounds of differently pigmented rice varieties and their changes during domestic cooking. *Food Chem.* 187: 338-347.

Zhang, L., Du, L., Shi, T., Xie, M., Liu, X., and Yu, M. 2022. Effects of pulsed light on germination and gamma-aminobutyric acid synthesis in brown rice. *J. Food Sci.* 87(4): 1601-1609.

Zhang, Q., Liu, N., Wang, S., and Pan, L. 2020. Effects of germination and aeration treatment following segmented moisture conditioning on the γ-aminobutyric acid accumulation in germinated brown rice. *Int. J. Agric. Biol. Eng.* 13(5): 234-240.

Zhang, Q., Liu, N., Wang, S., Liu, Y., and Lan, H. 2019. Effects of cyclic cellulase conditioning and germination treatment on the γ-aminobutyric acid content and the cooking and taste qualities of germinated brown rice. *Food Chem*. 289: 232-239.

Zhang, Q., Xiang, J., Zhang, L., Zhu, X., Evers, J., van der Werf, W., and Duan, L. 2014. Optimizing soaking and germination conditions to improve gamma-aminobutyric acid content in japonica and indica germinated brown rice. *J. Funct. Foods* 10: 283-291.

Zhang, Q., Xing, B., Sun, M., Zhou, B., Ren, G., and Qin, P. 2020. Changes in bio-accessibility, polyphenol profile and anti-oxidants of quinoa and djulis sprouts duringin vitrosimu-lated gastrointestinal digestion. *Food Sci. Nutr.* 8(8): 4232-4241.

Zhao, M., Ma, Y., Wei, Z., Yuan, W., Li, Y., Zhang, C., Xue, X., and Zhou, H. 2011. Determination and Comparison of gamma-Aminobutyric Acid (GABA) Content in Pu-erh and Other Types of Chinese Tea. *J. Agric. Food Chem*. 59(8): 3641-3648.

Zhao, R., Ghazzawi, N., Wu, J., Le, K., Li, C., Moghadasian, M. H., Siow, Y. L., Apea-Bah, F. B., Beta, T., Yin, Z., and Shen, G. X., 2018. Germinated brown rice attenuates atherosclerosis and vascular inflammation in low-density lipoprotein receptor-knockout mice. *J. Agric. Food Chem.* 66(17): 4512-4520.

Zhao, S., Ding, J., and Xiong, S. 2019. Development Of Gamma-Aminobutyric Acid–Enriched Germinated Rice Products. In: Feng, H., Nemzer, B., and Devries, J. W. (eds), *Sprouted Grains: Nutritional value, production and application.* AACC International Press, pp.175-190.

Zheng, X., Zhu, G., Lu, S., Xu, H., and Sun, J. 2017. Ventilation improving drying uniformity of germinated brown rice under continuous microwave conditions. *Transact. Chinese Soc. Agric. Eng.* 33(13): 280-286.

Zhou, H., Rao, Z., Zhang, Z., and Zhou, J. 2023. Function of the GABAergic System in diabetic encephalopathy. *Cell. Mol. Neurobiol.* 43(2): 605-619.

Zhou, X., Zhao, G., Sun, S., and Li, J. 2019. Antihypertensive effect of giant embryo brown rice and pre- germinated giant embryo brown rice on spontaneously hypertensive rats. *Food Sci. Nutr*. 307(9): 2888-2896.

Zhou, Y. and Danbolt, N. C. 2013. GABA and glutamate transporters in brain. *Front. Endocrinol.* 4:165.

Zhou, Z., Robards, K., Helliwell, S., and Blanchard, C. 2002. Ageing of stored rice: changes in chemical and physical attributes. *J. Cereal Sci.* 35(1): 65-78.

Zhou, Z., Robards, K., Helliwell, S., Blanchard, C., and Baxterb, G. 2003. Rice ageing. I. effect of changes in protein on starch behaviour. *Starch-Stärke* 55(3-4): 162-169.

APPENDICES

APPENDIX I

Score card for assessing the organoleptic qualities of cooked rice

***(Jyothi /Njavara/Chitteni*)**

No	Parameters	Treatments									
		T_0	T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9
1	**Appearance**										
2	**Colour**										
3	**Flavour**										
4	**Texture**										
5	**Taste**										
7	**Overall acceptability**										

9 point hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like or dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Date: **Signature:**

Name:

APPENDIX II

Score card for the selection of the best suitable condition for GABA enrichment

Title : Medicinal properties and process optimisation for GABA enrichment in rice			
GABA content	Total antioxidant activity	Organoleptic qualities	Total
			1

APPENDIX III

Procedure for the preparation of processed rice products from GABA enriched rice

1. COOKED RICE

Ingredients:

GABA enriched rice – 100g

Water – As required

Procedure:

Add washed GABA enriched rice to a sufficient amount of boiling water. Cook it well. After cooking, strain the excess water to get the cooked rice.

2. RICE PORRIDGE

Ingredients

GABA enriched rice flour– 50 g

Water – As required

Procedure:

Add GABA enriched rice flour to a 150 ml boiling water. Stir continuously to get the thickness of the porridge.

3. RICE FLAKES

Ingredients:

GABA enriched Paddy – 100 g

Salt- as required

Procedure:

Soak the paddy in excess water for 72 h and kept it for another 72 h for getting GABA enriched rice. Remove water, wet paddy was put into heated salt in an iron pan for 40-50 seconds with continuous stirring until it gets tender. The tender grains are rolled, then flattened using a flaking machine.

4. PUFFED RICE

Ingredients:

GABA enriched rice – 100 g

Salt – as required

Procedure :

Soak the paddy in excess water for 72 h and kept it for another 72 h for getting GABA enriched rice. Remove water, wet paddy was put into heated salt in an iron pan for 40-50 seconds with continuous stirring. Almost all the husk was separated from popped kernel, which was already detached during heating.

APPENDIX IV

Score card for assessing the organoleptic qualities of processed rice products of GABA enriched rice

No	Parameters	Treatments											
		Jyothi				***Njavara***				***Chitteni***			
		T_1	T_2	T_3	T_4	T_5	T_6	T_7	T_8	T_9	T_{10}	T_{11}	T_{12}
1	**Appearance**												
2	**Colour**												
3	**Flavour**												
4	**Texture**												
5	**Taste**												
7	**Overall acceptability**												

9 point hedonic scale

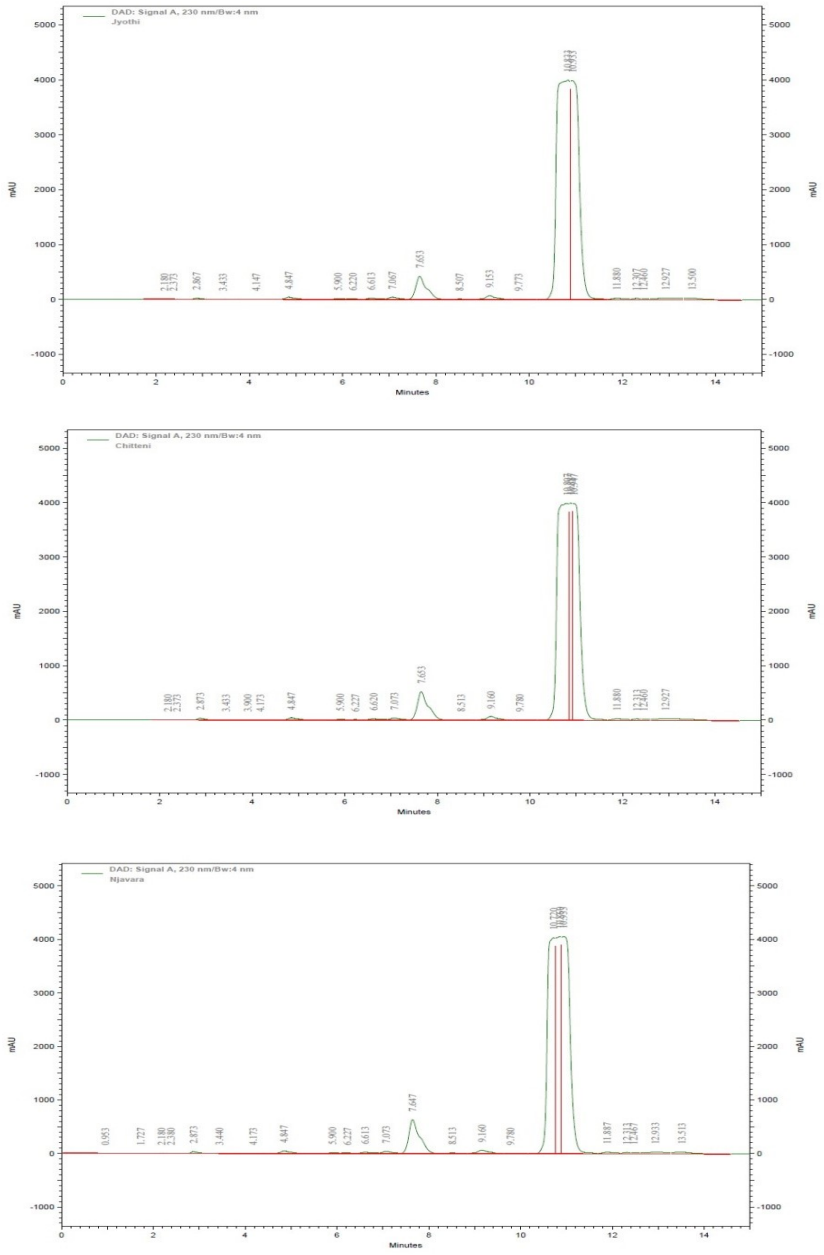
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like or dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Date: **Signature:**

Name:

APPENDIX V

HPLC chromatogram of GABA derivatized with 2-hydroxynaphthaldehyde for GABA enriched rice varieties of *Jyothi, Njavara* and *Chitteni*



Chromatogram of GABA derivatized with 2-hydroxynaphthaldehyde for 72 h soaked 72 h germinated **(a)** *Jyothi* **(b)** *Chitteni* **(c)** *Njavara*

APPENDIX VI

Organoleptic qualities of germinated and cooked *Jyothi/Njavara/Chitteni* rice

Treatments	Cultivar	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T_1 (24 h S + 24 h G)	*Jyothi*	7.38 (4.21)	7.66 (3.58)	7.47 (3.64)	7.54 (3.93)	7.59 (4.18)	7.59 (3.89)	7.53
T_2 (24 h S + 48 h G)		7.09 (3.04)	7.64 (3.46)	7.35 (3.14)	7.42 (3.18)	7.40 (3)	7.57 (3.29)	7.42
T_3 (24 h S+ 72 h G)		7.47 (4.86)	7.85 (4.19)	7.64 (4.50)	7.66 (4.50)	7.61 (4.32)	7.71 (4.50)	7.65
T_4(48 h S + 24 h G)		7.19 (3.21)	7.78 (4.35)	7.45 (3.36)	7.54 (3.79)	7.40 (3.36)	7.61 (3.82)	7.49
T_5 (48 h S + 48 h G)		7.5 (4.75)	7.97 (5.35)	7.59 (4.11)	7.59 (3.79)	7.52 (3.50)	7.66 (3.93)	7.63
T_6 (48 h S + 72 h G)		7.38 (4.14)	7.85 (4.88)	7.83 (5.68)	7.69 (4.82)	7.73 (4.96)	7.78 (5.18)	7.71
T_7 (72 h S + 24 h G)		7.40 (4.54)	7.95 (5.27)	7.73 (4.89)	7.85 (5.75)	7.80 (5.36)	7.76 (4.68)	7.74
T_8 (72 h S + 48 h G)		8.07 (7.57)	8.16 (6.26)	8.21 (7.61)	8.09 (7.04)	8.26 (7.82)	8.14 (7.39)	8.15
T_9 (72 h S + 72 h G)		8.33 (8.68)	8.28 (7.46)	8.30 (8.07)	8.38 (8.21)	8.40 (8.50)	8.35 (8.32)	8.34
W		0.525^{**}	0.295^{**}	0.522^{**}	0.451^{**}	0.570^{**}	0.465^{**}	
T_{10} (24 h S + 24 hG)		7.52 (3.43)	7.47 (4.04)	7.40 (3.96)	7.5 (4.43)	7.19 (3.71)	7.61 (4.32)	7.44
T_{11} (24 h S + 48 h G)		7.54 (4)	7.38 (2.93)	7.33 (3.25)	7.4 (3.29)	7.14 (3.11)	7.64 (4.11)	7.40

T_{12} (24 h S+ 72 h G)	*Njavara*	7.64 (4.25)	7.66 (4.61)	7.42 (3.93)	7.45 (3.86)	7.26 (3.79)	7.66 (4.25)	7.51
T_{13} (48 h S + 24 h G)		7.47 (3.46)	7.59 (4.25)	7.40 (3.57)	7.61 (4.86)	7.23 (3.71)	7.64 (3.93)	7.49
T_{14} (48 h S + 48 h G)		7.61 (4.14)	7.66 (4.46)	7.61 (4.86)	7.54 (4.14)	7.40 (4.89)	7.66 (4.36)	7.58
T_{15} (48 h S + 72 h G)		7.69 (5.07)	7.7s (4.93)	7.64 (5.21)	7.61 (5.18)	7.54 (5.71)	7.73 (4.89)	7.65
T_{16} (72 h S + 24 h G)		7.71 (5.25)	7.69 (4.75)	7.71 (5.57)	7.64 (4.79)	7.61 (6.14)	7.71 (4.71)	7.67
T_{17} (72 h S + 48 h G)		8.21 (7.43)	8.14 (7.43)	7.97 (7)	7.92 (6.86)	7.73 (6.32)	8.02 (6.64)	7.99
T_{18} (72 h S + 72 h G)		8.40 (7.96)	8.23 (7.61)	8.16 (7.64)	8.11 (7.61)	7.90 (7.61)	8.16 (7.79)	8.16
W		0.441^{**}	0.416^{**}	0.379^{**}	0.295^{**}	0.346^{**}	0.280^{**}	
T_{19} (24 h S + 2 G)	*Chitteni*	7.64 (4.29)	7.73 (4.89)	7.59 (4.25)	7.40 (4.11)	7.47 (4.32)	7.54 (4.18)	7.56
T_{20} (24 h S + 48 h G)		7.54 (3.57)	7.66 (4.46)	7.47 (3.64)	7.35 (3.64)	7.38 (3.36)	7.57 (4.43)	7.49
T_{21} (24 h S+ 72 h G)		7.66 (4.39)	7.73 (4.86)	7.54 (3.86)	7.45 (4.50)	7.40 (3.68)	7.59 (4.75)	7.56
T_{22} (48 h S + 24 h G)		7.59 (4.11)	7.64 (4.46)	7.52 (3.79)	7.40 (3.93)	7.40 (3.79)	7.52 (3.82)	7.51
T_{23} (48 h S + 48 h G)		7.66 (4.21)	7.69 (4.43)	7.69 (5)	7.38 (3.93)	7.59 (5.21)	7.69 (5.29)	7.61
T_{24} (48 h S + 72 h G)		7.69 (4.64)	7.71 (4.68)	7.66 (4.71)	7.54 (4.68)	7.61 (5)	7.59 (4.86)	7.63

T_{25} (72 h S + 24 h G)		7.71 (4.96)	7.73 (4.82)	7.71 (5.14)	7.76 (6.04)	7.83 (6.39)	7.69 (5.07)	7.73
T_{26} (72 h S + 48 h G)		8.09 (7.57)	7.90 (6)	8.19 (7.11)	7.88 (6.89)	7.90 (6.64)	7.78 (5.86)	7.95
T_{27} (72 h S + 72 h G)		8.11 (7.25)	7.97 (6.39)	8.21 (7.50)	7.92 (7.29)	7.88 (6.61)	7.92 (6.75)	8
W		0.350^{**}	0.101^{*}	0.339^{**}	0.300^{**}	0.269^{**}	0.13^{1**}	

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

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

S- Soaking, G-Germination

APPENDIX VII

Organoleptic qualities of cooked GABA enriched rice of *Jyothi, Njavara* and *Chitteni*

Treatments		Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
Jyothi	C	7.33 (1.71)	7.52 (2.11)	7.38 (2.14)	7.54 (2.46)	7.57 (2.71)	7.57 (2.46)	7.48
	T	8.38 (5.21)	8.33 (5.29)	8.35 (5.21)	8.38 (5.46)	8.35 (5.57)	8.40 (5.46)	8.36
Njavara	C	7.45 (2.04)	7.59 (2.32)	7.42 (2.14)	7.42 (2.18)	7.38 (2.07)	7.57 (2.18)	7.47
	T	8.40 (5.25)	8.23 (4.75)	8.16 (4.43)	8.16 (4.46)	7.90 (4.07)	8.16 (4.68)	8.16
Chitteni	C	7.54 (2.36)	7.66 (2.61)	7.54 (2.39)	7.52 (2.29)	7.54 (2.61)	7.61 (2.43)	7.56
	T	8.14 (4.43)	8.00 (3.93)	8.23 (4.68)	7.97 (4.14)	7.90 (3.96)	7.95 (3.79)	8.03
W		0.852**	0.630**	0.664**	0.611**	0.538**	0.611**	

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

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

C- control, T -treatment

APPENDIX VIII

Organoleptic qualities of cooked GABA enriched rice of *Jyothi, Njavara* and *Chitteni* during storage

Storage period / Character	*Jyothi*					
	Control			Treatment		
	Initial	3 MAS	6 MAS	Initial	3 MAS	6 MAS
Appearance	8	7.8	7.52	8.5	8.3	7.98
Colour	7.78	7.5	7.1	8.4	8.15	7.9
Flavour	7.3	7.21	7.07	8.25	8.12	7.89
Texture	7.4	7.25	7.02	8.14	8.08	7.98
Taste	7.1	6.8	6.6	8.4	8.14	8
Overall acceptability	7.3	7.2	7.1	8.48	8.25	7.98
Total mean score	7.48	7.29	7.06	8.36	8.17	7.95

Storage period / Character	*Njavara*					
	Control			Treatment		
	Initial	3 MAS	6 MAS	Initial	3 MAS	6 MAS
Appearance	7.9	7.78	7.5	8.25	8.19	8.1
Colour	7.77	7.32	7.2	8.14	8.14	8.05
Flavour	7.33	7.3	7.11	8.18	8	7.8
Texture	7.5	7.2	7	8	7.88	7.79

Taste	7.09	6.7	6.5	8.11	7.9	7.74
Overall acceptability	7.28	7.1	6.9	8.3	7.89	7.55
Total mean score	7.47	7.23	7.03	8.16	8	7.83

Storage period / **Character**	***Chitteni***					
	Control			**Treatment**		
	Initial	**3 MAS**	**6 MAS**	**Initial**	**3 MAS**	**6 MAS**
Appearance	8	7.95	7.5	8.11	8	7.9
Colour	7.8	7.6	7.26	7.94	7.88	7.7
Flavour	7.4	7.25	7	8.04	7.85	7.42
Texture	7.6	7.12	7.08	7.9	7.82	7.67
Taste	7.2	6.9	6.66	8.05	7.88	7.65
Overall acceptability	7.38	7.3	7.15	8.16	7.75	7.22
Total mean score	7.56	7.35	7.10	8.03	7.86	7.59

APPENDIX XI

Organoleptic qualities of processed products from GABA enriched rice of *Jyothi, Njavara* and *Chitteni*

Treatments	Appearance	Colour	Flavour	Texture	Taste	Overall acceptability	Total score
T_1 (GABA enriched *Jyothi* + Cooking)	8.40 (3.07)	8.33 (2.61)	8.33 (3.46)	8.38 (3.54)	8.35 (3.46)	8.40 (3.54)	8.36
T_2 (GABA enriched *Jyothi* + Pulverising)	8.00 (1.75)	8.11 (2.25)	7.95 (2.18)	7.95 (2.21)	7.92 (2.25)	8.02 (2.29)	7.99
T_3 (GABA enriched *Jyothi* + Flaking)	8.23 (2.46)	8.21 (2.50)	7.80 (1.71)	8.02 (2.43)	7.78 (1.75)	8.00 (2.14)	8.01
T_4(GABA enriched *Jyothi* + Puffing)	8.28 (2.71)	8.23 (2.64)	8.14 (2.64)	7.85 (1.82)	7.97 (2.54)	7.97 (2.04)	8.07
W	0.316^{**}	0.035^{*}	0.520^{**}	0.477^{**}	0.419^{**}	0.532^{**}	
T_5 (GABA enriched *Njavara* + Cooking)	8.38 (3.46)	8.21 (3.18)	8.19 (3.07)	8.16 (2.96)	7.92 (1.79)	8.16 (3.00)	8.17
T_6 (GABA enriched	7.83	7.78	7.90	7.83	8.02	7.90	7.88

Njavara + Pulverising)	(1.79)	(1.46)	(2.21)	(1.96)	(2.14)	(2.18)	
T_7 (GABA enriched *Njavara* + Flaking)	7.95 (2.11)	8.09 (2.68)	7.71 (1.82)	8.00 (2.43)	8.38 (3.50)	8.00 (2.61)	8.02
T_8 (GABA enriched *Njavara* + Puffing)	8.14 (2.64)	8.11 (2.68)	8.16 (2.89)	8.07 (2.64)	8.21 (2.57)	7.92 (2.21)	8.1
W	0.538^{**}	0.416^{**}	0.325^{**}	0.159^{**}	0.430^{**}	0.156^{**}	
T_9 (GABA enriched *Chitteni* + Cooking)	8.11 (3.00)	8.02 (2.86)	8.26 (3.54)	7.97 (3.25)	7.90 (3.07)	7.95 (2.79)	8.03
T_{10} (GABA enriched *Chitteni* + Pulverising)	7.80 (2.00)	8.02 (2.46)	7.83 (2.14)	7.47 (1.71)	7.42 (1.61)	7.95 (3.00)	7.75
T_{11} (GABA enriched *Chitteni* + Flaking)	8.21 (3.11)	7.97 (2.43)	7.80 (2.07)	7.78 (2.64)	7.59 (2.29)	7.76 (2.04)	7.85
T_{12} (GABA enriched *Chitteni* + Puffing)	7.78 (1.89)	7.92 (2.25)	7.88 (2.25)	7.69 (2.39)	7.88 (3.04)	7.78 (2.18)	7.82
W	0.433^{**}	0.063^{*}	0.455^{**}	0.357^{**}	0.378^{**}	0.219^{**}	

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

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

MEDICINAL PROPERTIES AND PROCESS OPTIMISATION FOR GABA ENRICHMENT IN RICE

By

SIMLA THOMAS

(2018-24-002)

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Community Science

(FOOD SCIENCE AND NUTRITION)

Faculty of Agriculture

DEPARTMENT OF COMMUNITY SCIENCE

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR - 680 656

KERALA, INDIA

2023

ABSTRACT

Rice, the grain of life is consumed worldwide and serves as the cornerstone of global food security. Brown rice in its whole form contains various nutritive and bioactive components and has numerous health benefits. Germination is an effective method to enhance the organoleptic, textural and nutritional qualities of rice grains, including functional compounds such as gamma amino butyric acid (GABA).

GABA is a four carbon non-protein amino acid and inhibitory neurotransmitter which has a great potential to prevent several diseases. Rice naturally contains GABA and factors such as cultivar, temperature, moisture, soaking period and germination time influence the GABA levels in rice. The quantification of GABA content and the possibilities for its enrichment have not been explored so far. Hence, the current study, "Medicinal properties and process optimisation for GABA enrichment in rice" was carried out with the objectives of optimisation of conditions for GABA enrichment and to evaluate the physicochemical characteristics and medicinal properties of GABA enriched rice. The study also aimed to analyse the effect of processing on GABA content and to identify the maximum retention of GABA in processed products. The popular rice variety *Jyothi*, the medicinal rice variety *Njavara* (yellow glumed) and the indigenous rice variety *Chitteni* were selected for the study.

The quantity of GABA in ungerminated *Jyothi* (19.18 mg/kg), *Njavara* (28.63 mg/kg) and *Chitteni* (26.67 mg/kg) was estimated. The GABA content in rice was enriched by optimising soaking (24 h, 48 h, 72 h) and germination (24 h, 48 h, 72 h) durations. A significant increase in GABA content was observed in germinated brown rice of *Jyothi,* soaked and germinated for 24 h (69.72 mg/kg) which increased further to 116.89 mg/kg after soaking and germination for 72 h. The GABA content of *Njavara* after soaking and germinating for 24 h was 74.85 mg/kg, which increased to 130.29 mg/kg after 72 h of soaking and germination. A similar increase of GABA content in *Chitteni* also was observed from 49.97 mg/kg (24 h soaking and germination) to 127.97 mg/kg (72 h soaking and 72 h germination). The methanolic rice extracts possessed total antioxidant activity by scavenging free radicals. In *Jyothi* rice variety, the highest antioxidant activity was observed in samples soaked for 48 h and germinated for 24 h (IC_{50} - 4.79 µg/ml). In *Njavara*, the highest antioxidant activity was found after 72 h

soaking and 48 h germination (IC_{50} - 4.28 µg/ml). In *Chitteni*, 24 h soaked and 48 h germinated rice samples possessed the highest antioxidant activity (IC_{50} -5.45 µg/ml), The highest total mean score in organoleptic qualities was observed after 72 h soaking and 72 h germination in *Jyothi* (8.34), *Njavara* (8.16) and *Chitteni* (8.0). Based on the GABA content, total antioxidant activity and sensory qualities, the most suitable conditions for GABA enrichment were standardised as 72 h soaking and 72 h germination and selected for further studies.

The nutritional composition of GABA enriched rice of *Jyothi, Njavara* and *Chitteni* was analysed and compared with the control, the moisture content significantly increased in a range of 13.19 to 13.58 per cent. The protein content of GABA enriched grains ranged from 12.43 to 14.28 per cent. After GABA enrichment, the fat content (2.04 to 3.0 %) was found to be significantly increased. The starch content in the GABA enriched rice ranged from 71.52 to 75.76 per cent and a significant decrease in starch content was observed in all three rice varieties. Germination led to a considerable decrease in the amylose content (22.88 to 23.87 %). The carbohydrate (60.43 to 63.23 %) and energy content (324.88 to 326.30 Kcal) showed significant reduction after germination. The *in vitro* starch digestibility of GABA enriched rice ranged from 75.13 to 78.18 per cent. The mineral content in GABA enriched rice was observed as calcium (11.35 to 13.11 mg/100g), zinc (2.33 to 3.37 mg/100g), iron (2.25 to 3.12 mg/100g) and phosphorous (300.77 to 332.58 mg/100g). The thiamine content was noted as 0.64 to 0.80 mg/100g. The riboflavin (0.13 to 0.18 mg/100g) and vitamin E content (4.61 to 5.11 µg/g) increased in GABA enriched rice compared to their ungerminated counterparts. Among the minerals, the *in vitro* availability of phosphorous (55.89 to 59.95 %) was the highest, followed by calcium (44.09 to 49.55 %), zinc (34.54 to 38.14 %) and iron (30.09 to 33.24 %), after GABA enrichment in *Jyothi*, *Njavara* and *Chitteni* respectively.

The GABA enriched grains possessed antioxidant and antiproliferative activity. The methanolic extract of GABA enriched rice exhibited antioxidant activity by moderate reducing power and scavenging the DPPH (1,1-diphenyl 1,2- picrylhydrazyl) radicals, superoxide and hydroxyl groups. The highest antioxidant activity was possessed by GABA enriched *Njavara*, *Chitteni* and *Jyothi* in ferric oxide reducing

power activity with the projected IC_{50} values of 47.30 µg/ml, 52.15 µg/ml and 53.81 µg/ml respectively.

The bioactive compounds in GABA enriched rice mainly included phenols and flavonoids. The phenol content of GABA enriched rice ranged from 35.92 to 42.50 GAE µg/mg and the flavonoid content varied from 13.8 to 17.5 QE µg/mg.

The antiproliferative activity of GABA enriched rice was also assessed in the hepatic cancer cell line (HepG2). The viability of cancer cells was inhibited by the rice extract of GABA enriched *Jyothi*, *Njavara* and *Chitteni* with an IC_{50} value of 92.75 µg/ml, 56.53 µg/ml and 76.10 µg/ml respectively.

The GABA enriched grains were packed in polyethylene bags and kept for storage studies for six months. Though, a slight decrease in the physico chemical properties and organoleptic qualities was observed by the end of the storage period, the microbial population was within the permissible limits. The GABA enriched rice was found to be shelf stable for six months.

Processed products like cooked rice, parboiled rice, pulverised rice, flaked rice and puffed rice were prepared from GABA enriched rice of *Jyothi, Njavara* and *Chitteni*. The retention of GABA was observed after cooking (72.15 to 73.05 %), parboiling (79.96 to 84.34 %), pulverising (72.45 to 95.97 %), flaking (84.03 to 90.45 %) and puffing (81.34 to 86.26 %). After processing, the total antioxidant activity was found to be slightly decreased. All the processed rice products were organoleptically acceptable with an average mean score of more than 7.75.

The present study revealed that, GABA content in rice can be enhanced by adopting optimum soaking and germination durations. In GABA enriched rice, the antioxidant, antiproliferative activities, nutritional benefits and sensory qualities were found to be higher than ungerminated rice. The developed processed rice products showed good sensory qualities and retained GABA content. Germination is an effective and low cost strategy to transform rice into a functional food.

സംക്ഷിപ്തം

ജീവധാന്യമായ അരി ലോകമെമ്പാടും ഉപഭോഗം ചെയ്യപ്പെടുകയും ആഗോള ഭക്ഷ്യസുരക്ഷയുടെ ആണിക്കല്ലായി പ്രവർത്തിക്കുകയും ചെയ്യുന്നു. തവിടു കളയാത്ത അരിയിൽ വിവിധ പോഷക ഘടകങ്ങളും ബയോ ആക്റ്റീവ് ഘടകങ്ങളും കൂടാതെ നിരവധി ആരോഗ്യ ഗുണങ്ങളും ഉണ്ട്. അരിയുടെ പോഷക ആരോഗ്യ ഗുണങ്ങൾ വർദ്ധിപ്പിക്കുന്നതിനുള്ള ഫലപ്രദമായ മാർഗമാണ് മുളപ്പിക്കൽ. ഗാമാ അമിനോ ബ്യൂട്ടിറിക് ആസിഡ് (ഗാബ) പോലുള്ള ആരോഗ്യ ഘടകങ്ങളും മുളപ്പിച്ച അരിയിൽ അടങ്ങിയിരിക്കുന്നു. കൂടാതെ മുളപ്പിക്കൽ വഴി അരിയുടെ രുചിഗുണങ്ങൾ വർദ്ധിക്കുകയും ചെയ്യുന്നു.

ജ്യോതി, ചിറ്റേനി, ഞവര എന്നി മൂന്ന് നെല്ലിനങ്ങളിലാണ് പഠനം നടത്തിയത്. മുളപ്പിയ്ക്കാത്ത ജ്യോതി (19.18 mg/kg), ഞവര (28.63 mg/kg), ചിറ്റേനി (26.67 mg/kg) എന്നിവയിൽ ഗാബയുടെ അളവ് കണക്കാക്കിയിട്ടുണ്ട്. കുതിർക്കൽ (24 മണിക്കൂർ, 48 മണിക്കൂർ, 72 മണിക്കൂർ), മുളപ്പിക്കൽ (24 മണിക്കൂർ, 48 മണിക്കൂർ, 72 മണിക്കൂർ) എന്നിവ നിജപ്പെടുത്തികൊണ്ട് അരിയിലെ ഗാബയുടെ തോത് സമ്പുഷ്ടീകരിച്ചു. 24 മണിക്കൂർ (69.72 mg/kg) കുതിർത്ത് മുളപ്പിച്ച ജ്യോതിയുടെ തവിടു കളയാത്ത അരിയിൽ ഗാബയുടെ അളവിൽ ഗണ്യമായ വർദ്ധനവ് കാണപ്പെട്ടു, ഇത് 72 മണിക്കൂർ കുതിർത്ത് മുളപ്പിച്ചതിന് ശേഷം 116.89 mg/kg ആയി വർദ്ധിച്ചു. 24 മണിക്കൂർ കുതിർത്ത് മുളപ്പിച്ചതിന് ശേഷമുള്ള ഞവരയിലെ ഗാബയുടെ അളവ് 74.85 mg/kg ആയിരുന്നു, ഇത് 72 മണിക്കൂർ കുതിർത്ത് മുളപ്പിച്ചപ്പോൾ 130.29 mg/kg ആയി വർദ്ധിച്ചു. ചിറ്റേനിയിലെ ഗാബ അളവിലും വർദ്ധനവ് 49.97 mg/kg (24 മണിക്കൂർ കുതിർക്കലും മുളപ്പിക്കലും) എന്നതിൽ നിന്ന് 127.97 mg/kg (72 മണിക്കൂർ കുതിർക്കലും 72 മണിക്കൂർ മുളപ്പിക്കലും) ആയി ഉയർന്നു. മുളപ്പിച്ച അരിയുടെ മെത്തനോളിക് സത്ത് ഫ്രീ റാഡിക്കലുകളെ തടയുന്നതായും ആന്റി ഓക്സിഡൻറ് ഘടകങ്ങൾ അടങ്ങിയിരിക്കുന്നതായും കണ്ടെത്തി. ജ്യോതി നെല്ലിനത്തിൽ, 48 മണിക്കൂർ കുതിർത്തതും 24 മണിക്കൂർ മുളപ്പിച്ചതുമായ സാമ്പിളുകളിൽ (IC_{50} - 4.79 µg/ml) ഏറ്റവും ഉയർന്ന ആന്റിഓക്സിഡൻറ് പ്രവർത്തനം നിരീക്ഷിക്കപ്പെട്ടു. ഞവര അരി, 72 മണിക്കൂർ കുതിർത്തതിനും 48 മണിക്കൂർ മുളപ്പിച്ചതിനും ശേഷം ഏറ്റവും ഉയർന്ന ആന്റിഓക്സിഡൻറ് പ്രവർത്തനം ഉണ്ടായതായി കണ്ടെത്തി (IC_{50} - 4.28 µg/ml). 24 മണിക്കൂർ കുതിർത്തു 48 മണിക്കൂർ മുളപ്പിച്ച ചിറ്റേനി അരിയുടെ സാമ്പിളുകളിൽ ഏറ്റവും ഉയർന്ന ആന്റിഓക്സിഡൻറ് പ്രവർത്തനം (IC_{50} -5.45 µg/ml)

ഉണ്ടായിരുന്നു, 72 മണിക്കൂർ കുതിർത്തതിനും 72 മണിക്കൂർ മുളച്ചതിനും ശേഷം ജ്യോതിയിലും (8.34), ഞവരയിലും (8.16), ചിറ്റേനിയിലും (8.0) രുചി, നിറം, ഗന്ധം, പദാർത്ഥ സ്വഭാവം തുടങ്ങിയ സെൻസറി ഗുണങ്ങളിൽ ഏറ്റവും ഉയർന്ന ശരാശരി സ്കോർ നിരീക്ഷിക്കപ്പെട്ടു. ഗാബ യുടെ അളവ്, മൊത്തം ആന്റിഓക്സിഡന്റ് പ്രവർത്തനം, സെൻസറി ഗുണങ്ങൾ എന്നിവയെ അടിസ്ഥാനമാക്കി, തവിടു കളയാത്ത അരിയിൽ ഗാബ സമ്പുഷ്ടീകരണത്തിനു ഏറ്റവും അനുയോജ്യമായ വ്യവസ്ഥകൾ 72 മണിക്കൂർ കുതിർക്കുന്നതും 72 മണിക്കൂർ മുളപ്പിക്കുന്നതും ആണെന്ന് കണ്ടെത്തി തുടർപഠനങ്ങൾ നടത്തി.

ജ്യോതി, ഞവര, ചിറ്റേനി എന്നിവയുടെ ഗാബ സമ്പുഷ്ടമായ അരിയുടെ പോഷകഘടന വിശകലനം ചെയ്യുകയും, അവയുടെ തന്നെ മുളപ്പിക്കാത്ത അരിയുമായി താരതമ്യപ്പെടുത്തുകയും ചെയ്തു മുളപ്പിച്ച അരിയിൽ ഈർപ്പത്തിന്റെ അളവ് 13.19 മുതൽ 13.58 ശതമാനം വരെ വർദ്ധിച്ചതായി കണ്ടെത്തി. ഗാബ സമ്പുഷ്ടമായ ധാന്യങ്ങളുടെ പ്രോട്ടീൻറെ വർധനയുടെ തോത് 12.43 മുതൽ 14.28 ശതമാനം വരെയാണ്. ഗാബ സമ്പുഷ്ടീകരണത്തിനു ശേഷം, കൊഴുപ്പിന്റെ അളവ് (2.04 മുതൽ 3.0 % വരെ) വർദ്ധിച്ചതായി കണ്ടെത്തി ഗാബ സമ്പുഷ്ടമായ അരിയിലെ അന്നജത്തിന്റെ അംശം 71.52 മുതൽ 75.76 ശതമാനം വരെയാണ്, കൂടാതെ മൂന്ന് നെല്ലിനങ്ങളിലും അന്നജത്തിന്റെ അളവ് ഗണ്യമായി കുറഞ്ഞു അരി മുളപ്പിക്കുന്നത് അമൈലോസിന്റെ അളവിൽ (22.88 മുതൽ 23.87 % വരെ) ഗണ്യമായ കുറവുണ്ടാക്കി. കാർബോഹൈഡ്രേറ്റും (60.43 മുതൽ 63.23 % വരെ) ഊർജ്ജത്തിന്റെ അളവും (324.88 മുതൽ 326.30 Kcal വരെ) മുളച്ച് കഴിഞ്ഞപ്പോൾ കാര്യമായ കുറവ് കാണിച്ചു. ഗാബ സമ്പുഷ്ടമായ അരിയുടെ ഇൻ വിട്രോ സ്റ്റാർച്ച് ഡൈജസ്റ്റബിലിറ്റി 75.13 മുതൽ 78.18 ശതമാനം വരെയാണ്. ഗാബ സമ്പുഷ്ടമാക്കിയ അരിയിലെ ധാതുക്കളുടെ അളവ് കാൽസ്യം (11.35 മുതൽ 13.11 mg/100g), സിങ്ക് (2.33 to 3.37 mg/100g), ഇരുമ്പ് (2.25 മുതൽ 3.12 mg/100g), ഫോസ്ഫറസ് (300.77 മുതൽ 332 05 mg/100g വരെ) എന്നിങ്ങനെയാണ്. തൈമിന്റെ അളവ് 0.64 മുതൽ 0.80 മില്ലിഗ്രാം / 100 ഗ്രാം വരെ രേഖപ്പെടുത്തിയിട്ടുണ്ട് ഗാബ സമ്പുഷ്ടമായ അരിയിൽ റൈബോഫ്ലേവിനും (0.13 മുതൽ 0.18 mg/100g) വിറ്റാമിൻ ഇ യുടെ അളവും (4.61 to 5.11 µg/g) അവയുടെ മുളപ്പിക്കാത്ത അരികളെ അപേക്ഷിച്ച് വർദ്ധിച്ചു ഗാബ സമ്പുഷ്ടമായ ജ്യോതി, ഞവര, ചിറ്റേനി എന്നിവയുടെ അരിയിൽ, കാൽസ്യം (44.09 മുതൽ 49.55 %), സിങ്ക് (34.54 മുതൽ 38.14 %), ഇരുമ്പ് (30.09 മുതൽ 33.24 വരെ) എന്നിവയുടെ ഇൻ

വിട്രോ ലഭ്യത താരതമ്മ്യം ചെയ്യുമ്പോൾ ഫോസ്ഫറസിന്റെ ഇൻ വിട്രോ ലഭ്യത (55.89 മുതൽ 59.95 %) ഉയർന്നതാണ്.

ഗാബ സമ്പുഷ്ടമാക്കിയ അരിക്ക് ആന്റിഓക്സിഡന്റും ആന്റി പ്രൊലിഫെറേറ്റീവ് പ്രവർത്തനവും ഉണ്ടായിരുന്നു. ഗാബ സമ്പുഷ്ടമാക്കിയ അരിയുടെ മെത്തനോളിക് സത്തിൽ മിതമായ രീതിയിൽ ഡി പി പി എച്ച് (1,1-ഡിഫെനൈൽ 1,2- പിക്രിൽഹൈഡ്രാസിൽ) റാഡിക്കലുകൾ, സൂപ്പർ ഓക്സൈഡ്, ഹൈഡ്രോക്സൈൽ ഗ്രൂപ്പുകൾ എന്നിവയുടെ ശക്തി കുറയ്ക്കുകയും നീക്കം ചെയ്യുകയും ചെയ്തുകൊണ്ട് ആന്റിഓക്സിഡന്റ് പ്രവർത്തനം നടക്കുന്നതായി നിരീക്ഷിച്ചു. ഫെറിക് ഓക്സൈഡ് കുറയ്ക്കുന്ന ഊർജ്ജ പ്രവർത്തനത്തിൽ ഗാബ സമ്പുഷ്ടമാക്കിയ ഞവര, ചിറ്റേനി, ജ്യോതി എന്നിവ ഏറ്റവും ഉയർന്ന ആന്റിഓക്സിഡന്റ് പ്രവർത്തനം യഥാക്രമം IC_{50} മൂല്യം 47.30 µg/ml, 52.15 µg/ml, 53.81 µg/ml എന്നിങ്ങനെ ആണ്.

ഗാബ സമ്പുഷ്ടമാക്കിയ അരിയിലെ ബയോ ആക്റ്റീവ് സംയുക്തങ്ങളിൽ പ്രധാനമായും ഫിനോളുകളും ഫ്ലേവനോയ്ഡുകളും ഉൾപ്പെടുന്നു. ഗാബ സമ്പുഷ്ടമാക്കിയ അരിയിലെ ഫിനോളിന്റെ അളവ് 35.92 മുതൽ 42.50 GAE µg/mg വരെയും ഫ്ലേവനോയിഡിന്റെ അളവ് 13.8 മുതൽ 17.5 QE µg/mg വരെയും വ്യത്യാസപ്പെടുന്നു.

ഗാബ സമ്പുഷ്ടമായ അരിയുടെ കരൾ കാൻസർ സെൽ ലൈനിൽ (HepG2) ഉള്ള ആന്റി പ്രോലൈഫെറേറ്റിവ് പ്രവർത്തനം വിലയിരുത്തി. ഗാബ സമ്പുഷ്ടമായ ജ്യോതി, ഞവര, ചിറ്റേനി എന്നിവയുടെ അരിയുടെ സത്ത് IC_{50} മൂല്യങ്ങൾ 92.75 µg/ml, 56.53 µg/ml, 76.10 µg/ml എന്നിങ്ങനെ ഉള്ളവയാണെന്നു കണ്ടെത്തി. ഇത് കരൾ കാൻസർ രോഗങ്ങളുടെ വ്യാപനത്തെ തടയുന്നുണ്ടെന്നു നിരീക്ഷിച്ചു.

ഗാബ സമ്പുഷ്ടമാക്കിയ അരി പോളിഎത്തിലീൻ ബാഗുകളിൽ പായ്ക്ക് ചെയ്യുകയും ആറ് മാസത്തേക്ക് സൂക്ഷിപ്പ് കാലയളവ് പഠനത്തിന് വിധേയമാക്കി. സംഭരണ കാലയളവിന്റെ അവസാനത്തോടെ ഭൗതിക രാസഗുണങ്ങളിലും, രുചി, നിറം, ഗന്ധം, പദാർത്ഥ സ്വഭാവം തുടങ്ങിയ സെൻസറി ഗുണങ്ങളിലും നേരിയ കുറവുണ്ടായെങ്കിലും, സൂക്ഷ്മജീവികളുടെ എണ്ണം അനുവദനീയമായ പരിധിക്കുള്ളിലായിരുന്നു. ഗാബ സമ്പുഷ്ടമായ അരി ആറ് മാസത്തേക്ക് സൂക്ഷിപ്പ് മേൻമയുള്ളതായി കണ്ടെത്തി.

ജ്യോതി, ഞവര, ചിറ്റേനി എന്നിവയുടെ ഗാബ സമ്പുഷ്ടമായ അരിയിൽ നിന്നാണ് ചോറ്, പുഴുങ്ങിയ അരി, പൊടിച്ച അരി, ഫ്ലേക്കേഡ് റൈസ്, പഫ്ഡ്

റൈസ് തുടങ്ങിയ സംസ്കരിച്ച ഉൽപ്പന്നങ്ങൾ തയ്യാറാക്കിയത്. പാചകം ചെയ്യതിന് ശേഷവും (72.15 മുതൽ 73.05 %), പാർബോയിലിംഗിന് ശേഷവും (79.96 മുതൽ 84.34 %), അരി പൊടിച്ചത്തിനു ശേഷവും (72.45 മുതൽ 95.97 %), ഫ്ലേക്കിംഗ് (84.03 മുതൽ 90.45 %), പഫിംഗ് (86.34 %) എന്നിവയ്ക്ക് ശേഷവും നല്ല ഒരു ശതമാനം ഗാബ നിലനിൽക്കുന്നതായി നിരീക്ഷിക്കപെട്ടു. സംസ്കരണത്തിന് ശേഷം, മൊത്തം ആന്റിഓക്സിഡന്റ് പ്രവർത്തനം ചെറിയ തോതിൽ കുറഞ്ഞതായി കണ്ടെത്തി മുളപ്പിച്ച അരി ഉപയോഗിച്ചു തയ്യാറാക്കിയ എല്ലാ അരി ഉൽപന്നങ്ങളും 7.75 ലധികം ശരാശരി സെൻസറി സ്കോർ ഉള്ളതിനാൽ സ്വീകാര്യമായിരുന്നു

കുതിർക്കലും മുളയ്ക്കുന്ന സമയവും നിജപ്പെടുത്തുന്നതിലൂടെ അരിയിലെ ഗാബയുടെ അളവ് വർദ്ധിപ്പിക്കാൻ കഴിയുമെന്ന് നിലവിലെ പഠനം വെളിപ്പെടുത്തുന്നു ഗാബ സമ്പുഷ്ടമായ അരിയിൽ, ആന്റിഓക്സിഡന്റ്, ആന്റിപ്രൊലിഫെറേറ്റീവ് പ്രവർത്തനങ്ങൾ, പോഷക ഗുണങ്ങൾ, സെൻസറി ഗുണങ്ങൾ എന്നിവ മുളപ്പിക്കാത്ത അരിയെക്കാൾ ഉയർന്നതായി കണ്ടെത്തി. മുളപ്പിച്ച അരി ഉപയോഗിച്ചു തയ്യാറാക്കിയ അരി ഉൽപന്നങ്ങൾ നല്ല സെൻസറി ഗുണങ്ങൾ കാണിക്കുകയും ഗാബയുടെ അളവ് നിലനിർത്തുകയും ചെയ്തു. അരിയിലെ പോഷകഗുണങ്ങൾ വർധിപ്പിച്ചു കൊണ്ട്, ആരോഗ്യ ദായകമായ ഭക്ഷണമാക്കി മാറ്റുന്നതിനുള്ള ഫലപ്രദവും ചെലവുകുറഞ്ഞതുമായ മാർഗ്ഗമാണ് മുളപ്പിക്കൽ