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ORIGINAL PAPER

Profiling of amino acids in traditional and improved rice (*Oryza sativa* L.) varieties of Sri Lanka and their health promoting aspects

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Abstract

Investigation made on the variation in amino acid compositions among selected traditional and newly improved rice varieties cultivated in Sri Lanka is presented. The free amino acid (FAA) composition including gamma-amino butyric acid (GABA) and protein bound total amino acids (TAAs) of eleven traditional and seven improved rice cultivars grown at *Batalagoda* across the two major cultivating seasons in the country were evaluated. Significant variations ($p < 0.05$) in GABA levels, individual and total amino acids in free and protein bound form were observed among the studied varieties. Both traditional and improved varieties reported comparable mean total FAAs and TAA levels. However, significantly higher levels of GABA, which has health promoting functional properties, were found in newly improved rice varieties in comparison to the traditional varieties. Significant impact of seasonal variation on total amino acid levels was observed in majority of the varieties with most of the varieties reporting higher amino acid levels when cultivated during the "*Maha*", which is the major cultivation season in the country. The *Behethheenati* rice out of the traditional, while *Bg 300 rice* out of the newly improved, was found the most beneficial among the investigated varieties. The potential significant contribution obtainable with several local traditional and improved varieties in fulfilling the majority of the recommended essential amino acid nutrient requirement in the daily diet by the world health organization is highlighted.

Keywords Amino acids · GABA · Rice · Sri Lanka

Introduction

Rice (*Oryza sativa* L.) is the dietary staple of more than half of the world's population with a vast majority of spread across Asia including Sri Lanka. It has a wide genetic diversity with thousands of varieties grown around the world. Owing to the differences arising inherent to the cultivar, postharvest conditions, agronomic traits and environmental conditions, the nutrient composition of rice is significantly varied (Kamara et al. 2010; Juliano and Villareal 1993).

Being the secondary most abundant nutrient next to carbohydrates, protein plays a significant role as a major determinant of its nutritional value and in determining the functional properties, texture, pasting capacity and the sensory characteristics of rice (Lyon et al 1999; Martin and Fitzgerald 2002; Xie et al. 2008). Since amino acids are the building blocks of proteins, from the nutritional perspective, the amino acid composition of rice becomes important in defining the characteristics related to the protein quality of rice.

Besides the carbohydrates and the proteins which are present as the major components, lipids, fibers, minerals, sugars and free amino acids (FAAs) are also found in rice as minor constituents. In addition to being incorporated into

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the seed-storage proteins, as the precursors for the synthesis of organic acids, sugars, osmolytes, phytohormones and secondary metabolites involved in plant cell wall protection (Amir et al. 2018), FAAs play a central role in seeds. In addition, the apparent relationship observed in the levels of FAAs with the organoleptic characteristics in food has inspired the scientific research toward the analysis of FAA composition in food (Kasumyan 2016; Gunlu and Gunlu 2014; Nishimura and Kato 1988). Hence, the investigation of the variation in the FAAs which inherent unique characteristic tastes in food becomes interesting.

In addition to the proteinogenic FAA pool, rice is also enriched with gamma-aminobutyric acid (GABA) which is a non-proteinogenic amino acid. GABA aids the regulation of several physiological functions such as neurotransmission (Jakobs et al. 1993), diuretic effects, inducing relaxation effects (Mody et al. 1994), reducing blood pressure (Inoue et al. 2003) and is a secretagogue of insulin and thereby assist to control the diabetic conditions (Imam et al. 2012). Inhibition of cancer cell proliferation has also been observed with the consumption of GABA enriched food (Park and Oh 2007) and hence, from the nutritional aspect, the investigation of GABA levels in food becomes inevitably important. In addition, investigation of formation of GABA in each rice variety is imperative for the selection of varieties to be popularized and using desirable traits in breeding.

Further to the relationship of FAAs with sensory perception, the FAA; asparagine together with soluble sugars is reported to be associated with the formation of acrylamide (Curtis and Halford 2016) a compound which is classified as a group 2A carcinogen declared by the International Agency for Research on Cancer (IARC 1994). Hence, the level of free asparagine present in food products is an important quality assurance parameter which needs to be considered in food to control the levels of acrylamide formation during processing. Therefore, the impact caused by FAAs in food cannot be underestimated.

Being the dietary staple, many rice varieties including more than thousands of different traditional rice varieties (Ranbukwella and Priyadarshan 2016) inheriting health promoting functional properties have been reported in Sri Lanka (Abeysekara et al. 2015; Premakumara et al. 2013). Sri Lanka is recognized as one of the most important geographical origins of many useful rice traits by the International Rice Research Institute (IRRI) (Arachchi and Wijerathna 2008). With a widespread across lowland to upland conditions, Sri Lanka reserves a wide diversity in rice germplasm. Out of the total paddy land in the country, 98.8% of lands are cultivated with newly improved varieties (Rambukwella and Priyadarshana 2016). Nowadays, due to the low productivity and difficulties associated with the cultivation, relatively a lesser number of traditional rice varieties are grown in the country. Owing to the inherent health promotional functional properties, Sri Lankan rice varieties are drawing the global recognition and hence, the physicochemical properties as well as the nutritional composition of rice have been conscientiously studied (Hettiarachchi et al. 2016; Abeysekara et al. 2015; Premakumara et al. 2013). However, except for the work by Gunaratne et al. (2013), little attention has been drawn toward a comprehensive analysis on the variation of amino acid profiles of the rice varieties grown in the country. Therefore, in this context, the aim of the present study was to investigate the varietal and seasonal variations in the amino acid profiles among selected traditional and newly improved rice varieties to provide a reference to their potential nutritional benefits and for future screening of varieties in breeding programs.

Materials and methods

Materials

Chemicals and reagents

Amino acid reference standards (purity > 98%) and all the other chemicals and solvents were purchased from Sigma Aldrich.

Grain samples

Eighteen Sri Lankan rice varieties including traditional and newly improved red and white varieties were obtained from Rice Research Development Institute (RRDI) at *Batalagoda*. They were grown experimentally in plots using randomized block design during 2017–2018 *Yala* and *Maha* seasons. The authentication of rice varieties was performed with the assistance of the RRDI. The selection of the varieties was based considering the consumer preference, production yield and the health benefits associated with the varieties. Further, the selection of the rice varieties was made to represent the varietal spread, different grain characteristics such as pericarp color and different agronomic traits such as number of days to maturity.

Eleven Sri Lankan traditional rice varieties: *Dikwee, Kaluheenati, Murungakayan, Pachchaperumal, Rathel, Herath Banda, Sulai, Kuruluthuda, Dahanala, Suwandel, Dewaraddiri, Behethheenati, Suduru samba, Madathawalu* and seven improved rice varieties: *Bg 403, At 307, Bg 352, Bg 300, Bg 94–1, Bg 359* and *At 306* cultivated in the RRDI at *Batalagoda* were subjected to the study (Table S1). The new improved varieties which were developed using locally developed breeding lines, exotic lines or varieties, and few which were developed from traditional varieties were selected for the study.

The cultivars selected for the study were cultivated during the two major cultivation seasons; *Yala* which lasts from March to September and *Maha* which starts from October to February the following year. There are differences in the environmental conditions between the two major seasons with typically higher rainfall figures (Suppiah and Yoshino 1986) and relatively short bright sunshine hours observed in the *Maha* season compared to the *Yala* season. The rice varieties cultivated during the 2017 *Yala* and the 2017/2018 *Maha* seasons were analyzed.

Sample preparation

The paddy was dehulled using a De-huller (Model THU-35B, Satake, Japan) at 1900 rpm, was finely grounded using a laboratory grinder (IKA-MF 10 basic Microfine grinder drive) and was sieved through a 0.3 mm sieve prior to analysis.

Free amino acid and GABA analysis

The free amino acids (FAAs) including gamma-amino butyric acid (GABA) were analyzed using the method as described by Liyanaarachchi et al. (2018). The detection of FAAs and GABA was performed using an Eksigent 100XL liquid chromatograph coupled to ABSciex QTrap 4500 tandem mass spectrometer (LC–MS/MS) in electrospray ionization (ESI) mode with chromatographic separation on an Agilent Zorbax Eclipse Plus C18 column (4.6 mm × 10 mm, 5 µm) using the chromatographic and instrumental conditions described by Liyanaarachchi et al. (2018, 2020a).

Total amino acid analysis

For total amino acid (TAA) analysis, 0.2 g of the sample prepared, as mentioned above, were hydrolyzed using 6 M HCl at 110 °C (ISO 13903; Liyanaarachchi et al. 2020b) for 22 h. The digested samples after adjustment of pH to 2.2 with 1 M HCl were filtered through 0.45 µm syringe filters prior to injection to HPLC. The quantitative analysis of TAAs was performed with an Agilent 1100 high-performance liquid chromatograph (HPLC) using automated pre-column derivatization with o-phthalaldehyde (OPA) and Fluorenylmethyloxycarbonyl chloride (FMOC). The 16 amino acids were separated on an Agilent Zorbax Eclipse AAA column (4.6 × 150 mm, 5 micron) with diode array detection (DAD) with gradient elution (Henderson et al . 2000).

Statistical evaluation

The statistical analysis was performed using the statistical software package, SAS for Windows V 9.1 (SAS Institute Inc., NC, USA). The level of significance was $p < 0.05$. The FAAs quantified in six replicates from each variety were analyzed for a particular variety. The obtained results were analyzed using ANOVA, Duncan multiple range test and Pearson's correlation analysis.

Results

Variation in FAA composition

According to the FAA profiles, glutamic acid, aspartic acid, asparagine, glycine, tryptophan and alanine were the predominant ones in the experimented rice cultivars while hydroxyproline, phenylalanine, isoleucine, methionine, glutamine and leucine being less present (Table S2 and Table S3). The mean asparagine content which is reported to have an established connection with acrylamide formation (Curtis and Halford 2016) ranged between 6.7 mg/100 g and 19.9 mg/100 g.

The mean total free amino acid (TFAA) content in the studied cultivars ranged between 46.1–82.4 mg/100 g of rice on dry weight basis. Significant differences ($p < 0.05$) in mean values obtained for the individual and TFAA content were observed among the varieties. The improved *Bg 300* variety reported the highest TFAA content followed by *At 307* which is also an improved variety. The lowest TFAA level was observed in the traditional *Dahanala* rice. However, no significant difference in the mean TFAA levels between the traditional and improved rice varieties was reported.

Among the investigated varieties, the rice with the white pericarp had significantly higher mean TFAA levels compared to the varieties with the red pericarp color. However, when Pearson's correlation analysis was performed, extremely weak negative correlation (0.29) was observed between the pericarp color and the TFAA levels.

The GABA content also significantly varied among the cultivars ranging from 0.7 mg/100 g to 5.9 mg/100 g (Supplementary Table S2 and S3). The improved *Bg 300* reported the highest GABA content while the lowest was detected in the traditional *Madathawalu* variety, respectively. Newly improved rice varieties had significantly higher mean levels of GABA compared to the traditional varieties. On the other hand, rice varieties with red pericarp reported significantly lower mean levels of GABA in comparison to the varieties with white pericarp color.

During the experimented period, comparative mean rainfall, relative humidity, total bright sunshine hours and potential evaporation were reported during the two seasons. However, in the *Yala* season, higher total rainfall of 999 mm, compared to the total rainfall of 560 mm received during the *Maha* season was reported. Further, during the experimented period, the mean minimum and maximum temperatures of *Maha* and *Yala* seasons remained at 21.7–23.9 °C and 30.2–31.6 °C, respectively. The mean minimum and maximum temperatures during the *Yala* season were significantly higher compared to that recorded for the *Maha* season. When the mean TFAA levels were analyzed for a particular cultivar, TFAA levels significantly varied across the seasons for all the investigated cultivars except for *Bg 300*, *Dahanala* and *Dewaraddiri* (Table 1). For majority of the varieties, in comparison to the rice cultivated during the *Yala* season, rice cultivated during the *Maha* season reported significantly higher TFAA levels.

Variation in TAA composition

Significant variations in aspartic acid, glutamic acid, serine, glycine, alanine, leucine and proline levels were observed among the varieties. Except for individual aspartic acid, glutamic acid and TAA levels, impact of the season was found to be significant for the rest of the amino acids. Glutamic acid, aspartic acid, alanine and glycine were the predominant protein bound amino acids in the rice varieties (Table S4 and Table S5).

The mean TAA levels observed in rice ranged between 70.3–104.4 g/kg. The mean TAA contents significantly varied among the varieties. The two improved varieties: *At 306* and *Bg 352* were reported with the overall highest and the lowest mean TAA levels, respectively. As observed in case of FAAs, no significant difference between the mean TAA levels in traditional and improved varieties was observed. On the other hand, rice varieties with red and white pericarp color had comparative TAA levels with no significant difference.

Similarly to TFAA levels, for majority of the cultivars, mean TAA levels observed for a particular variety for *Maha* season significantly exceeded the mean TAA levels detected for the same variety in the *Yala* season (Table 1).

Table 1 Seasonal variation in free and total amino acid contents in rice varieties

Cultivar	TFAA (mg/100 g)		TAA (g/kg)	
	Yala	*Maha*	*Yala*	*Maha*
Kaluheenati	89.6 ± 1.1^{a}	75.3 ± 0.7^{b}	94.6 ± 0.5^{a}	86.7 ± 0.7^{b}
Murungakayan	29.9 ± 0.7^{b}	75.0 ± 0.8^{a}	66.1 ± 0.6^{b}	83.7 ± 0.9^{a}
Rathel	79.1 ± 0.9^{a}	61.6 ± 0.6^{b}	63.5 ± 0.8^{b}	78.1 ± 1.0^{a}
Herathbanda	29.0 ± 0.4^{b}	71.1 ± 0.5^{a}	$64.6 \pm 0.4 \backslash^{b}$	77.9 ± 1.2^{a}
Sulai	40.0 ± 0.4^{b}	76.2 ± 0.8^{a}	68.7 ± 0.5^{b}	88.1 ± 0.6^{a}
Kuruluthuda	33.5 ± 0.7^{b}	67.4 ± 0.5^{a}	$70.4 \pm 1.0^{\ b}$	87.1 ± 0.4^{a}
Dahanala	27.9 ± 0.5^{a}	64.2 ± 0.8^{a}	67.7 ± 0.6^{b}	81.3 ± 0.7^{a}
Suwandel	44.1 ± 0.5^{a}	90.3 ± 0.8^{b}	93.3 ± 0.5^{b}	95.1 ± 0.6^{a}
Dewaraddiri	66.1 ± 0.7^{a}	61.3 ± 0.6^{a}	81.6 ± 1.1^{a}	76.9 ± 0.8^{b}
Behethheenati	47.1 ± 0.4^{b}	79.5 ± 0.6^{a}	95.0 ± 0.9^{a}	91.4 ± 0.7^{b}
Madathawalu	68.0 ± 0.4^{b}	78.5 ± 0.9^{a}	78.5 ± 0.9^{a}	68.0 ± 0.4^{b}
At 307	84.5 ± 0.5^{a}	74.0 ± 1.0^{b}	73.3 ± 0.7^{b}	79.7 ± 0.6^{a}
Bg 352	37.3 ± 0.7^{b}	62.2 ± 0.6^{a}	68.7 ± 0.6^{b}	71.9 ± 0.7^{a}
Bg 300	87.1 ± 1.2^{a}	87.0 ± 1.1^{a}	69.7 ± 0.6^{b}	85.3 ± 0.9^{a}
Bg 94–1	68.6 ± 1.0^{b}	76.6 ± 1.5^{a}	69.1 ± 1.1^{b}	86.8 ± 0.9^{a}
Bg 359	33.5 ± 0.8^{b}	63.9 ± 0.7^{a}	74.2 ± 1.1^{b}	86.5 ± 1.1^{a}
At 306	73.2 ± 0.6^{b}	98.0 ± 1.5^{a}	94.8 ± 0.8^{b}	114.0 ± 0.5^{a}
Bg 403	47.6 ± 0.4^{b}	61.3 ± 1.1^{a}	73.6 ± 0.7^{b}	82.5 ± 0.9^{a}

Values in a particular category within a row followed by different letters are significantly different at $p < 0.05$ according to t test, (number of replicates $= 6$)

TFAA Total free amino acid; *TAA* Total protein bound amino acids

Discussion

Variation in FAA composition

The observed TFAA levels in the present study were comparable to the findings made by Kamara et al. (2010) and Komatsuzaki, et al. (2007) for rice varieties found in Asia. The two main subspecies of Asian cultivated rice known as *indica* and *japonica* have clearly demonstrated diverged morphological characteristics, physiological and biochemical characteristics, (Yang et al. 2014). Even though the ranges of TFAA levels detected in the present study were comparable with the research findings reported in *indica* rice varieties in Asia, there are significant changes in the individual FAA levels and the profiles observed in rice (Kamara et al. 2010). In comparison to *indica* varieties reported in their study, the individual FAA levels present for glutamine, leucine and tyrosine were relatively lower for the studied varieties in the present study. In addition, glutamic acid, asparagine, aspartic acid, alanine and glycine levels observed in the present study were also comparatively higher than the mean values reported for rice which belongs to *indica* and *japonica*/*javanica* groups. However, Komatsuzaki, et al. (2007) have reported comparable FAA compositions in *japonica* varieties as observed in the present study.

Further, the FAA compositions observed in the rice were significantly lower than the FAA composition reported for other cereal grains of wheat, rye, barley (Mustafa et al 2007; Nagaoka 2005) and maize (Culea et al 2015). Therefore, this marked difference in FAA composition enables differentiation of rice from other cereal grains based on the FAA composition.

In comparison to asparagine levels reported in cereal grains such as rye, wheat, barley and maize (Zilic et al. 2017; Fredrikson et al. 2004), investigated rice varieties have significantly lower asparagine levels. Therefore, as described in the literature, during the food processing subjected with heating, tendency toward acrylamide formation is comparatively lesser in rice compared to the other cereals reported with high free asparagine contents. On the other hand, based on the knowledge generated with respect to free asparagine levels reported with the cultivars, varieties which have less tendency toward free asparagine generation can be utilized in food processing industries.

As previously highlighted, the differences in the environmental conditions such as total rainfall and mean minimum and maximum temperature between the two major seasons could possibly have impacted for the noticeable significant variation observed in the TFAA values within the same cultivar. The significant variations observed in TFAA levels among the two seasons for the same variety have accounted for larger standard deviations reported for a particular variety (Table S2, Table S3). Since majority of the varieties exhibited significantly higher TFAA levels during the *Maha* season, it could be considered that, for majority of the varieties cultivated at *Batalagoda*, the mean temperature conditions prevailed during the grain filling period of rice appear to be in favor with the amino acid formation pathways accounting for higher TFAA levels in rice seeds. Therefore, these findings further establish the significant influence caused by the environmental conditions on the compositional changes in amino acids in crops.

On the other hand, the varieties such as *Dahanala, Dewaraddiri and Bg 300* which shows stability on TFAA levels over the two seasons needs to be identified and further investigated as probable environmental resilient varieties in future breeding programs.

Variation in TAA composition

Similarly to the findings made by Kamara et al. 2010, Pro which is the precursor that accounts for the characteristic popcorn aroma in rice were highest in the *At 306* which is a long slender rice variety with a pleasant smell while the second highest was reported in *Suwandel* which is also an aromatic rice variety. Similarly as observed in the present study, Sekhar and Reddy (1982) also reported comparatively higher protein contents in scented rice varieties compared to the non-scented ones.

In the literature, several studies reported similar compositions of Glu, Asp and Val in rice (Ning et al 2010; Rita et al. 2009; Park et al. 2009; Liu et al. 2017). However, in comparison to those studies, relatively higher levels of Ala and Gly have been reported in the local rice varieties. The significant changes observed in the individual as well as TAA compositions among the cultivars could be attributed to the varietal changes inherent with the rice varieties (Chandrasekhar et al. 1970; Kamara et al 2010; Verma et al. 2017).

As stated with mean TFAA levels, significantly higher mean TAA levels detected for the same variety in the *Maha* season compared to the *Yala* season which was observed for majority of the varieties could be attributed to the seasonal impact arising from the climatic differences inherent with the two seasons. The significant variations observed in amino acid levels among the two seasons for the same variety has accounted for the larger standard deviations reported for a particular variety (Table S4, Table S5). Similarly as observed with the TFAAs, specifically the total rainfall figures and the mean minimum temperature prevailed during the *Maha* season, demonstrate to be in favor with the formation of total protein bound amino acids in majority of the varieties. The stability in TAA formation over the two seasons was observed in none of the varieties, demonstrating lack of environmental resilience over TAA formation.

Health promoting aspects arising from the presence of amino acids in local traditional and improved rice varieties

The nutritional properties of rice which vary significantly among different varieties cultivated in different regions of the world (Calingacion et al. 2014; Sompong et al. 2011) are important for the rice consumers. Due to the high nutritional quality and the presence of bioactive compounds, global recognition and attention have been drawn toward the Sri Lankan traditional rice varieties. Among the traditional varieties (*Behethheenati, Kaluheenati and Suwandel)* and *At 306* improved variety reported higher TAA contents depicting higher nutritional quality with respect to amino acids.

Withanawasam (2017) reports that traditional rice varieties of *Kaluheenati, Beheth heenati* and *Suwandel* are recommended to control diabetes. As per the findings of the present study, these three varieties reported the highest TAA contents among the traditional varieties. Therefore, it could possibly be considered that in addition to demonstrating medium GI values (Hafeel et al 2016; Prasantha 2018), the presence of the significantly high TAA contents and particularly high levels of amino acids which have an established relationship of lowering diabetes such as arginine, alanine and serine levels (Miczke et al 2015) could be attributed to the diabetes controlling ability of these traditional rice varieties. In addition to abovementioned amino acids, both *Kaluheenati* and *Suwandel* rice also contained significantly higher amounts of GABA levels which aids the regulation several physiological functions in human body including lowering of blood glucose levels (Imam et al. 2012). Along with lowering blood glucose levels, GABA participates in neurotransmission (Jakobs et al. 1993) and reportedly take part in inducing relaxation effects (Mody et al. 1994), reducing blood pressure (Inoue et al. 2003) and inhibition of cancer cell proliferation (Park and Oh, 2007). The GABA levels observed in the local *indica* rice varieties were greater than the *japonica/javanica* and *indica* varieties reported by Kamara et al. (2010). However, comparable GABA levels and genotype variations have been observed in

Table 2 Comparison of essential individual mean amino acid levels* of rice varieties against the daily recommended allowance specified by the Food and Agricultural Organization (FAO) for an average adult with 50 kg body weight

Amino acid content given in (g/300 g)

Cultivar	Histidine	Threonine	Valine	Methionine	Phenylalanine	Isoleucine	Leucine	Lysine
Kaluheenati	0.46±0.06	0.61±0.06	2.00±0.06	0.40±0.06	1.37±0.06	0.99±0.06	2.09±0.06	1.17±0.06
Murungakayan	0.34±0.07	0.50±0.09	1.74±0.10	0.38±0.09	1.15±0.10	0.91±0.09	1.75±0.09	0.99±0.09
Rathel	0.33±0.07	0.49±0.09	1.65±0.09	0.33±0.08	1.13±0.09	0.82±0.09	1.60±0.09	1.08±0.09
Herathbanda	0.35±0.08	0.45±0.09	1.63±0.09	0.33±0.07	1.15±0.08	0.86±0.09	1.60±0.09	0.94±0.09
Sulai	0.28±0.05	0.53±0.05	1.81±0.05	0.27±0.05	1.17±0.05	0.83±0.05	1.87±0.05	0.85±0.05
Kuruluthuda	0.38±0.08	0.53±0.07	1.85±0.10	0.40±0.07	1.29±0.08	0.93±0.09	1.76±0.09	1.14±0.09
Dahanala	0.41±0.09	0.51±0.07	1.73±0.09	0.35±0.08	1.14±0.07	0.88±0.09	1.66±0.09	0.99±0.09
Suwandel	0.46±0.07	0.64±0.07	2.15±0.09	0.42±0.07	1.46±0.08	1.10±0.07	2.00±0.07	1.23±0.07
Dewaraddiri	0.44±0.06	0.51±0.08	1.68±0.07	0.41±0.06	1.17±0.08	1.36±0.06	1.72±0.06	1.02±0.06
Behethheenati	0.50±0.06	0.63±0.06	2.10±0.06	0.43±0.06	1.42±0.08	1.05±0.06	2.09±0.06	1.25±0.06
Madathawalu	0.32±0.06	0.48±0.06	1.72±0.06	0.34±0.06	1.19±0.08	0.88±0.06	1.65±0.06	1.06±0.06
At 307	0.40±0.07	0.53±0.07	1.75±0.07	0.40±0.07	1.18±0.07	0.86±0.07	1.68±0.07	1.09±0.07
Bg 352	0.41±0.06	0.45±0.06	1.52±0.06	0.46±0.06	1.06±0.06	1.26±0.06	1.44±0.06	0.99±0.06
Bg 300	0.60±0.01	0.59±0.01	1.81±0.05	0.58±0.05	1.42±0.02	1.02±0.02	1.65±0.02	1.27±0.02
Bg 94–1	0.34±0.08	0.53±0.08	1.75±0.08	0.37±0.07	1.25±0.08	0.89±0.08	1.70±0.08	1.11±0.08
Bg 359	0.45±0.07	0.52±0.07	1.77±0.07	0.51±0.07	1.20±0.07	1.37±0.07	1.67±0.07	1.04±0.07
At 306	0.49±0.08	0.67±0.08	2.27±0.08	0.39±0.07	1.68±0.08	1.20±0.08	2.41±0.08	1.24±0.08
Bg 403	0.44±0.07	0.51±0.07	1.68±0.07	0.49±0.07	1.16±0.07	1.25±0.07	1.67±0.07	1.07±0.07
WHO Requirement	0.5 g	0.75 g	1.3 g	0.52 g	1.25 g	1 g	1.95 g	1.5 g

WHO: World Health Organization

*The levels were calculated considering the consumption of an average of 100 g of raw rice per meal thrice a day, number of replicates (*n*) = 6

brown *indica* rice varieties cultivated in Thailand (Karladeea and Suriyonga 2012) and in China (Shen et al. 2015). The improved rice varieties, *Bg 300* followed by *At 306* reported the highest GABA content while, *Kaluheenati* and *Suwandel* were among the traditional rice varieties which constitute the third and fourth among the tested list. Hence, irrespective of whether the variety being traditional or improved, the nutritional significance of local rice varieties significantly varied. Hence, from the nutritional perspective, with respect to comprising more nutritional amino acids, among the traditional varieties, *Kaluheenati, Behethheenati and Suwandel* as well as *At 306* and *Bg 300* among the improved varieties, stand out from the rest of the varieties. These findings on the genetic diversity of each rice variety's ability to synthesize GABA will provide guidance on the selection of varieties to be popularized and in the manipulation of desirable traits in breeding to improve nutritional and functional properties of rice.

The composition of amino acid reflects the protein quality of rice varieties. Even though rice contains lesser protein compared to other cereals, rice comprises the highest digestible protein content with the highest biological value in comparison to other cereals. Rice has relatively a good balance of amino acids. Being the first limiting essential amino acid in cereals, lysine content in rice is higher compared to wheat, corn and sorghum (Eggum et al. 1982). Among the investigated local varieties, leucine and valine were the highest essential amino acids present with methionine and histidine present in relatively lesser quantities. Table 2 summarizes the mean essential amino acid levels observed in the analyzed varieties in comparison to the daily intake for each individual essential amino acids recommended by the WHO/Food and Agricultural Organization (FAO), 2007. The levels present in each rice variety were calculated considering the consumption of 100 g of raw rice per meal thrice a day. All the rice varieties comprised the recommended daily requirement for valine, however, were below the recommended requirement for lysine and histidine. *Behethheenati* and *Bg 300* consisted of five out of eight essential amino acids within the recommended daily requirement, while *Suwandel* and *At 306* comprised of four out of eight essential amino acids within the daily requirement recommended by the WHO (2007). Findings indicated that except for tryptophan which was not analyzed, consumption of 100 g of local rice varieties per meal (calculated for uncooked rice) thrice a day is sufficient to provide more than half of the daily requirement of essential amino acids recommended by the WHO. Hence, in aiding to strengthen the national strategies toward ensuring food and nutrient security, through the findings of the study, an insight is provided for the selection of rice varieties with high nutritional quality to be popularized in the breeding programs.

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Compliance with ethical standards

Conflict of interest We wish to confirm that there are no known conflicts of interest associated with this publication.

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