Production and characterization of fermented rice flour containing gamma-aminobutyric acid (GABA)

Divyashri G.[1], Prapulla S.G.[2*]
[1]Academy of Scientific and Innovative Research (AcSIR), New Delhi
[1,2]Microbiology and Fermentation Technology department, CSIR-Central Food Technological Research Institute (CFTRI), Mysore 570 020
*E-mail: prapullasg@yhaoo.co.in

Abstract— *Fermented foods provides therapeutical attributes beyond their basic nutritional value and are known to reduce disease risk. Broken rice was fermented using Enterococcus faecium NCIM 5593 and its fermentation characteristics was studied. Attempts were made to formulate gamma-aminobutyric acid (GABA) containing fermented rice flour (GFRF) by lactic acid fermentation. Fermentation enhanced the level of GABA and antioxidant phenolics. GFRF exhibited potential antioxidant capacity evaluated against DPPH (77.89±1.85 mg vitamin C equivalent/g dry matter) and ABTS (163.21±2.81 mg vitamin C equivalent/g dry matter) radicals. Fermentation significantly increased the levels of proteins and reduced carbohydrate content. Microstructure of GFRF was also influenced, where its starch granules where released from its enclosed structure after fermentation. In addition, fermentation enhanced the whiteness of the flour. This investigation shows evidence that fermentation modified the functionality of GFRF and can be used as a functional food ingredient. Further studies are directed towards studying the effect of GFRF extract to ameliorate neurotoxin induced oxidative dysfunctions and neurotoxicity in mice model.*

Keywords— Fermentation, Fermented foods, Gamma-aminobutyric acid, Microstructure, Antioxidant.

I. INTRODUCTION

Rice (*Oryza sativa*), being one of the most produced and consumed grains in the world, is a rich source of bioactive compounds, including many phenolic antioxidants (Mira et al., 2008; Zhang et al., 2010). One of the main byproducts of rice processing is broken rice. Broken rice is separated from head rice during rice milling process and is known to occur to approximately 1 to 13% of milled rice (Lee et al., 2011). Most broken rice is mainly used as animal feed and low-priced rice cakes as it is inadequate for regular consumption. One way of utilizing such broken rice is by fermentation process. It has been used to produce lactic acid (Nakano et al., 2012) and ethanol (Gohel and Duan, 2012) with high fermentation efficiency. It thus may serve as an important raw material for fermentation. Lactic acid bacteria (LAB) are industrially important microbes that are used world over. Consumption of lactic acid fermented foods is common in many Asian countries. Fermented foods offer an array of health benefits as they improve organoleptic quality. Fermentation also results in improved digestibility and nutritive value (Arici and Daglioglu, 2002). Health benefits from the fermented plant materials are usually direct, through interaction of ingested live microorganisms with the host (probiotic effect) or indirect as a result of the ingestion of microbial metabolites, which are synthesized during fermentation (biogenic effect) (Gobbetti et al., 2010). Cereal fermentation leads to improvement in product shelf life, nutritional value, and digestibility and significantly lowers antinutritional contents in the final products (Karovicova, 2007).

Gamma-aminobutyric acid (GABA) is a four carbon non-protein amino acid that acts as a major inhibitory neurotransmitter in central nervous system (CNS) (Kinnersley and Turano, 2000). Numerous studies have proclaimed the role of GABA as an antihypertension, anticancer, anti-inflammatory and hepatoprotection agents (Watanabe et al., 2006; Kang et al., 2011; Ali et al., 2013). These aspects have stimulated the interest to formulate GABA-containing natural product (Ali et al., 2013). Thus the purpose of the study was to formulate GABA containing fermented rice flour (GFRF) using a potent GABA producer, *E. faecium* NCIM 5593. The reason to enhance GABA levels by microbial fermentation is because of its varied health benefits: GABA promotes fat loss by the stimulation of human growth hormone production, increases sleep cycle by giving deeper rest, boosts immune system, lowers blood pressure, inhibits development of cancer cells and also assists in the treatment of

anxiety disorders (Oh and Oh, 2014; Ito and Ishikawa, 2004). In addition, Ito and Ishikawa, (2004) suggested that GABA has preventive effects on Alzheimer's disease and other cerebral related disorders, such as amnesia and dementia.

II. MATERIALS AND METHODS

2.1 Bacterial strain and growth conditions

Bacterial strain, *Enterococcus faecium* NCIM 5593 used in the study has earlier been reported to be a potent GABA producer (Gangaraju et al., 2014). The strain was isolated from indigenous fermented food and identified using genomic techniques (Divyashri and Prapulla, 2015a). The laboratory GABA production process using *E. faecium* NCIM 5593 is optimized and the strain has shown its potential to produce GABA under simulated gastro-intestinal conditions (Divyashri and Prapulla, 2015a & b). The strain was maintained at -80 OC de Man Rogosa and Sharpe (MRS) broth with 10% (v/v) glycerol and was normally cultured in MRS broth at 37 °C for regular experiments.

2.2 Preparation of rice flour medium

Briefly, broken rice (1001) was obtained from Bannur rice millers, Bannur, Karnataka, India. It was cleaned, destonned and pulverized to make flour. Rice flour medium was prepared by suspending various percentage of rice flour (1, 3, 5 and 7% w/v) in distilled water. The glutamate (0.5% w/v) was supplemented to the prepared medium, sterilized at 121 °C for 20 mins, inoculated with *E. faecium* NCIM 5593 and incubated at 37 °C, 120 rpm for 48 h. The effect of glutamate concentration on rice flour medium was studied by varying its concentration (0.25, 0.5, 0.75, 1, 3, 5% w/v) in rice flour medium (3% w/v). To analyze GABA, fermented slurry was centrifuged at 8000 rpm for 20 mins and the resulting water extract was subjected to HPLC analysis as described in our earlier publication (Divyashri and Prapulla, 2015b).

2.3 Preparation of GABA containing fermented rice flour (GFRF)

Rice flour medium (3% w/v) supplemented with glutamate (1% w/v) was sterilized at 121 °C for 20 mins, inoculated with *E. faecium* NCIM 5593 and incubated at 37 °C, 120 rpm for 48 h. Similar set was experiments performed without inoculating the strain served as control. The control and fermented slurry was thermally treated at 70 °C for 10 mins and centrifuged at 8000 rpm for 20 mins and the resulting water extract was lyophilized at -80 °C to yield GFRF extract. Organic acids was analyzed using HPLC equipped with a UV detector as described by Lei et al., (2015). The pH was measured by using a digital pH meter (Analab Scientific Instruments, Gujarat, India). Titratable acidity was determined as described by Shori et al., (2013).

2.4 Scanning Electron Microscopy (SEM) and Color Measurement

SEM analysis was performed using scanning electron microscope (SEM-Leo 435 VP, Leo Electron Microscopy Ltd, Zeiss, Cambridge, UK) and the changes in color was determined according to the method of Lu et al., (2012) using color measurement systems (Hunter Ultrascan XE).

2.5 Proximate analysis, total phenolics and flavonoids

Moisture, protein, fat and ash contents was determined according to the methods of Association of Official Analytical Chemists (AOAC, 2012). Total carbohydrate was calculated by difference. The total phenolic and flavonoid content was determined as described by Hossain and Shah, (2015). Total phenolic and flavonoid contents were expressed as mg gallic acid (GAE) and quercetin (QE) per gram of dry matter (DM), respectively. Phenolic acid profiling was performed using HPLC (Sreerama et al., 2010).

2.6 *In vitro* antioxidant assays and anti-nutritional factors

2,2′-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging activities was assayed according to Polthum and Ahromrit, (2014). Ferric reducing antioxidant power (FRAP) assay was performed as described by Thaipong et al., (2006). The phytate extracted using 2.4% HCl was assayed (Liang et

al., 2007). The trypsin inhibitor activity and α-amylase inhibition assay was determined according to the method of Mubarak, (2005) and Shori and Baba, (2014), respectively.

III. RESULTS AND DISCUSSION

3.1 Preparation of GABA containing rice flour (GFRF)

To study the effect of rice flour concentration on GABA production, rice flour was varied as 1, 3, 5 and 7% w/v. No significant variation was observed from 3% and above and hence 3% was considered optimum (Fig. 1a). The effect of glutamate concentration was studied in 3% rice flour medium by varying its concentration (0.25, 0.5, 1, 3 and 5%). GABA concentration increased with increase in glutamate concentration upto 1% and thereafter a gradual decrease was observed (Fig. 1b). Rice flour (3% w/v) and glutamate (1% w/v) were revealed to be the optimum points for maximum GABA production. Time course of GABA production in optimized medium was studied (Fig. 1c). GABA production increased with increase in fermentation time upto 60 h, later no change was observed. At 60 h of fermentation, a GABA concentration of 750.55±26.03 mg/100g DM was determined. GABA content in control RF was found to be 0.86±0.02 mg/100 DM. Kradangar and Songsermpong, (2015) reported highest GABA (36.82 mg/100 g DM) by naturally fermenting rice flour for 3 days at 35 °C. Therefore our results are noteworthy for maximum GABA production in rice flour medium using *E. faecium* NCIM 5593. pH of the fermented slurry was dropped to 4.1 at the end the fermentation from an initial value of 6.6. Short chain fatty acids (SCFAs) produced as a result of carbohydrate fermentation will not only inhibit the growth of undesirable microorganisms in the product but also inhibit their growth in intestinal tract (Henningsson et al., 2001). Titratable acidity of 68.13±3.47% was determined in GFRF and lactic acid (363.12±4.15 mmoles/100 g DM) was found to be the major acid produced followed by butyric, formic and acetic acid. Kradangar and Songsermpong, (2015) similarly noted increase in lactic acid content during fermentation. Lactic acid play role in acid-probiotic effects in the intestine and suppress neoplastic characteristics of tumor cells (Lei et al., 2015). Butyric acid may contribute significantly in maintaining the integrity of colonic mucosa (Johansson et al., 1998). Acetic acid enters the peripheral circulation and propionic acid will be largely taken up by liver (Wong et al., 2006). Trace amounts of propionic acid (0.0323±0.004 mmoles/100 g DM) was also detected in GFRF (Table. 1).

TABLE 1
PH, TITRATABLE ACIDITY AND ORGANIC ACID PROFILING

	RF	GFRF
pH	6.6±0.09	**4.1±0.12***
Titratable acidity (% total acids)	3.46±0.17	**68.13±3.47***
Acids (mmoles/100 g DM)		
Lactic acid	ND	363.12±4.15
Butyric acid	ND	247.35±3.01
Formic acid	ND	230.39±6.94
Acetic acid	ND	211.76±3.85
Propionic acid	ND	0.0323±0.004

The results are mean of three independent experiments ± SE. *The mean differences were significant at 95% confidence interval (α≤0.05) through unpaired t test. ND-Not detected.

SEM and color analysis of RF and GFRF were performed to examine its microstructure and color, respectively (Fig. 1d & e). Starch is the main component of rice flour and is responsible for its functional properties. Consequently, starch reduction and changes in its structure as a result of fermentation may contribute to the alterations in physicochemical and functional properties of GFRF (Ilowefah et al., 2015). The micrograph (SEM) of RF indicated smooth surface with no pores. This is because the starch granules in RF are strongly packed in closed form (Ilowefah et al., 2015). Unlike in GFRF, there were large number of pores which indicates starch breakdown by fermenting microorganism. Said et al., (2015) reported similar

observation with fermented and non-fermented Indonesian rice flour. Fermentation improved whiteness, with a higher W value compared to RF (Table. 2). The results are in line with the reports on increased whiteness upon fermentation (Lu et al., 2005), which is a critical factor affecting the whiteness of flour.

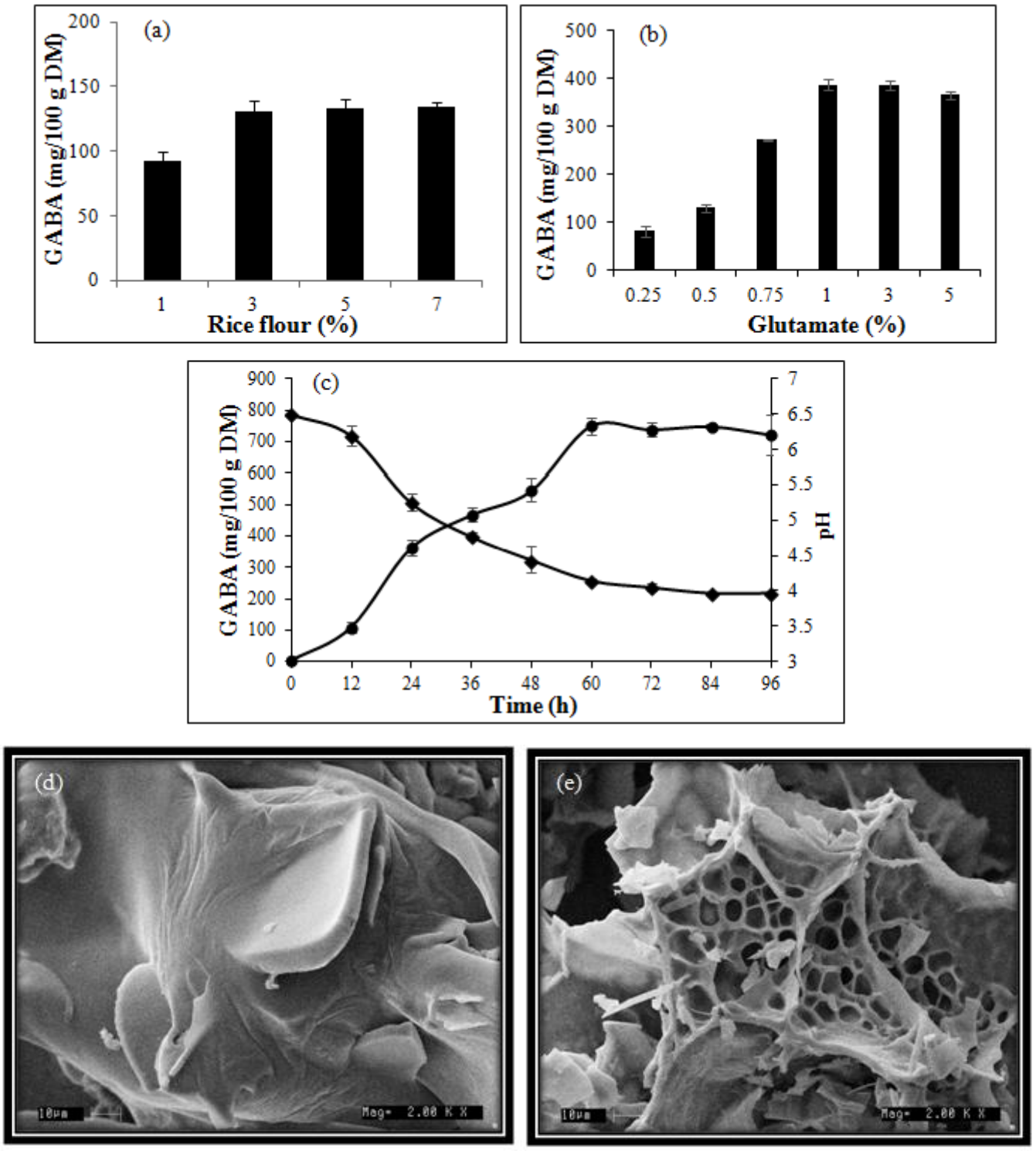

FIG. 1: GABA ENRICHED FERMENTED RICE FLOUR. (a) EFFECT OF RICE FLOUR PERCENTAGE ON GABA. (b) EFFECT OF GLUTAMATE CONCENTRATION ON GABA. (c) TIME COURSE OF GABA PRODUCTION IN OPTIMIZED RICE FLOUR MEDIUM. (d) SCANNING ELECTRON MICROGRAPH OF RF (f) SCANNING ELECTRON MICROGRAPH OF GFRF

TABLE. 2
COLOR VALUES

	L*	a*	b*	W
GFRF	74.35±0.03	-3.84±0.01	19.32±0.02	67.66±0.01*
RF	72.67±0.05	-2.46±0.01	21.43±0.01	65.19±0.03

The results are mean of three independent experiments ± SE. *The mean differences were significant at 95% confidence interval (α≤0.05) through unpaired t test.

3.2 Proximate analysis, total phenolics and flavonoids

Change in proximate composition are presented in Table. 3. Moisture content of GFRF was not significantly higher than that of RF. However, fermentation brought about a significant ($p<0.05$) increase (28.64%) in protein content. This is attributed to the increased protein synthesis by the fermenting microorganism (Chinma et al., 2014). Furthermore, Mackay and Baldwin, (1990) also reported significant improvement in protein quality of fermented cereal products. Marginal decrease in fat and ash content was observed in GFRF. Reduction in ash content may be due to the leaching of the soluble inorganic salts during fermentation (Sade, 2009). Total carbohydrate content in GFRF was significantly ($p<0.05$) lower (3.36%). Significant decrease in total carbohydrate content in GFRF is attributed to the utilization of carbohydrates for growth and metabolism (Igbabul et al., 2014).

TABLE 3
PROXIMATE COMPOSITION

Constituents (%DM)	RF	GFRF
Moisture	11.84±0.4	12.67±0.8
Crude Protein	9.46±0.3	**12.17±0.5***
Fat	1.36±0.2	1.14±0.5
Ash	2.36±0.1	2.04±0.2
[a]Total carbohydrates	87.14±0.5	**84.33±0.6***
[b]Energy (kcal/g)	398.64	396.26

The results are mean of three independent experiments ± SE. *The mean differences were significant at 95% confidence interval (α≤0.05) through unpaired t test.

The antioxidant phytochemicals in rice is receiving increased attention for their potential role in prevention of diseases as well as in food quality improvement (Sreerama et al., 2010). Fig. 2a shows the total phenolic and flavonoid content expressed as ug gallic acid (GAE)/g extract and mg QE/mg DM, respectively. Fermentation significant increased ($p<0.05$) total phenolics and flavonoids. Phenolic acids were detected in RF and GFRF at various concentrations (Fig. 2b & c). HPLC analysis showed enhancement in ferulic acid (78.88%), protocatechuic acid (49.74%), p-coumaric acid (48.31%), syringic acid (46.55%), gallic acid (38.86%), cinnamic acid (13.66%) and sinapic acid (3.28%) showed an increase in their content in GFRF (Table. 4). Degradation of bound ferulic acid by the enzyme ferulic acid esterase can be correlated with the increase in ferulic acid (Rashid et al., 2015). Increase in ferulic acid during lactic acid fermentation has also been observed by Rashid et al., (2015). The increase in other phenolic acids in GFRF may be attributed to the production of specific enzymes by fermenting microorganism capable of hydrolyzing ester linkage to release the bound phenolics (Estrada et al., 2015). Consequently, the released phenolic acids may improve the nutraceutical value of cereals and bioavailability (Rashid et al., 2015). Caffeic acid has been detected only in GFRF. Among the phenolic compounds, reduction in the amounts of p-hydroxybenzoic acid and vanillic acid was observed upon fermentation. Reduction in the amounts of p-hydroxybenzoic acid and vanillic acid in GFRF may be attributed to its conversion into other metabolites (Huynh et al., 2014). The change in the profile of phenolic compounds during fermentation process is attributed to the production of esterase, decarboxylase and β-glucosidase during the growth of the fermenting microorganisms (Marazza et al., 2009; Hur et al., 2014).

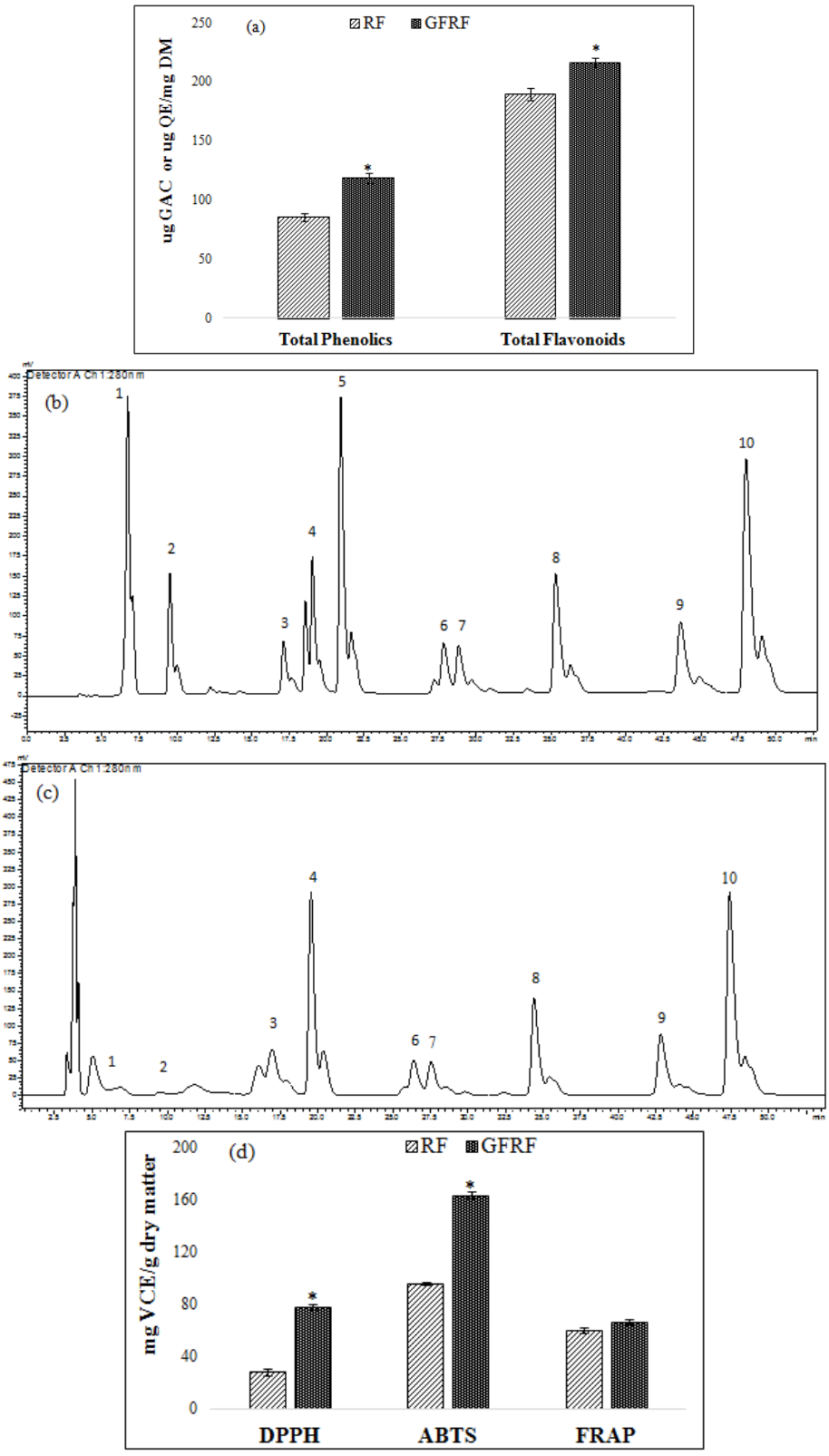

FIG. 2: PHENOLICS AND IN VITRO ANTIOXIDANT ASSAY. (a) TOTAL PHENOLIC AND FLAVONOIDS CONTENT. THE VALUES ARE MEAN ± SE. *THE MEAN DIFFERENCES WERE SIGNIFICANT AT 95% CONFIDENCE INTERVAL ($\alpha \leq 0.05$) THROUGH UNPAIRED T TEST. (b) HPLC CHROMATOGRAM OF PHENOLIC ACIDS IN GFRF. (c) HPLC CHROMATOGRAM OF PHENOLIC ACIDS IN RF. (d) IN VITRO ANTIOXIDANT ASSAYS. THE VALUES ARE MEAN ± SE. *THE MEAN DIFFERENCES WERE SIGNIFICANT AT 95% CONFIDENCE INTERVAL ($\alpha \leq 0.05$) THROUGH UNPAIRED T TEST.

TABLE 4
PHENOLIC ACID PROFILING

Phenolic acid	Percent increase/decrease
Gallic acid	(+) 38.86
Protocatechuic acid	(+) 49.74
p-Hydroxybenzoic acid	(-) 07.32
Vanillic acid	(-) 38.48
Syringic acid	(+) 46.55
p-Coumaric acid	(+) 48.31
Ferulic acid	(+) 78.88
Sinapic acid	(+) 03.28
Cinnamic acid	(+) 13.66

3.3 *In vitro* antioxidant assays and anti-nutritional factors

Dietary antioxidants have ability to stimulate cellular defense and may protect cellular components against oxidative damage (Rahman, 2007). DPPH, ABTS and FRAP assays were performed to examine the antioxidant potential of RF and GFRF (Fig. 2d). GFRF exhibited significant ($p<0.05$) increase in their ability to scavenge DPPH and ABTS radicals but no significant change was observed for FRAP assay. The ability of GFRF to show significant increase in antioxidant activity could be due to increase in total phenolic and flavonoid during fermentation. In addition, fermentation induces structural breakdown of substrates, leading to the liberation or synthesis of various antioxidant compounds (Hur et al., 2014). Effect of fermentation on phytate content, trypsin inhibitory activity and α-amylase inhibition is presented in Table. 5. No significant decrease in phytate content was observed in GFRF. The observed reduction of phytates in GFRF is attributed to phytases and phosphatases production which hydrolyze phytates (Roger et al., 2015). Trypsin inhibitors are responsible for reducing protein digestibility, pancreatic hypertrophy and poor growth performance (Osman et al., 2003). Fermentation caused significant ($p<0.05$) reduction in trypsin inhibitory activity (35.77%). Reduction in trypsin inhibitor activity during lactic acid fermentation of cereals has been reported (Ejigui et al., 2005). GFRF showed significantly ($p<0.05$) greater inhibition (12.82±1.01%) of α-amylase than RF (8.97±0.83%). The consumption of fermented foods rich in α-amylase inhibitors can be regarded as a practical dietary approach to manage hyperglycemia (Shori and Baba, 2015).

TABLE 5
ANTI-NUTRITIONAL FACTORS

	RF	GFRF
Phytate (mg/100 g DM)	356.23±8.26	348.65±7.37
Trypsin inhibitor activity (IU/mg DM)	2.46±0.07	**1.58±0.05* (35.77)**
α-amylase inhibition (%)	8.97±0.83	**12.82±1.01***

The values are mean ± SE. Statistical analysis was performed using one way ANOVA followed by post-hoc Turkey's test. 'a' and 'b' represents statistically significant against control and ACR treated mice.

Taken together, our finding suggests that fermentation of broken rice flour could produce significant levels of GABA and enhance antioxidant phenolics. GFRF displayed better antioxidant ability in comparison to control rice flour. This investigation shows evidence that fermentation modified the functionality of GFRF and can be used as a functional food ingredient. Further studies are directed towards studying the effect of GFRF extract to ameliorate neurotoxin induced oxidative dysfunctions and neurotoxicity in mice model.

ACKNOWLEDGEMENT

Gangaraju Divyashri is grateful to Council of Scientific and Industrial Research (CSIR), New Delhi, India, for the award of a Research Fellowship. The **Director, CFTRI**, is acknowledged for supporting the research work.

REFERENCES

[1] Association of Official Analytical Chemists, (2012). AOAC Official methods of analysis (19th ed., Vol. I and II). Washington, DC.

[2] Arici, M., & Daglioglu, O. (2002). Boza: a lactic acid fermented cereal beverage as a traditional Turkish food. *Food Reviews International*, *18*(1), 39-48.

[3] Chinma, C. E., Ilowefah, M., Shammugasamy, B., Ramakrishnan, Y., & Muhammad, K. (2014). Chemical, antioxidant, functional and thermal properties of rice bran proteins after yeast and natural fermentations.*International Journal of Food Science & Technology*, *49*(10), 2204-2213.

[4] Divyashri, G., & Prapulla, S. G. (2015a). An insight into kinetics and thermodynamics of gamma-aminobutyric acid production by *Enterococcus faecium* CFR 3003 in batch fermentation. *Annals of Microbiology*, *65*(2), 1109-1118.

[5] Divyashri, G., & Prapulla, S. G. (2015b). Mass transfer characterization of gamma-aminobutyric acid production by *Enterococcus faecium* CFR 3003: encapsulation improves its survival under simulated gastro-intestinal conditions. *Bioprocess and biosystems engineering*, *38*(3), 569-574.

[6] Ejigui, J., Savoie, L., Marin, J., & Desrosiers, T. (2005). Beneficial changes and drawbacks of a traditional fermentation process on chemical composition and antinutritional factors of yellow maize (Zea mays). *J Biol Sci*, *5*(5), 590-6.

[7] Estrada, B. A. A., Gutiérrez-Uribe, J. A., & Serna-Saldívar, S. O. (2014). Bound phenolics in foods, a review. *Food chemistry*, *152*, 46-55.

[8] Gangaraju, D., Murty, V. R., & Prapulla, S. G. (2014). Probiotic-mediated biotransformation of monosodium glutamate to γ-aminobutyric acid: differential production in complex and minimal media and kinetic modelling.*Annals of microbiology*, *64*(1), 229-237.

[9] Gobbetti, M., Cagno, R. D., & De Angelis, M. (2010). Functional microorganisms for functional food quality. *Critical reviews in food science and nutrition*, *50*(8), 716-727.

[10] Gohel, V., & Duan, G. (2012). Conventional process for ethanol production from Indian broken rice and pearl millet. *Bioprocess and biosystems engineering*, *35*(8), 1297-1308.

[11] Henningsson, Å. M., Margareta, E., Nyman, G. L., & Björck, I. M. (2001). Content of short-chain fatty acids in the hindgut of rats fed processed bean (Phaseolus vulgaris) flours varying in distribution and content of indigestible carbohydrates. *British Journal of Nutrition*, *86*(03), 379-389.

[12] Hossain, M. A., & Shah, M. D. (2015). A study on the total phenols content and antioxidant activity of essential oil and different solvent extracts of endemic plant Merremia borneensis. *Arabian Journal of Chemistry*, *8*(1), 66-71.

[13] Hur, S. J., Lee, S. Y., Kim, Y. C., Choi, I., & Kim, G. B. (2014). Effect of fermentation on the antioxidant activity in plant-based foods. *Food chemistry*, *160*, 346-356.

[14] Huynh, N. T., Van Camp, J., Smagghe, G., & Raes, K. (2014). Improved release and metabolism of flavonoids by steered fermentation processes: a review. *International journal of molecular sciences*, *15*(11), 19369-19388.

[15] Igbabul, B., Hiikyaa, O., & Amove, J. (2014). Effect of fermentation on the proximate composition and functional properties of mahogany bean (Afzelia africana) flour. *Current Research in Nutrition and Food Science Journal*, *2*(1), 01-07.

[16] Ilowefah, M., Bakar, J., Ghazali, H. M., Mediani, A., & Muhammad, K. (2015). Physicochemical and functional properties of yeast fermented brown rice flour. *Journal of food science and technology*, *52*(9), 5534-5545.

[17] Ito, S., & Ishikawa, Y. (2004, February). Marketing of value-added rice products in Japan: germinated brown rice and rice bread. In *FAO rice conference Rome, Italy* (pp. 12-13).

[18] Johansson, M. L., Nobaek, S., Berggren, A., Nyman, M., Björck, I., Ahrne, S., ... & Molin, G. (1998). Survival of Lactobacillus plantarum DSM 9843 (299v), and effect on the short-chain fatty acid content of faeces after ingestion of a rose-hip drink with fermented oats. *International journal of food microbiology*, *42*(1), 29-38.

[19] Kang, Y. M., Qian, Z. J., Lee, B. J., & Kim, Y. M. (2011). Protective effect of GABA-enriched fermented sea tangle against ethanol-induced cytotoxicity in HepG2 cells. *Biotechnology and bioprocess engineering*, *16*(5), 966-970.

[20] Karovicova, Z. K. J. (2007). Fermentation of cereals for specific purpose.*Journal of Food and Nutrition Research*, *46*(2), 51-57.

[21] Kinnersley, A. M., & Turano, F. J. (2000). Gamma aminobutyric acid (GABA) and plant responses to stress. *Critical Reviews in Plant Sciences*, *19*(6), 479-509.

[22] Kradangar, P., & Songsermpong, S. (2015). Optimization of Fermentation Process on the GABA Content and Quality of Fermented Rice Flour and Dry Fermented Rice Noodles. *Journal of Food Processing and Preservation*,*39*(6), 1183-1191.

[23] Lee, J. H., Cho, J. H., Lee, J. Y., Yeo, U. S., Song, Y. C., Oh, S. H., ... & Kang, H. W. (2011). Varietal Difference of Eating Quality after Storage in Room Temperature. *Korean Journal of Breeding Science*, *43*(4).

[24] Lei, M., Dai, X., & Liu, M. (2015). Biological Characteristics and Safety Examination of Five Enterococcal Strains from Probiotic Products. *Journal of Food Safety*, *35*(3), 324-335.

[25] Lei, M., Dai, X., & Liu, M. (2015). Biological Characteristics and Safety Examination of Five Enterococcal Strains from Probiotic Products. *Journal of Food Safety*, *35*(3), 324-335.

[26] Liang, J., Han, B. Z., Han, L., Nout, M. J., & Hamer, R. J. (2007). Iron, zinc and phytic acid content of selected rice varieties from China. *Journal of the Science of Food and Agriculture*, *87*(3), 504-510.

[27] Lu, Z. H., Li, L. T., Min, W. H., Wang, F., & Tatsumi, E. (2005). The effects of natural fermentation on the physical properties of rice flour and the rheological characteristics of rice noodles. *International journal of food science & technology*, *40*(9), 985-992.

[28] Lu, Z. H., Li, L. T., Min, W. H., Wang, F., & Tatsumi, E. (2005). The effects of natural fermentation on the physical properties of rice flour and the rheological characteristics of rice noodles. *International journal of food science & technology*, *40*(9), 985-992.

[29] Marazza, J. A., Garro, M. S., & de Giori, G. S. (2009). Aglycone production by Lactobacillus rhamnosus CRL981 during soymilk fermentation. *Food microbiology*, *26*(3), 333-339.

[30] McKay, L. L., & Baldwin, K. A. (1990). Applications for biotechnology: present and future improvements in lactic acid bacteria. *FEMS Microbiology reviews*, *7*(1-2), 3-14.

[31] Mira, N. V. M. D., Barros, R. M. C., Schiocchet, M. A., Noldin, J. A., & Lanfer-Marquez, U. M. (2008). Extraction, analysis and distribution of phenolic acids in pigmented and non-pigmented genotypes of rice (Oryza sativa L.). *Food Science and Technology (Campinas)*, *28*(4), 994-1002.Zhang,

[32] Mohd Ali, N., Mohd Yusof, H., Long, K., Yeap, S. K., Ho, W. Y., Beh, B. K., ... & Alitheen, N. B. (2012). Antioxidant and hepatoprotective effect of aqueous extract of germinated and fermented mung bean on ethanol-mediated liver damage. *BioMed research international*, *2013*.

[33] Mubarak, A. E. (2005). Nutritional composition and antinutritional factors of mung bean seeds (Phaseolus aureus) as affected by some home traditional processes. *Food Chemistry*, *89*(4), 489-495.

[34] Nakano, S., Ugwu, C. U., & Tokiwa, Y. (2012). Efficient production of d-(−)-lactic acid from broken rice by Lactobacillus delbrueckii using Ca (OH) 2 as a neutralizing agent. *Bioresource technology*, *104*, 791-794.

[35] Oh, C. H., & Oh, S. H. (2004). Effects of germinated brown rice extracts with enhanced levels of GABA on cancer cell proliferation and apoptosis. *Journal of medicinal food*, *7*(1), 19-23.

[36] Osman, M. A., Reid, P. M., & Weber, C. W. (2003). The effect of feeding tepary bean (Phaseolus acutifolius) proteinase inhibitors on the growth and pancreas of young mice. *Pakistan Journal of Nutrition*, *2*(3), 111-115.

[37] Polthum, P., & Ahromrit, A. (2014). GABA content and Antioxidant activity of Thai waxy corn seeds germinated by hypoxia method♣. *Songklanakarin Journal of Science & Technology*, *36*(3).

[38] Rahman, K. (2007). Studies on free radicals, antioxidants, and co-factors.*Clinical interventions in aging*, *2*(2), 219.

[39] Rashid, N. Y. A., Razak, D. L. A., Jamaluddin, A., Sharifuddin, S. A., & Long, K. (2015). Bioactive compounds and antioxidant activity of rice bran fermented with lactic acid bacteria. *Malaysian journal of microbiology*, 11(2): 156-162.

[40] Roger, T., Ngouné Léopold, T., & Carl Moses Funtong, M. (2015). Nutritional Properties and Antinutritional Factors of Corn Paste (Kutukutu) Fermented by Different Strains of Lactic Acid Bacteria. *International journal of food science*, *2015*.

[41] Sade, F. O. (2009). Proximate, antinutritional factors and functional properties of processed pearl millet (Pennisetum glaucum). *Journal of Food Technology*, *7*(3), 92-97.

[42] Said, H. N., Harijono., & Kusnadi, J. (2015). Influence of natural fermentation on the morphology and physicochemical properties of Indonesian rice flour and their effect on rice paper. International *Journal of chemtech research*, 7(4), 1951-1959.

[43] Shori, A. B. (2013). Antioxidant activity and viability of lactic acid bacteria in soybean-yogurt made from cow and camel milk. *Journal of Taibah University for Science*, *7*(4), 202-208.

[44] Shori, A. B., & Baba, A. S. (2014). Comparative antioxidant activity, proteolysis and in vitro α-amylase and α-glucosidase inhibition of Allium sativum-yogurts made from cow and camel milk. *Journal of Saudi Chemical Society*, *18*(5), 456-463.

[45] Sreerama, Y. N., Sashikala, V. B., & Pratape, V. M. (2010). Variability in the distribution of phenolic compounds in milled fractions of chickpea and horse gram: evaluation of their antioxidant properties. *Journal of agricultural and food chemistry*, *58*(14), 8322-8330.

[46] Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of food composition and analysis*, *19*(6), 669-675.

[47] Watanabe, N., Endo, Y., Fujimoto, K., & AOKI, H. (2006). Tempeh-like fermented soybean (GABA-tempeh) has an effective influence on lipid metabolism in rats. *Journal of Oleo Science*, *55*(8), 391-396.

[48] Wong, J. M., De Souza, R., Kendall, C. W., Emam, A., & Jenkins, D. J. (2006). Colonic health: fermentation and short chain fatty acids. *Journal of clinical gastroenterology*, *40*(3), 235-243.

[49] Zhang, M. W., Zhang, R. F., Zhang, F. X., & Liu, R. H. (2010). Phenolic profiles and antioxidant activity of black rice bran of different commercially available varieties. *Journal of agricultural and food chemistry*, *58*(13), 7580-7587.