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RESEARCH ARTICLE

Total and free amino acid contents of popular rice varieties (*Oryza sativa* L.) consumed in the capital city of Sri Lanka

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Abstract: This study investigated the amino acid composition of eight local and three imported rice varieties frequently consumed by consumers in the capital of Sri Lanka. Investigation included twenty free amino acids (FAAs) analysed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) and sixteen protein bound amino acids analysed using high performance liquid chromatography with diode array detection (HPLC-DAD). The mean total amino acid (TAA) in rice ranged between 64.5 ± 1.9 g/kg and 96.0 ± 5.1 g/kg on dry basis (db). *Ponni, Basmathi, Rathu Nadu* and Fragrant rice which were parboiled varieties, reported the overall highest mean TAA contents while imported *Ponni* and *Basmathi* exhibited the highest mean essential amino acid (EAA) contents depicting high quality protein. Red rice varieties of *Nadu*, *Kekulu* and *Kekulu Samba* reported significantly ($p < 0.05$) higher mean TAA contents than their white counterpart varieties. The parboiled varieties; *Rathu Nadu*, *Sudu Nadu*, *Keeri Samba* and *Samba* reported the overall highest gamma amino butyric acid (GABA) contents (0.9 ± 0.3–5.0 ± 1.7 mg/100g). Completely polished *Sudu Kekulu* reported the overall lowest mean TAA, EAA and GABA contents. Except for unanalysed tryptophan, findings revealed that disregarding the nutritional loss encountered during cooking irrespective of variety, consumption of approximately 100 g of raw rice (after cooking) three times a day will provide an average adult (of 50 kg body weight) more than 50 % of the daily intake of essential individual amino acids recommended by the Food and Agricultural Organization (FAO).

Keywords: Essential amino acids, GABA, rice, Sri Lanka, total amino acids.

INTRODUCTION

Rice (*Oryza sativa* L.) is the dietary staple of more than half of the world's population, which significantly contributes to the daily nutritional requirement (Kennedy & Burlingame, 2003).

As the dietary staple, rice accounts for approximately 45 % of the total calorie intake and around 40 % of the total protein requirement of an average Sri Lankan (Kennedy *et al*., 2003). The per capita consumption of rice fluctuated around 105 kg per year throughout the past three decades (Jayatissa *et al*., 2014). There are two major types of rice varieties mainly grown in the country termed short grain (*Samba*) and long grain (*Nadu*) among which the consumption of long grain predominates with an average of 81 % where as the short grain accounts for only around 19 % of the total rice consumption. Rice is also categorised as parboiled or raw (*Kekulu*) based on the method of processing. The percentage consumption of parboiled rice (54.3 %) surpasses the percentage of non-parboiled rice consumed in the country. The selection of the variety for consumption is significantly influenced by the taste, aroma, texture and ease of cooking while the appearance characteristics such as colour, shape, size and cleanliness are among the secondary important factors that can be attributed to the selection of the variety

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(Rambukwella *et al*., 2017). Owing to the consumption habits related to urbanisation and ethnic differences, significant variations in rice consumption by varieties have been reported (Department of census and statistics, 2016). According to the survey report, *Sudu Kekulu, Sudu Kekulu Samba, Rathu Kekulu, Rathu Kekulu Samba, Samba, Rathu Nadu, Sudu Nadu* and *Basmathi* were among the highly consumed rice varieties in the country.

As the second most abundant nutrient next to carbohydrates, protein plays a significant role in determining the functional properties, texture, pasting capacity and the sensory characteristics of rice (Lyon *et al*., 1999; Martin & 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 protein- bound amino acids present as major components, minor amounts of free amino acids (FAAs) are also found in rice. The apparent relationship reported with the levels of FAAs in food to the organoleptic characteristics has inspired the scientific research towards analysis of FAA composition in food (Nishimura & Kato, 1988; Gunlu & Gunlu, 2014; Kasumyan, 2016).

In addition to the proteogenic FAA pool, rice is also enriched with gamma-aminobutyric acid (GABA) which is a non proteogenic amino acid that aids 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), controlling diabetic conditions (Imam *et al*., 2012) and inhibiting cancer cell proliferation (Park & Oh, 2007). Hence, from the nutritional aspect, the investigation of GABA levels present in rice becomes inevitably important.

The aim of the present study was to determine the levels and the variation patterns of the amino acid profiles including GABA present in rice consumed by the local population of the major city of the country, with a view to provide reference for selection of varieties to be included in the food consumption patterns and to identify varieties to be popularised in future breeding programmes.

METHODOLOGY

Chemicals and reference standards

The analytical reference standards of amino acids including GABA with purity > 98 % were obtained from Sigma Aldrich Chemicals, St. Louis, MO and the internal standard; L-theanine with purity > 98% was purchased from Baxter Smith Labs, USA. The other chemicals used including the solvents were of either LCMS grade or HPLC grade purchased from Sigma Aldrich Chemicals, Switzerland. The derivatising agents, *o*-phthalaldehyde 3-mercaptopropionic acid (OPA-3MPA), 9-Fluorenylmethoxycarbonyl chloride (FMOC) and borate buffer (pH–10.2) were purchased from Agilent Technologies, USA.

Table 1: Basic characteristics and the average monthly consumption of rice per person for the varieties used in the study

Variety	Grain size	Ecotype	Parboiled/ non-parboiled	Pericarp colour	Local/ imported	% of average monthly consumption per person*
Rathu Kekulu Samba	Short grain	*indica*	Non-parboiled	Red	Local	1.0
Sudu Kekulu Samba	Short grain	*indica*	Non-parboiled	White	Local	2.5
Rathu Kekulu	Long grain	*indica*	Non-parboiled	Red	Local	22.9
Sudu Kekulu	Long grain	*indica*	Non-parboiled	White	Local	18.5
Rathu Nadu	Long grain	*indica*	Parboiled	Red	Local	3.6
Sudu Nadu	Long grain	*indica*	Parboiled	White	Local	34.4
Keeri Samba	Short grain	*indica*	Parboiled	White	Local	na
Samba	Short grain	*indica*	Parboiled	White	Local	15.1
Basmathi	Long grain	*indica*	Parboiled	White	Imported	0.2
Ponni	Short grain	*indica*	Parboiled	White	Imported	na
Fragrant Rice	Short grain	*indica*	Parboiled	White	Imported	na

na: data not available

* Percentage of average monthly consumption per person out of the total rice consumed was adapted from the household income and expenditure survey (Department of Census and statistics, 2016)

Grain samples

Eight locally grown varieties: *Rathu Kekulu Samba, Sudu Kekulu Samba, Rathu Kekulu, Sudu Kekulu, Rathu Nadu, Sudu Nadu, Keeri Samba, Samba* and three imported rice varieties; *Basmathi, Ponni*, fragrant rice, which are highly consumed and popular among the Sri Lankan consumers were selected for the study (Table 1). These varieties included rice with different grain characteristics. In addition, the investigated rice included varieties which were subjected to different post harvest treatments of milling and parboiling.

Representative six samples from each variety collected from the leading supermarkets and the open economic centres located at Nugegoda, Narahenpita, Kirulapona, Pettah, Dematagoda, and Wellawatta representing the Colombo municipal area were analysed.

Sample preparation for FAA analysis including GABA

The finely ground (IKA-MF 10 basic Microfine grinder drive) uncooked rice samples were sieved through a 0.3 mm sieve prior to analysis. The FAAs in rice were extracted using the validated method described in the previous study carried out by the authors (Liyanaarachchi *et al*., 2018). The summary of the method performance characteristics of FAA analysis are given as a supplementary table. For the FAA analysis including GABA, 100 µL from each IS; L-theanine and L-Nor (concentration 100g/L) were added to 0.2 g of finely ground and sieved rice samples. The extraction of FAAs was performed by shaking the weighed rice sample with 10 mL of methanol:water (40:60, v/v) mixture for 10 min in a mechanical shaker at 125 rpm followed by centrifugation at 15,000 rpm for 10 min. After filtration

Table 2: Method conditions for LC-MS/MS analysis

Amino Acid	RT (min)	ESI Mode	Q* (Da)	Q1 (Da)	Q2 (Da)	DP (V)	EP (V)	CE (V)	CXP (V)
Asn	3.96	Pos	133	116	74	31,41	10	13,25	8,22
Phe	7.34	Pos	166	120	103	31	10	21,35	12,28
Val	4.49	Pos	118	72	55	36	10	13,29	10,6
Lys	3.69	Pos	147	130	84	16,21	10	13,23	6,8
Ala	4.01	Pos	90	44	-	20	10	19	6
Tyr	4.88	Pos	182	165	136,123	46,51	10	19,13,25	6,16
His	3.73	Pos	156	110	93	41	10	17,31	14,8
Trp	9.46	Pos	205	188	146	41	7	15,24	9,39
Ser	3.94	Pos	106	60	42	31	10	15,31	6,4
Asp	3.99	Pos	134	88	70	26,36	10	13,25	14,6
Met	4.68	Pos	150	133	104	21	10	13,15	8,12
Glu	4.06	Pos	148	102	84	21	10	15,23	4,12
Pro	4.27	Pos	116	70	-	8	7	23	9
Gly	3.92	Pos	76	30	-	46	10	17	8
Ile	5.45	Pos	132	86	69	40	7	15,24	14,9
Leu	6.15	Pos	132	86	69	36	10	23	8,20
Arg	3.74	Pos	175	116	70,60	46,56,41	10	19,35,19	6,8,6
Thr	4.03	Pos	120	74	56	46,86	10	15,23	8,6
Gln	2.84	Pos	147	84	102	21,31	10	23,19	6,12
GABA	3.05	Pos	104	69	87	31,26	10	25,5	6
Nor (IS)	6.00	Pos	132	86	68	31	10	25	8
Theanine (IS)	4.01	Pos	175	84	158	35	10	27,16	24,39

RT: Retention time; ESI: Electron spray ionisation; Q*: Parent mass; Q1: Quantifier mass; Q2: Qualifier mass; DP: Declustering; potential; CE: Collision energy; EP: Exit potential; CXP: Collision cell exit potential

Asp: Aspartic acid; Glu: Glutamic acid; Asn: Asparagine; Ser: Serine; Gln: Glutamine; His: Histidine; Gly: Glycine; Thr: Threonine; Arg: Arginine; Ala: Alanine; Tyr: Tyrosine; Val: Valine; Met: Methionine; Lys: Lysine; Trp: Tryptophan; Phe: Phenylalanine; Ile: Isoleucine; Leu: Leucine; Pro: Proline; Nor: Norleucine IS: Internal standard; GABA: Gamma amino butyric acid

through 0.22 µm nylon syringe filter, the supernatant solution was subjected to LC-MS/MS analysis.

The moisture content of the rice samples were determined by oven drying the rice at 130 °C for 2 h as specified in the ISO 712 method (ISO 712, 2009).

Instrumentation and analytical LC-MS/MS method for FAA analysis

The detection of FAAs was performed using an Eksigent Expert Ultra LC 100 XL (Eksigent, Netherlands) UPLC system, coupled to an ABSciex (QTrap) 4500 series triple quadrupole linear ion trap mass spectrometer (Sciex, USA) in electro spray ionisation (ESI) with multiple reaction monitoring (MRM) transitions (Table 2). The ion spray voltage was set at 5500 V while the source temperature was maintained at 500 ºC. Both the nebulizer and the heater gas were operated at 50 kPa.

The FAAs were separated on an Agilent Zorbax Eclipse C18 (4.6 x 100 mm, 5 micron) column using gradient elution within 12.5 min (Figure 1a). The gradient elution started with 90 % A for 0 min; ramped to 30 % B within next 6.5 min at a flow rate of 0.4 mL/min; ramped to 100 % B in 7 min and was kept at 100 % B till 8 min; ramped to 90 % A in 8.5 min and kept at 90 % till 12.5 min at a flow rate of 0.4 mL/min. The column was operated at 40 °C throughout the total runtime. Data acquisition and processing were performed using Analyst software (version 1.6.2) from Sciex Corporation, USA.

Sample preparation for TAA analysis

To 0.2 g of the sieved rice sample placed inside a screw capped glass tube, of 5.00 mL of the hydrolysis mixture (6 mol/L HCI containing 1 g of phenol per liter) was added. The sample after vortexing for 5 min was placed inside a drying oven set at 110 °C for 22 h to complete the

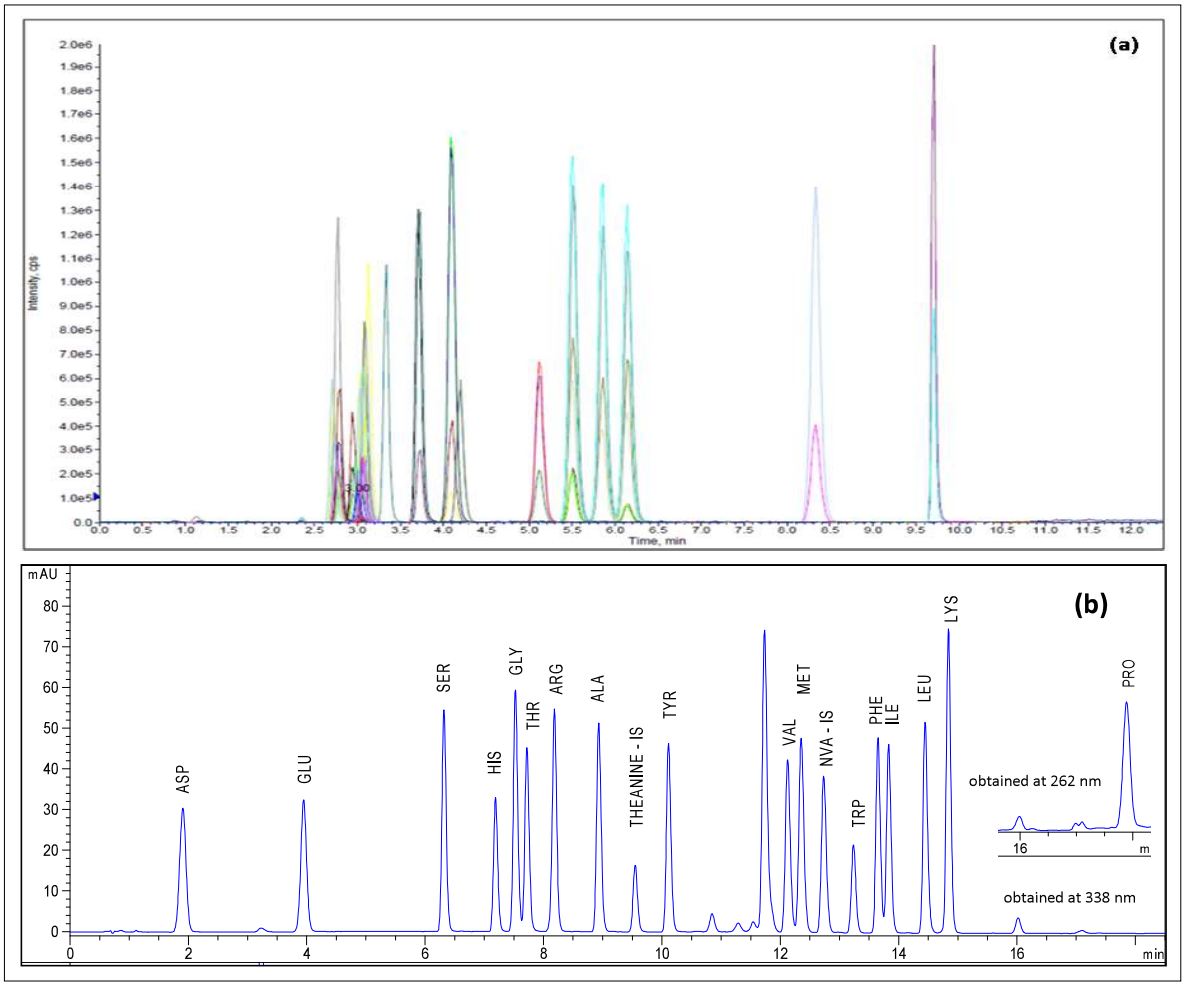

Figure 1: a) LC-MS/MS chromatogram of amino acid standards, b) HPLC chromatogram of amino acid standards

hydrolysis (ISO 13903). In order to minimise building up of pressure due to the evolution of gaseous substances, the screw capped tube containing the hydrolysis mixture was tightened only after it was kept open for an hour inside the oven. After completion of the hydrolysis, the mixture was transferred to an ice bath and the pH of the hydrolysed mixture was adjusted to 2.2 using 10 M sodium hydroxide solution. Final pH adjustments were done with 1M sodium hydroxide solution while keeping the temperature of the solution below 40 ^{o}C. The pH adjusted solution was transferred to a 25 mL volumetric flask of 100 µL from each 50 nmol/L IS's L-Nva and L-theanine. The resulting solution was made up to the mark with ultra pure water acidified to pH 2.2 with HCl.

Instrumentation and analytical HPLC method for TAA analysis

The analysis was performed using an Agilent 1100 HPLC system (Agilent Technologies, Palo Alto, CA) which consisted of a diode array detector (DAD) (G1321A) and a programmable auto sampler (G1313A). The chromatographic separation of amino acids was achieved using an Agilent Zorbax Eclipse AAA column (4.6 mm x 150 mm, 5 micron) with gradient elution (Figure 1b). The gradient elution started with 100 % A for 1.9 min; ramped to 57 % B within next 18.1 min; ramped to 100 % B in 18.6 min and kept at 100 % B till 22.3 min; then ramped to 100 % A in 23.2 min and kept with 100 % A till 26 min. The column was operated at 40 °C and the flow rate of the method was set at 2 mL/min throughout the runtime (Henderson *et al*., 2000). The automated pre-column derivatisation with *o*-phthalaldehyde 3-mercaptopropionic acid OPA-3MPA and fluorenylmethyloxycarbonyl chloride (FMOC) was performed prior to injection with a programmed injector using the injector settings as described in Table 3 (Henderson *et al*., 2000). The primary amino acids which were derivatised using OPA-3MPA were monitored at 338 nm while the secondary amino acid, Pro, which was derivatised using FMOC was monitored at 262 nm using the DAD detector. Agilent Chemstation software version B.04.03 was used for the data acquisition and analysis.

Table 3: Injector programme for pre-column online derivatisation for TAA analysis

Line	Function	Amount	Vial position	Reagent
1	Draw	2.5 µL	1	borate buffer
2	Draw	0.5 µL	7 onwards*	sample
3	Mix	3.0 µL in air max speed 2 times		
4	Wait	0.5 min		
5	Draw	0 µL	2	Water - (needle wash using uncapped vial)
6	Draw	0.5 µL	3	OPA
7	Mix	3.5 µL in air max speed 6 times		
8	Draw	0.5 µL	4	FMOC
9	Mix	4.0 µL in air max speed 6 times		
10	Draw	0 µL	2	Water- (needle wash using water in uncapped vial
11	Draw		6	acetonitrile
12	Draw	32 µL	5	water (capped vial)
13	Mix	18 µL in air max speed 2 times		
14	Inject			

TAA: Total amino acids; * vial position of the sample in the tray

Table 4: Free amino acid composition of the rice varieties (mg/100 g on dry basis)

Cultivar/ Amino acid		Essential amino acids												Non essential amino acids								
		Phe	Val	Lys	Ile	His	Trp	Met	Leu	Thr	Tyr	Ala	Ser	Asp	Asn	Glu	Pro	Gly	Arg	Gln	GABA	Total
Rathu Kekulu Samba	Mean	0.2 ^{d}	0.5 ^{def}	ND	0.2 ^{de}	ND	0.6 ^{d}	ND	0.2 ^{d}	0.4 ^{a}	0.6 ^{efg}	4.6 ^{cde}	ND	7.7 ^{ab}	10.5^{b}	9.9 ^{bc}	0.3 ^{de}	1.0 ^{b}	0.3 ^{d}	ND	1.1^{cd}	38.1^{de}
	SD	0.05	0.01		0.03		0.06		0.05	0.01	0.03	0.08		1.2	2.8	2.5	0.07	0.2	0.03		0.1	7.1
Sudu Kekulu Samba	Mean	0.3 ^{cd}	0.6 ^{def}	0.2 ^{c}	0.2 ^{cd}	ND	0.9 ^{cd}	ND	0.3 ^{d}	0.8 ^{cde}	0.6 ^{def}	4.1 ^{def}	0.4 ^{cd}	8.3 ^{a}	12.2 ^{b}	10.1 ^{bc}	0.6 ^{bc}	1.0 ^{b}	0.6 ^{cd}	0.7 ^{de}	1.5 ^{cd}	43.4 ^{cd}
	SD	0.08	0.1	0.1	0.05		0.2		0.07	0.2	0.2	0.8	0.1	2	2.1	0.7	0.2	0.4	0.3	0.3	0.8	7.5
Rathu Kekulu	Mean	0.4 ^{bcd}	0.6 ^{def}	0.2 ^{c}	0.3 ^{cd}	ND	0.6 ^{d}	ND	0.3 ^{d}	1.5 ^{cd}	0.7 ^{de}	5.6 ^{c}	0.3 ^{cd}	6.1 ^{bc}	10.7 ^{b}	9.6 ^{bc}	0.7 ^{bc}	1.5 ^{b}	0.6 ^{cd}	0.3 ^{e}	2.1 ^{c}	42.1 ^{cd}
	SD	0.07	0.3	0.05	0.05		0.03		0.08	0.2	0.2	1.4	0.4	2.4	1	1.1	0.2	0.3	0.06	0.1	0.7	8.1
Sudu Kekulu	Mean	0.3 ^{ef}	0.5 ^{ef}	ND	0.2 ^{de}	ND	0.3 ^{e}	ND	0.2 ^{d}	0.5 ^{de}	0.4 ^{efg}	3.8 ^{de}	0.2 ^{cd}	5.9 ^{bcd}	9.8 ^{bc}	8.4 ^{c}	0.4 ^{cde}	1.2 ^{b}	0.3 ^{d}	ND	0.9 ^{d}	33.3 ^{ef}
	SD	0.05	0.2		0.06		0.2		0.04	0.2	0.06	1.4	0.2	1.9	1.6	1.9	0.1	0.7	0.08		0.3	8.5
Rathu Nadu	Mean	1.0 ^{a}	1.2 ^{bc}	0.5 ^{b}	0.6 ^{a}	0.2 ^{d}	0.9 ^{cd}	0.1 ^{cd}	1.2 ^{a}	1.0 ^{cde}	1.4 ^{b}	5.3 ^{cd}	0.2 ^{cd}	4.1 ^{def}	6.8 ^{cd}	12.9 ^{a}	1.3 ^{a}	1.0 ^{b}	1.3 ^{b}	1.1 ^{cd}	5.0 ^{a}	47.1 ^{c}
	SD	0.1	0.2	0.2	0.07	0.02	0.09	0.03	0.2	0.1	0.08	0.2	0.1	0.6	0.3	2.3	0.1	0.2	0.4	0.2	1.7	5.6
Sudu Nadu	Mean	1.0 ^{a}	1.4 ^{b}	0.6 ^{ab}	0.6 ^{a}	0.4 ^{ab}	1.2 ^{ab}	0.3 ^{ab}	1.1 ^{ab}	1.9 ^{b}	1.5 ^{b}	7.2 ^{b}	0.9 ^{b}	2.7 ^{f}	12.1 ^{b}	10.8 ^{b}	1.3 ^{a}	2.5 ^{a}	1.9 ^{a}	3.4 ^{b}	4.9 ^{a}	57.7 ^{b}
	SD	0.2	0.08	0.06	0.07	0.06	0.3	0.06	0.2	0.2	0.2	1.4	0.4	0.6	1	1.6	0.2	0.7	0.6	1.1	0.5	8.8
Keeri Samba	Mean	1.7 ^{a}	1.9 ^{a}	0.8 ^{a}	0.7 ^{a}	0.4 ^{a}	1.4 ^{a}	0.4 ^{a}	1.3 ^{a}	2.4 ^{a}	2.0 ^{a}	9.3 ^{a}	1.4 ^{a}	6.5 ^{abc}	16.5 ^{a}	11.1 ^{ab}	1.4 ^{a}	2.6 ^{a}	2.1 ^{a}	4.2 ^{a}	4.6 ^{ab}	72.7 ^{a}
	SD	0.6	0.6	0.4	0.2	0.1	0.3	0.3	0.7	1.3	0.5	1.1	0.6	1.4	5.1	1.7	0.3	1.2	0.8	1.2	0.9	18.3
Samba	Mean	0.7 ^{b}	1.0 ^{c}	0.5 ^{b}	0.4 ^{b}	0.3 ^{bc}	1.4 ^{cd}	0.2 ^{bc}	0.7 ^{c}	1.2 ^{c}	1.2 ^{bc}	7.6 ^{b}	0.3 ^{a}	3.5 ^{f}	9.6 ^{bc}	10.6 ^{b}	1.4 ^{a}	1.2 ^{b}	1.4 ^{b}	1.7 ^{c}	3.9 ^{b}	48.8 ^{c}
	SD	0.2	0.3	0.2	0.1	0.2	0.4	0.1	0.3	0.5	0.5	2.1	0.03	1.4	3.5	1.5	0.4	0.3	0.6	0.9	0.8	13.2
Basmathi	Mean	0.2 ^{cd}	0.3 ^{f}	ND	0.1 ^{e}	ND	0.1 ^{ef}	ND	ND	0.4 ^{e}	0.2 ^{g}	2.9 ^{fg}	0.1 ^{cd}	2.9 ^{f}	9.7 ^{bc}	5.9 ^{d}	0.2 ^{e}	1.0 ^{b}	0.3 d	ND	2.0 ^{c}	26.3 ^{f}
	SD	0.06	0.2		0.04		0.1			0.2	0.05	0.4	0.1	1.4	2.3	1.1		0.4	0.2		0.9	6.5
Ponni	Mean	5.0 ^{bc}	0.9 ^{cd}	0.4 ^{b}	0.3 ^{bc}	0.3 ^{bc}	1.0 ^{bc}	0.3 ^{ab}	0.8 ^{bc}	1.0 ^{cde}	0.9 ^{cd}	3.4 ^{efg}	1.0 ^{b}	5.3 c^{de}	16.6 ^{a}	5.6 ^{d}	0.8 a	2.2 ^{a}	1.0 ^{bc}	0.5 de	1.2 ^{cd}	48.5 ^{cd}
	SD	0.4	0.4	0.2	0.2	0.05	0.2	0.09	0.3	0.4	0.5	0.9	0.5	2.5	3.2	1.5	0.4	0.7	0.4	0.2	0.3	12.4
Fragrant Rice	Mean	0.3 ^{cd}	0.7 ^{de}	0.2 ^{c}	0.2 ^{cd}	0.2 ^{cd}	ND	0.2 ^{bc}	0.3 ^{d}	0.4 ^{e}	0.3 ^{fg}	2.1 ^{g}	0.5 ^{c}	2.6 ^{f}	4.5 ^{d}	3.1 ^{e}	0.6 ^{bcd}	1.0 b	0.2 ^{d}	0.2 e	1.2 ^{cd}	18.8 ^{g}
	SD	0.1	0.2	0.04	0.07	0.01		0.02	0.08	0.1	0.08	0.1	0.1	0.8	0.6	1	0.1	0.08	0.04	0.08	0.1	3.5

SD: Standard Deviation; n (number of replicates) = 6; ND: Not detected (Respective limit of determination is given in the supplementary Table); Values within a column followed by different letters are significantly different at $p < 0.05$ according to Duncan multiple range test

Asp: Aspartic acid; Glu: Glutamic acid; Ser: Serine; His:Histidine; Gly: Glycine; Thr: Threonine; Arg: Arginine; Alanine; Tyr: Tyrosine; Val: Valine; Met: Methionine; Trp: Tryptophan;

Phe: Phenylalanine Ile: Isoleu;cine; Leu:Leucine; Lys: Lysine; Pro: Proline; Asn:Asparagine; Gln: Glutamin; Trp: Tryptophan; GABA: Gamma amino butyric acid; Tot: Total free amino acids

Table 5: Total individual amino acid composition of the rice varieties (g/kg on dry basis)

Cultivar/		Non essential amino acids									Essential amino acids							
Amino acid		**Asp**	**Glu**	**Ser**	**Pro**	**Gly**	**Thr**	**Arg**	**Ala**	**Tyr**	**Val**	**Met**	**Phe**	**Ile**	**Leu**	**Lys**	**His**	**Total**
Rathu Kekulu Samba	**Mean**	6.8^{cde}	14.2^{cd}	5.3^{bc}	3.2^{bc}	6.7^{b}	1.7^{bc}	4.0^{cd}	8.3^{b}	1.5^{bc}	6.0^{c}	1.3^{cde}	4.1^{bc}	3.0^{bc}	5.9^{bc}	3.5^{ab}	1.3^{c}	76.8^{bc}
	SD	0.2	0.5	0.3	0.6	0.4	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.1	0.2	0.8	0.1	2.5
Sudu Kekulu Samba	**Mean**	6.0^{fg}	12.4^{ef}	4.8^{cd}	3.1^{bc}	5.8^{cd}	1.5^{cd}	3.5^{ef}	6.9^{c}	1.3^{cd}	5.3^{d}	1.2^{e}	3.5^{de}	2.6^{d}	5.2^{cd}	3.0^{bc}	1.2^{c}	67.2^{de}
	SD	0.6	0.7	0.4	0.8	0.4	0.1	0.2	0.5	0.1	0.6	0.1	0.4	0.3	0.6	0.5	0.1	4.5
Rathu Kekulu	**Mean**	6.1^{efg}	13.3^{de}	5.1^{bcd}	3.5^{bc}	6.4^{bc}	1.7^{bcd}	3.9^{cde}	8.0^{b}	1.4^{bcd}	5.8^{cd}	1.2^{cde}	3.8^{cd}	2.9^{c}	5.7^{bc}	3.1^{bc}	1.2^{c}	73.2^{cd}
	SD	0.8	1.5	0.6	0.5	0.7	0.2	0.4	0.8	0.2	0.6	0.1	0.4	0.3	0.7	0.6	0.1	6.5
Sudu Kekulu	**Mean**	5.7^{g}	11.6^{f}	4.6^{d}	3.0^{c}	5.6^{d}	1.5^{d}	3.4^{f}	7.1^{c}	1.3^{d}	4.7^{e}	1.2^{de}	3.2^{e}	2.5^{d}	4.9^{d}	3.2^{bc}	1.2^{c}	64.5^{e}
	SD	0.1	0.4	0.3	0.5	0.3	0.1	0.1	0.5	0	0.4	0.1	0.1	0.2	0.5	0.4	0.1	1.9
Rathu Nadu	**Mean**	7.3^{bc}	17.0^{b}	6.6^{a}	3.7^{ab}	8.1^{a}	2.0^{a}	5.1^{a}	10.1^{a}	1.8^{a}	7.2^{a}	1.6^{a}	4.9^{a}	3.7^{a}	7.4^{a}	3.2^{bc}	1.6^{a}	91.2^{a}
	SD	0.5	1.4	0.8	1.6	0.9	0.2	0.8	0.7	0.3	0.6	0.4	0.3	0.4	0.6	0.5	0.3	6.8
Sudu Nadu	**Mean**	6.4^{defg}	14.0^{cd}	5.1^{bcd}	3.3^{bc}	6.3^{bcd}	1.7^{bcd}	3.6^{def}	7.9^{b}	1.4^{bcd}	6.0^{c}	1.4^{bcd}	4.0^{bc}	3.0^{bc}	5.8^{bc}	3.2^{bc}	1.2^{c}	74.4^{bc}
	SD	0.1	0.6	0.2	0.5	0.2	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.6	0.1	1.1
Keeri samba	**Mean**	7.1^{bcd}	15.0^{c}	5.5^{b}	3.8^{ab}	6.9^{b}	1.8^{b}	4.1^{c}	8.5^{b}	1.6^{b}	6.4^{bc}	1.4^{cde}	4.4^{b}	3.2^{bc}	6.2^{b}	3.3^{abc}	1.3^{c}	80.5^{b}
	SD	0.2	0.4	0.2	0.6	0.3	0.1	0.2	0.5	0.1	0.2	0.1	0.3	0.2	0.4	0.4	0	2.9
Samba	**Mean**	6.6^{cdef}	14.9^{c}	5.1^{bcd}	3.8^{ab}	6.9^{b}	1.7^{bc}	3.9^{cde}	8.4^{b}	1.4^{bcd}	6.0^{c}	1.4^{abc}	4.2^{bc}	3.1^{bc}	5.9^{bc}	2.8^{c}	1.3^{c}	77.4^{bc}
	SD	0.3	1.3	0.7	0.5	0.7	0.3	0.3	0.9	0.1	0.7	0.2	0.4	0.3	1	0.5	0.1	6.6
Basmathi	**Mean**	7.3^{bc}	18.2^{ab}	6.4^{a}	4.3^{a}	7.8^{a}	2.0^{a}	4.9^{ab}	9.6^{a}	1.9^{a}	7.3^{a}	1.6^{ab}	5.3^{a}	3.7^{a}	7.5^{a}	3.9^{a}	1.5^{ab}	93.2^{a}
	SD	1.3	1.3	0.3	0.4	0.3	0.1	0.3	0.6	0.1	0.2	0.1	0.2	0.2	0.1	0.5	0.1	3.7
Ponni	**Mean**	8.4^{a}	18.9^{a}	6.7^{a}	4.3^{a}	8.3^{a}	2.1^{a}	4.9^{ab}	10.0^{a}	1.8^{a}	7.3^{a}	1.4^{abc}	5.3^{a}	3.6^{a}	7.6^{a}	3.8^{a}	1.5^{ab}	96.0^{a}
	SD	0.5	0.9	0.4	0.4	0.6	0.1	0.3	0.6	0.1	0.5	0.2	0.3	0.2	0.5	0.6	0.1	5.1
Fragrant Rice	**Mean**	7.8^{ab}	17.8^{ab}	6.5^{a}	3.8^{ab}	8.2^{a}	2.0^{a}	4.6^{b}	9.5^{a}	1.8^{a}	6.8^{ab}	1.3^{cde}	5.0^{a}	3.3^{b}	7.3^{a}	3.0^{bc}	1.4^{bc}	90.0^{a}
	SD	1.1	2.6	0.8	0.3	1	0.2	0.5	1.2	0.3	0.8	0.1	0.7	0.3	1	0.4	0.1	10.1

SD: Standard deviation; n (number of replicates) = 6;Values within a column followed by different letters are significantly different at $p < 0.05$ according to duncan multiple Range test

Asp: Aspartic acid; Glu: Glutamic acid; Ser: Serin; His: Histidine; Gly: Glycine; Thr: Threonine; Arg: Arginine; Ala: Alanine; Tyr: Tyrosine; Val: Valine; Met: Methionine; Trp: Tryptophan; Phe: Phenylalanine; Ile: Isoleucine; Leu:Leucine; Lys: Lysine; Pro: Proline;

The two methods incorporated for the analysis of amino acids were validated as per the guidelines described in FDA, AOAC International and Eurachem Method validation guidelines (Eurachem, 2014; AOAC, 2002) and found in compliance with the international method validation requirements (Supplementary Table).

The individual levels of the free and total amino acids present in the samples were quantified using the calibration plots constructed by fitting the analyte concentrations of the calibrators versus the peak area ratios of the analyte to IS with line regression over six calibration levels.

Statistical evaluation

The statistical analysis was performed using the statistical software package, SAS for Windows V 9.1 (SAS Institute Inc., NC, USA). The amino acids were quantified in six replicates from each of the varieties and were analysed using ANOVA, Duncan multiple range test (DMRT) analysis. The level of significance was $p < 0.05$.

RESULTS AND DISCUSSION

Variation in FAA composition

Table 4 summarises the mean individual and total free amino acid (TFAA) contents observed in the studied rice varieties. According to the FAA profiles, asparagine, glutamic acid, aspartic acid and alanine were among the amino acids with the highest content while methionine and histidine were the least present. The mean TFAA content ranged between 18.8 ± 3.5 and 72.7 ± 18.3 mg/100 g of rice (db). The observed TFAA levels are comparable to the findings made by Kamara *et al*. (2010) and Komatsuzaki *et al*. (2007) for rice varieties found in Asia. Further, it was also found that the TFAA composition observed in rice is significantly lower than the FAA composition reported for other cereal grains such as wheat, rye, barley (Nagaoka, 2005; Mustafa *et al.*, 2007) and corn (Culea *et al.*, 2015).

Significant differences in mean values obtained for individual and TFAA content in rice varieties were observed. The highest mean TFAA level was observed in *Keeri Samba* (72.7 ± 18.3 mg/100 g) variety while the lowest was found in the fragrant rice with an average value of 18.8 ± 3.5 mg/100 g. Unlike the TAA compositions, no marked influence was observed on TFAA levels from either the processing techniques or grain characteristics. Influence caused by the environmental and the agronomic practices could be attributed for these variations in the composition of amino acids observed for a particular cultivar (Juliano & Villareal, 1993; Kamara *et al*., 2010).

The GABA content also significantly varied among the cultivars ranging from 0.9 ± 0.3 – 5.0 ± 1.7 mg/100 g (db) (Table 4). The GABA levels observed in the experimented *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 few brown *indica* rice varieties cultivated in China (Shen *et al*., 2015) and several rice varieties grown in Thailand (Karladeea & Suriyonga, 2012).

The highest GABA content was detected in *Rathu Nadu* and *Sudu Nadu* while the lowest amount was observed in *Sudu Kekulu* rice. Interestingly, all the varieties which reported the highest were parboiled rice varieties. The enzymatic activities induced during the germination step carried out prior to boiling and the heating step involved with the production of parboiled rice, which accounts for considerable increment in the GABA content, significantly contributes towards the raised nutritive value of parboiled rice over the non-parboiled varieties (Liu *et al*, 2005). In addition, the inward diffusion during the parboiling steps which results in the retention of minerals and water-soluble vitamins (Battacharya, 1985) accounts for the comparatively higher nutritive value in parboiled rice relative to the milled rice. Therefore, these findings in conjunction with the health promoting activity associated with GABA in reducing diabetes (Imam *et al*., 2012), high blood pressure (Inoue *et al*., 2003) and reported anti cancer activity (Park & Oh, 2007) can inevitably be utilised in driving the local food consumption patterns towards consumption of parboiled rice. Further, these findings on the genetic diversity of the ability of each rice variety to synthesise GABA will provide guidance for the selection of varieties to be popularised and in the manipulation of desirable traits in breeding to improve nutritional and functional properties of rice.

Asparagines present in free form together with soluble sugars are reported to be associated with the formation of acrylamide (Curtis & Halford, 2016), a compound which is classified as a group 2A carcinogen declared by the International Agency for Research on Cancer (IARC, 1994). Among the varieties, the mean asparagine content was found highest in *Ponni* (16.6 ± 3.2 mg/100g) and *Keeri Samba* (16.5 ± 5.1 mg/100g) (db) varieties, while the lowest was reported in fragrant rice. As given in Table 4, the mean asparagine content detected among the rice varieties ranged between 4.5 ±

0.6 – 16.6 ± 3.2 mg/100g (db). Curtis *et al*. (2016) in their study done with wheat have established that if the wheat grain contains 25.08 mg/100 g of asparagine, the acrylamide-forming potential will be 1.69 µg/kg. This is well below the benchmark levels set by the European Commission for rice-based products, which is 150 µg/kg (EU 2017/2158). The asparagine values detected in the rice varieties in the present study were significantly lower than the contents detected in other cereal grains such as rye, wheat, barley and maize (Fredriksson *et al*., 2004; Zilic *et al*., 2017). Therefore, during food processing, acrylamide formation promoted by heating is comparatively lower in rice compared to the other cereals reported with high free asparagine contents suggesting rice as a safer option over the rest of the cereals.

Variation in TAA composition

The summary of mean individual and mean TAA levels observed in the rice cultivars under study are given in Table 5. Glutamic acid was the most predominant amino acid which ranged between 11.6 ± 0.4 – 18.9 ± 0.9 g/kg (db). Comparatively higher levels of aspartic acid, glycine, alanine, aeucine, valine and serine were found in the rice cultivars while hydroxyproline, phenylalanine, isoleucine, methionine, glutamine and leucine were the least present. The mean TAA content in the studied cultivars ranged between 64.5 ± 1.9 g/kg and 96.0 ± 5.1 g/kg (db) of rice.

Several studies (Park *et al*., 2009; Rita *et al*., 2009; Ning *et al*, 2010; Liu *et al*., 2017) have reported similar compositions of glutamic acid, aspartic acid and valine contents in rice. However, comparatively higher levels of alanine and glycine were found in the investigated rice varieties.

Significant differences were observed in the mean values obtained for the individual and TAA content (Table 5). The variations observed for mean TAA content, total mean essential amino acids (EAA) and total mean non essential amino acid levels (NEAA) among the varieties are depicted in Figure 2. The total essential amino acids (EAA) included the eight indispensable amino acids, namely, valine, lysine, leucine, isoleucine, methionine, histidine, threonine and phenylalanine, while the total non essential amino acid levels (NEAA) is indicative of the sum of glutamic acid, aspartic acid, proline, alanine, glycine, serine, arginine and tyrosine.

The highest mean TAA content was detected in *Ponni* followed by *Basmathi, Rathu Nadu* and fragrant rice varieties, whereas the lowest TAA levels were observed in the *Sudu Kekulu* variety. All the cultivars reporting the highest TAA contents were parboiled varieties depicting the fact that in terms of nutritive value with respect to protein content, the parboiled varieties are superior over the non-parboiled varieties in the market. Another key finding was that out of the four with the highest mean TAA levels, three of the rice varieties, namely, *Ponni*, *Basmathi* and fragrant rice were imported whereas *Red Nadu* being the only local variety. Moreover, the levels of lysine, the limiting amino acid in cereal crops considered as an indicator of the protein quality was highest in the imported rice varieties *Basmathi* and *Ponni* with the least in the samba variety. Proline, the amino acid which accounts for the popcorn smell of rice was highest in the aromatic rice varieties *Ponni, Basmathi* and fragrant rice (Table 5).

In all experimented varieties, the mean TAA content in rice with the red pericarp were significantly higher than the rice varieties with the white pericarp. For example, as given in the Table 5, the mean TAA content in *Rathu Nadu, Rathu Kekulu* and *Rathu Kekulu Samba* were significantly higher compared to *Sudu Nadu, Sudu Kekulu* and *Sudu Kekulu Samba*, respectively. In unpolished rice, protein is more concentrated in the aleurone layer, the embryo, and the subaleurone layer of the endosperm compared to the deeper starchy endosperm (Ellis *et al*., 1987). Lower values in white rice varieties could possibly be due to the removal of significant amounts of amino acids present in the pericarp of rice during milling.

Figure 3 depicts the comparison of mean TAA content reported in the experimented varieties against the recommended daily intake for each individual essential amino acids (WHO/FAO), 2007. The levels present in each rice variety were calculated considering the consumption of three meals per day with an average of 100 g of raw rice per meal after cooking, disregarding the losses in amino acids encountered during cooking. Consumption of the above mentioned amount fulfills the nutritional requirement of the essential amino acid valine (Figure 3). However, the mean content of lysine, methionine, threonine and histidine in the experimented varieties was below the recommended daily intake. However, inclusion of *Rathu Nadu, Ponni* and *Basmathi* varieties in the meals thrice a day will provide the daily recommended allowance of leucine, isoleucine, valine and phenylalanine (WHO/FAO), 2007. Hence, in conjunction with the findings of the close relationship with rapid increase of fast food consumption and obesity and diabetes among the young generation in the country, the findings outlined in the study can be utilised by nutritionists and policy makers to come up with timely

interventions for redefining the consumption patterns of the people and in the popularisation of rice consumption for three meals per day in driving the generations towards a healthy nation. Further, as a remedial measure in combating malnutrition in the country, findings of this study can be utilised by the rice breeders in identification of traits in breeding varieties rich with amino acids such as lysine.

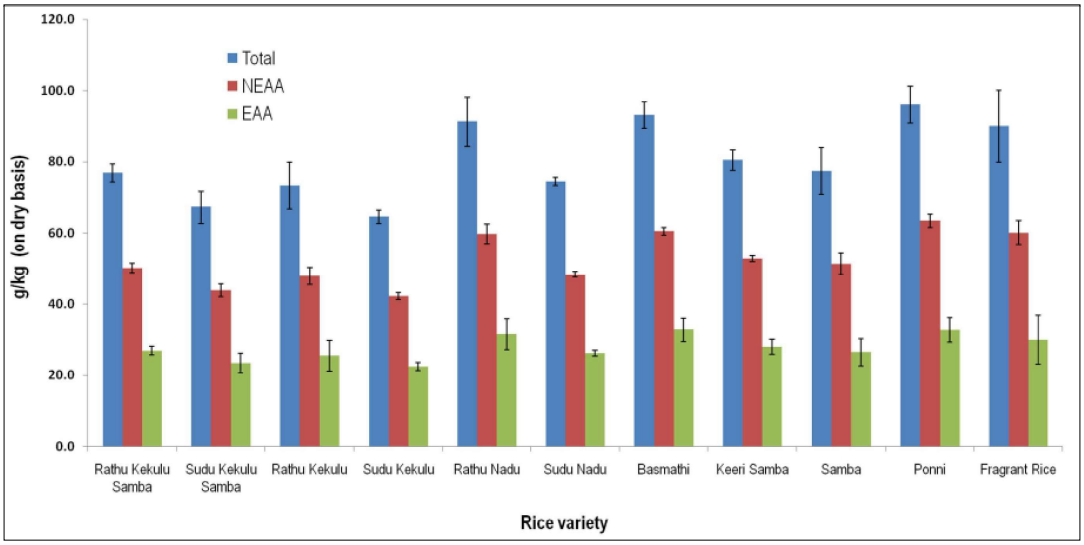

Figure 2: Variation in mean total amino acid content, essential amino acid (EAA) content and non-essential amino acid (NEAA) content in rice expressed as g/kg (on dry basis)
Number of replicates (n) = 6

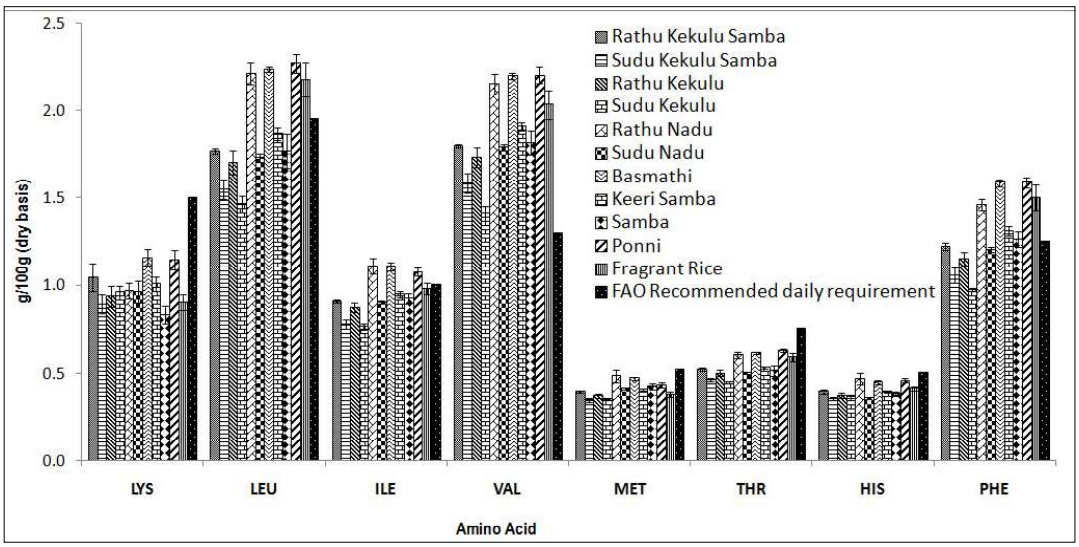

Figure 3: Comparison of essential individual mean amino acid levels* of rice varieties against the daily recommended allowance specified by the Food and Agricultural Organization (FAO) for an average adult of 50 kg body weight
* The levels were calculated considering the consumption of an average of 100 g of raw rice per meal thrice a day after cooking, disregarding the losses encountered during cooking; number of replicates (n) = 6

CONCLUSION

Owing to the wide genetic diversity and the processing techniques employed, significant variations in individual FAAs including GABA as well as TFAA levels were observed among the studied varieties.

Except for the unanalysed tryptophan, findings revealed that, disregarding the nutritional loss encountered during cooking and irrespective of variety, for an average adult of 50 kg body weight, consumption of approximately 100 g of raw rice per meal after cooking, three times a day will provide more than 50 % of the standard daily requirement of essential individual amino acids recommended by the Food and Agriculture Organization (FAO).

Conflict of Interest Statement

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

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Supplementary table: Method validation parameters for free amino acid analysis

Amino acid	Precision (% RSD)			% Recovery			Working range mg/100g	% Expanded Uncertainty (k = 2)	R^2	LOD mg/100g	LOQ mg/100g
	Low	Mid	High	Low	Mid	High					
Glu	3.1	0.3	0.3	86 ± 9	100 ± 3	103 ± 4	0.2 - 100	13	0.999	0.1	0.2
Asp	7.0	1.3	2.0	99 ± 8	98 ± 2	103 ± 6	0.4 - 100	13	0.999	0.2	0.4
Asn	2.3	0.1	0.2	92 ± 8	93 ± 2	104 ± 5	0.4 - 100	9	0.999	0.2	0.4
Ala	3.0	0.2	0.2	105 ± 8	105 ± 1	107 ± 6	0.4 - 100	13	0.999	0.2	0.4
Lys*	2.1	2.3	1.3	80 ± 3	85 ± 1	90 ± 2	0.3 - 100	4	0.999	0.1	0.3
Val	1.3	1.2	1.4	96 ± 2	93 ± 2	93 ± 1	0.2 - 100	4	0.999	0.1	0.2
Phe	2.4	1.0	1.2	96 ± 2	93 ± 1	95 ± 1	0.8 - 100	5	0.999	0.6	0.8
Tyr*	0.3	0.2	0.1	99 ± 2	99 ± 2	98 ± 2	0.2 - 100	4	0.999	0.1	0.2
His	2.6	2.0	1.4	95 ± 1	95 ± 1	101 ± 2	0.2 - 100	4	0.999	0.1	0.2
Ser	2.2	2.9	2.2	98 ± 1	94 ± 1	100 ± 3	0.1 - 100	5	0.999	0.05	0.1
Thr	2.1	2.3	1.8	94 ± 1	91 ± 2	98 ± 1	0.4 - 100	6	0.999	0.2	0.4
Pro	1.3	2.1	2.1	96 ± 2	98 ± 1	103 ± 4	0.2 - 100	5	0.999	0.1	0.2
Ile	0.8	0.3	1.2	92 ± 1	89 ± 1	92 ± 4	0.1 - 100	4	0.999	0.05	0.1
Leu	2.5	2.5	1.5	80 ± 2	80 ± 1	82 ± 5	0.2 - 100	5	0.999	0.1	0.2
Gly	1.6	1.2	2.9	104 ± 3	98 ± 2	101 ± 3	0.4 - 100	5	0.999	0.2	0.4
Met	1.2	2.8	1.9	99 ± 1	99 ± 1	101 ± 1	0.1 - 100	5	0.999	0.05	0.1
Gln	1.7	1.7	2.9	92 ± 2	89 ± 1	97 ± 1	0.2 - 100	5	0.999	0.1	0.2
Arg*	2.6	1.5	1.3	85 ± 6	87 ± 2	92 ± 2	0.2 - 100	6	0.999	0.1	0.2
Trp	2.5	3.6	0.2	106 ± 3	110 ± 5	110 ± 4	0.2 - 100	8	0.999	0.1	0.2
GABA	2.7	0.8	1.1	91 ± 3	85 ± 1	80 ± 1	0.4 - 100	12	0.999	0.2	0.4

LOD: Limit of detection; LOQ: Limit of quantification; R^2: Regression coefficient; RSD: Relative standard deviation; Number of replicates (n) = 6; Coverage factor (k) = 2

Low, Mid and High represent, 20 %, 50 % and 80 % levels of the working range of the method, respectively

* Method performance characteristics were evaluated under matrix matched conditions

Standard deviation of recoveries are given followed by the ± symbol in the respective cells

Asp: Aspartic acid; Glu: Glutamic acid; Asn: Asparagine; Ser: Serine; Gln: Glutamine; His:Histidine; Gly: Glycine; Thr: Threonine; Arg: Arginine; Ala: Alanine; Tyr: Tyrosine; Val: Valine; Met: Methionine; Trp: Tryptophan; Phe: Phenylalanine; Ile: Isoleucine; Leu:Leucine; Lys: Lysine; Pro: Proline; GABA: Gamma amino butyric acid