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Essential Amino Acid Quality Profile in Neglected and Underutilized Legumes (NULs)

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Abstract
A large number of consumers use plant-based products instead of animal-based products for their nourishment. This calls for a thorough investigation of the capacity of NULs to supply nutrients such as amino acids. Five different NULs protein extracts were profiled to ascertain the presence of the essential amino acids. In order to evaluate the quality, the essential amino acids from each of the NULs were matched against the standard FAO/WHO dietary indispensable amino acids for infants, children and adults. It was found that two of the NULs; *Vigna sp.* and *Phaseolus sp.* were the most promising, out of the five studied. *Vigna sp.* could supply adequate quantities of histidine (26.2 mg/g) for all the three age groups. However, isoleucine (35.9 mg/g) and the aromatic amino acid (phenylalanine + tyrosine) (94 mg/g) can supply adequate quantities for only children and adults. Lysine (53.6 mg/g) and threonine (44 mg/g) on the other hand, could be adequately supplied by *Phaseolus sp.* for at least, the adults' requirement. If the digestibilities of the two NULs proteins were evaluated to ascertain the levels of the post-prandial amino acids, and the two NULs were complimented with cereals, the NULs flour could eventually supply the essential amino acids for consumers who use them as food.
Keywords: Essential amino acids, Quality, Neglected legumes,

1. Introduction

In the past, pre-historic man lived a substantially vegetarian life much against what was previously believed (Hardy *et al.* 2012). People still continue to maintain vegetarian life-styles, either for religious purposes or for health reasons (Singh *et al.* 2007). Many people, though, continue to derive their protein sources from eating meat (Ruby *et al.* 2016). In the recent past however, scientific studies have provided evidence in support of the risks associated with the consumption of animal products (Brinkman *et al.* 2014; Tuso *et al.* 2013; Key *et al.* 2004). It is this belief that drive consumers to search for alternative sources of protein. The search for alternative protein sources have led to a number of studies into single cell proteins (Saeed *et al.* 2016; Nasseri *et al.* 2011). The production of single cell proteins has its own problems such as indigestibility of some of the proteins, and high levels of nucleic acids, resulting in the production of uric acid (Adedayo *et al.* 2011). Other studies also suggest plant-based proteins as a possible alternative protein resource. However, plant products alone are not able to fully provide the quality and the quantities of amino acids needed to support the nutritional needs of consumers (Ghadge *et al.* 2008). It is therefore, no surprise how the question of eating or not eating meat, has divided many communities (Ruby *et al.* 2016). In spite of the seemingly inadequate protein quality of plant products, in many of our communities, consumers depend on NULs as a major food resource (Adu-Dapaah & Sangwan 2004). These indigenous NULs are cultivated and consumed in many forms, and the various end uses, are signals to the extent to which traditional legumes are deeply imbedded in the cultural diets of the people (Quasem *et al.* 2009). The cultivation of these indigenous legumes persists because they do not require any extra agricultural inputs that have cost implications for the subsistent farmer. It is for these reasons that the frontiers for protein resources should be expanded to cover these indigenous legumes which currently have very little industrial applications.

A number of processes for improvements of protein quality, such as genetic engineering and traditional crop breeding (Luciani *et al.* 2005; Roesler *et al.* 1997; White *et al.* 2001; Falco *et al.* 1995; Galili *et al.* 2002) have been developed. However, additional gains can be made when some resources are committed to study the factors impeding wider utilization of neglected and underutilized crops. Generally, to ascertain the quality of protein, the essential amino acid profile, together with the digestibility and bioavailability of amino acids need to be determined (FAO/WHO 1990). Several techniques are available that can evaluate the quality of proteins, these include chemical score and protein digestibility corrected amino acid score (PDCAAS)(Schaafsma 2000). Chemical score defines protein quality by comparing the most limiting amino acid to the same type of amino acid in a standard or reference protein. The PDCAAS is obtained when chemical scores are multiplied by protein digestibilities (%). These techniques are theoretical quantification methods that suggest potential availability of test amino acids.

As a food security resource, NULs are increasingly gaining acceptance because their production requires less water, land and energy, compared to farmed animals (Chivenge *et al.* 2015). This trend is largely influenced by a growing consumer market demand for plant-based products (MIG 2013) such as meat substitutes and non-

diary milk from high-protein plants. It is known that soybeans have amino acid profile comparable to that of animal protein (Hasler 2002). However, the essential amino acid score of most NULs are yet to be determined. Since a large population of consumers rather consume greater quantities of plant proteins (58%), compared to animal proteins(42%) (FAOSTAT 2009), it is important to devote attention to study the amino acid profile of NULs to provide information to support their quality and quantities required for sustenance of different age groups that consume them. The objectives of the study were to profile and match the potential quality of the essential amino acids of five types of NULs against the recommended thresholds of dietary indispensable amino acids for infants, children and adults.

2. Materials and methods

2.1 Materials

Purchases of O-phthalaldehyde (OPA), Fluorenylmethyloxycarbonyl chloride (FMOC-Cl), 3-Mercapto propionic acid and 18 standard amino acids mixture were made from Fluka Sigma Aldrich (St Louis MO, USA). The seeds of five types of NULs were purchased from markets in towns in Brong Ahafo and Ashanti Regions; Techiman, Ejura, Amantin, Drobo, Abofuor and Mampong. The seeds were cleaned and solar-dried at an average temperature of 40 ^{o}C for two days. After pulverizing with Schulte-Buffalo Hammer mill (LLC, US) to 1 µm mesh size flour, they were packaged separately in plastic bags. Kjeldahl protein analyses had previously been determined (Table 1) on the five NULs to obtain protein contents of *Vigna sp.* (15.2%)*, Cajanus sp.* (21.3%)*, Mucuna sp.* (25.2%)*, Phaseolus sp.* (17.2%) and *Canavalia sp.* (17.4%).

2.2 Methods

2.2.1 Determination of amino acid composition

Accurately weighed 0.1 g quantities of each flour was separately digested in Pyrex test tube at 105 ^{o}C, for 24 h in 6 ml 6 N HCl (containing 0.1 % β-mercaptoethanol + 0.1 % phenol) (Pickering & Newton 1990). The digested samples were treated with OPA (Bartolomeo & Maisano 2006), and subsequently separated on HPLC with phosphate (constant phase) and acetonitrile/methanol /H_2O (gradient). A gradient of 0%, 0% through 53% to 100%, 100% and ending at 0%, 0% at definite time intervals (0, 2, 12, 16, 18, 20, 24 min) was effective. Specifically, an aliquot of 100 µl of digested samples was added to 100 ul of 0.4M borate buffer (pH=10.2) for 30 s. To this solution, 100 ul of OPA reagent and 15 Mm FMOC-Cl (in acetonitrile) each were added for derivatization to occur after which 600 ul of distilled water was added and 100 ul run on a reverse phase C18 silica column (4.6 x 150 mm with a particle size of 5 µm) HPLC system (Cecil Adept with Shimazu 10 Axl, UV detector (λ=338 nm) at 40 ^{o}C. A flow rate of 1 ml/min of mobile phase A (40mM NaH_2PO_4/Na_2HPO_4 (1:1) buffer pH=7.8) and mobile phase B (AcN/MeOH/H_2O (45/45/10 v/v/v). Tryptophan was determined, after alkaline hydrolysis at 120 °C for 12 h (Çevikkalp *et al.* 2016). The hydrolysates were filtered and the separation was performed using a mobile phase of acetonitrile and acetate buffer at pH 6.3 (1:9, v/v). The amino acids present were detected and quantified (Appendix 1) by matching their peaks with their standard retention time, and subsequently expressed as mg per g crude protein. The chemical scores of the essential amino acids (EAA) of each NUL seed was calculated, using (FAO/WHO 1990) reference amino acids as in Equation (2).

$$\text{EAA} = \frac{\text{mg of limiting EAA in NUL seed}}{\text{mg of limiting EAA in same standard/ reference protein}} \times 100 \qquad (2)$$

2.2.2 Statistical analysis

All amino acid analyses were done in triplicate. A one-way analysis of variance was done to determine if there were statistically significant differences among the mean mg/g amino acids of the samples of NULs under investigation. Statistical significance was set at $p \leq 0.05$. Fischer pairwise comparison test was also determined, using the Minitab software (Ryan *et al.* 1994). A one-sample t-test was run to test the means of the five NULs seeds against the mean thresholds of the dietary indispensable amino acids for infants, children and adults. The null hypothesis test was set to accept the mean (µ), if $\mu \geq$ hypothetical mean (reference thresholds), while the alternative hypothesis was set to reject the mean (µ), if $\mu \leq$ hypothetical mean (reference thresholds).

3. Results and discussion

3.1 Essential amino acid composition

The results (Table 1) show that, of the nine essential amino acids determined, only five showed adequate quantities when matched against dietary indispensable amino acids for infants (DIAA-I), children (DIAA-C) or older children/adolescent/adults (DIAA-A).

Table 1. Essential amino acid composition (mg/g, mean (± S.D.), n = 3) of five NULs samples matched with dietary indispensable and recommended amino acids(DIAA) for infants(I), children (C) and adults (A)

	Sources of protein					Reference amino acids		
EAA	*Cajanus sp.*	*Vigna sp.*	*Mucuna sp.*	*Canavalia sp.*	*Phaseolus sp.*	DIAA -I	DIAA -C	DIAA -A
His	8.9(±0.2)^{B}	26.2(±0.1)$^{A*^!}$	3.9(±0.2)^{D}	4.5(±0.3)^{C}	2.9(±0.2)^{E}	21.0	20.0	16.0
Ile	3.9(±0.1)^{C}	35.9(±0.3)$^{A^!}$	3.1(±0.3)^{D}	6.8(±0.2)^{B}	3.0(±0.1)^{D}	55.0	32.0	30.0
Leu	10.6(±1.3)^{C}	34.0(±0.1)^{A}	4.8(±0.1)^{D}	15.6(±3.5)^{B}	12(±1.2)^{C}	96.0	66.0	61.0
Lys	10.5(±0.3)^{C}	53.6(±3.3)$^{B!}$	3.7(±1.1)^{D}	13.3(±0.3)^{C}	63.1(±3.1)$^{A^!}$	69.0	57.0	48.0
Met + Cys	3.5(±0.2)^{C}	18.7(±0.5)^{A}	2.2(±0.3)^{CD}	11.3(±0.7)^{B}	2.6(±0.3)^{D}	33.0	27.0	23.0
Phe + Tyr	3.6(±0.2)^{D}	67.6(±5.5)$^{A^!}$	5.6(±0.3)^{D}	21.4(±3.3)^{B}	15.5(±1.4)^{C}	94.0	52.0	41.0
Thr	3.1(±0.3)^{C}	27.7(±0.3)$^{B!}$	2.1(±1.0)^{C}	2.1(±0.3)^{C}	56.4(±2.2)$^{A*^}$	44.0	31.0	25.0
Trp	1.1(±0.2)^{C}	2.6(±0.2)^{A}	1.1(±0.3)^{C}	1.2(±0.1)^{C}	1.8(±0.5)^{B}	17.0	8.5	6.6
Val	10.1(±2.3)^{B}	23.5(±0.3)^{A}	3.7(±0.2)^{C}	5.2(±0.3)^{C}	4.7(±0.3)^{C}	55.0	43.0	40.0

The EAA showing means with their standard deviations. Means with same alphanumeric along the row, show no significant differences (p>0.05). Notations: (*) significantly(p<0.05) ≥ DIAA-I (infant up to 6 months), (^) significantly (p<0.05) ≥ DIAA-C (children up to 3 years), (!) significantly (p<0.05) ≥ DIAA-A (older children, adolescents and adults)

By definition, essential amino acid (EAA) score is based on the comparison of the concentration of the first limiting essential amino acid in the test protein, to that amino acid in a reference or standard protein (Schaafsma 2000)**.** Based on this premise, the essential amino acid (EAA) scores showed that, all the NULs were limiting in tryptophan (Figure 1). Thus, depending on the reference amino acid used, EAA score increasingly improved as the DIAA threshold changed from infant through children to adult reference.

In all, the lowest EAA score, 6.5%, was found in *Cajanus sp.* and highest score of 39.4% was obtained in *Vigna sp.* when the adult and infant references were used respectively as the threshold. Similar chemical scores of between 28% and 36%, limited to the sulphur amino acids (methionine + cysteine) in *Phaseolus lunatus* proteins has been reported (Kathirvel & Kumudha 2011). However, the chemical score of protein extracts from *Vigna sp.* as 0.91%, limited to tryptophan has been reported (Yao *et al.* 2015). This lower level of chemical score of histidine compared to the levels obtained in this study for *Vigna sp* (15.3% (infants), 30.6% (children) or 39.4% (adult), makes the type of *Vigna sp.* in the study area better. The variations or uncertainties in the chemical scores or concentrations of amino acids obtained in this study relative to other studies might be attributed to factors such as genetic variations of legumes (Bressani & Elías 1980),edaphic factors (Norton *et al.* 1985) or even the procedure of the analysis of amino acids.

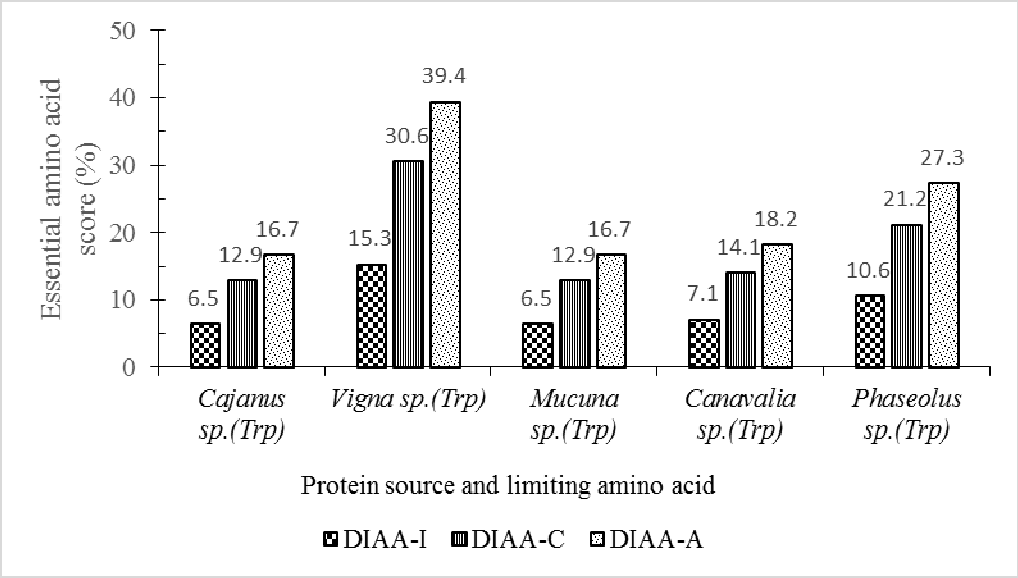

Figure 1. EAA scores of NULs based on their limiting amino acid (tryptophan), compared with same limiting amino acid in the three DIAA thresholds required by infants, children and adults

Histidine was found to be present in *Vigna sp.* at 26.2 mg/g (Table 1) which was significantly (p<0.05) higher compared to those found from the other NULs. However, the least amount of histidine was found in *Phaseolus sp.* (2.9 mg/g). The quantity of histidine determined in *Vigna sp.* was also significantly (p<0.05) greater or equal to the amounts required for all the thresholds; DIAA-I (21.mg/g), DIAA-C (20 mg/g) and

DIAA-A (16 mg/g), relative to the other NULs which were significantly lower (Table 1). Thus, levels of histidine determined in *Vigna sp*. would be adequate in supplying the amount required for infants, children and adults (FAO/WHO/UNU 2007). In contrast, the amount of histidine determined in the other NULs (Table 1) were significantly ($p>0.05$) lower, compared to the three reference thresholds. There are reports of values of between 29.9 mg/g and 44.4 mg/g protein of histidine in three accessions of *Mucuna pruriens var pruriens* (Fathima *et al.* 2010). There are other reports of histidine levels of 38.6 mg/g in *Vigna sp*. (Yao *et al.* 2015). It has also been reported (Ade-Omowaye *et al.* 2015) that histidine levels range between 5.95 mg/g and 8.92 mg/g, across legumes such as *Cassia sp., Canavalia sp., Vigna sp., Mallotus sp., Sphenostylis sp.* and *Cajanus sp*. This certainly show variations from what were obtained from this study.

The levels of isoleucine obtained in this study were significantly ($p<0.05$) different among the NULs (Table 1). The highest concentration of 35.9 mg/g, was determined in *Vigna sp*. and the lowest, 3.0 mg/g, was obtained in *Phaseolus sp*. The concentration was also significantly ($p<0.05$) equal or greater than both the adult (30 mg/g) and children thresholds (32 mg/g) but not the infants' threshold (55 mg/g). For studies involving *Canavalia sp.*, a concentration of 53 mg/g isoleucine has been reported (Sridhar & Seena 2006). Wide variations of isoleucine exist among NULs and this is shown in other reports of variable concentration of isoleucine. Values of isoleucine ranging between 59.4 mg/g and 69.4 mg/g among the assertions of *Mucuna pruriens var pruriens* have been reported (Fathima *et al.* 2010). There was also a report of 54.5 mg/g isoleucine in protein extracts from *Vigna sp.* (Yao *et al.* 2015). The results show that in this study (Table 1), leucine appears to be consistently lower than what was obtained in other studies. For instance, studies involving *Canavalia sp.* concluded on levels of leucine ranging between 100 mg/g and 120 mg/g (Sridhar & Seena 2006). Other studies have reported values of 102 mg/g in *Vigna sp*. (Yao *et al.* 2015) and levels of between 73.5 mg/g and 84.9 mg/g in *Canavalia sp.* and *Mucuna sp.* (Agbede & Aletor 2005). Therefore, the low levels of leucine obtained in this study puts the NULs in the study area as nutritionally less valuable. Interestingly, though leucine is an essential amino acid, its supplementation as a strategy to gain muscle mass may not be significant in post prandial muscle protein synthesis (van Vliet *et al.* 2015).

There were significantly ($p<0.05$) high levels of lysine in *Phaseolus sp.* (63.1 mg/g) and *Vigna sp.* (53.6 mg/g) relative to the other NULs studied (Table 1). The quantity of lysine obtained in *Phaseolus sp*. was significantly ($p<0.05$) equal to or greater than the threshold required for adults (48 mg/g) and children (57 mg/g), though the quantities were below what was required for infants (69 mg/g). Lysine levels in *Vigna sp.* had been reported as 80.2 mg/g (Yao *et al.* 2015) and the lysine content of *Canavalia gladiata* cotyledon and the whole seed flour also range between 53 mg/g and 58 mg/g (Ekanayake *et al.* 1999). However, a reported lysine levels of the accessions of *Canavalia sp.* ranging between 13 mg/g and 150 mg/g has been made (Sridhar & Seena 2006). Levels of lysine in *Phaseolus sp.* ranging between 74 mg/g to 75 mg/g in wild and cultivated seeds were also reported (Kathirvel & Kumudha 2011).

The total sulphur amino acids (methionine + cysteine) differed significantly ($p<0.05$) in the NULs studied (Table 1). Total sulphur amino acids ranged from a high of 18.7 mg/g in *Vigna sp.* to a low of 2.2 mg/g in *Mucuna sp*. These values are inadequate, compared to all the three thresholds of the sulphur amino acids required for proper sustenance of infants (33 mg/g), children (27 mg/g) and adults (23 mg/g) (FAO/WHO/UNU 2007). But the levels of the total sulphur amino acids are not surprising because low levels of methionine in plant-based proteins have been reported (Young & Pellet 1994). Wide-ranging values of between 56.8 mg/g and 12.4 mg/g for methionine, and also between 5.4 mg/g and 10.1 mg/g were reported for cysteine among the assertions of *Mucuna pruriens var pruriens* (Fathima *et al.* 2010). The peak value of 18.7 mg/g obtained in this study for *Vigna sp.* lies within the concentrations already reported (Fathima *et al.* 2010). However, it doesn't make them adequate to support sulphur amino acid levels required for infants, children and adults. Such low levels of amino acids must be complimented with cereals (Jukanti *et al.* 2012).

The aromatic amino acids were also significantly ($p<0.05$) higher in *Vigna sp.* (67.6 mg/g) relative to *Cajanus sp*. and *Mucuna sp*., which gave the least of 3.6 mg/g and 5.6 mg/g respectively. This makes the value obtained in *Vigna sp.,* significantly ($p<0.05$) greater than the aromatic amino acid threshold required for adults (41 mg/g) and children (52 mg/g) but not infants (94 mg/g). However, while one report (Adebowale *et al.* 2007) presented even higher quantities of the aromatic amino acids in *Mucuna sp.* as 84.4-121.3 mg/g, another report (Sridhar & Seena 2006) presented a rather wider range of values in *Canavalia sp*. of between 19 and 116 mg/g. Thus, by comparison, the concentrations of aromatic essential amino acids obtained in this study (67.6 mg/g) were low, though these levels can support adult and children's aromatic amino acid requirements.

Threonine level was as high as 56.4 mg/g in *Phaseolus sp.* and this is equal to or greater than both the threonine thresholds for infants (44 mg/g) and children (31 mg/g). Threonine concentration in *Vigna sp.* (27.7 mg/g) was significantly ($p<0.05$) lower than that determined in *Phaseolus sp.* (Table 1). Generally, levels of threonine obtained in this study were comparable to the levels (37 and 42 mg/g) obtained in *Canavalia gladiata* (Eknayake *et al.* 1999) and also in *Canavalia sp*. (10 and 50 mg/g) (Sridhar and Seena 2006). However, a higher level of 66.8 mg/g in *Mucuna sp*. has been reported (Adebowale *et al.* 2007).

The quantity of tryptophan in the five NULs were all below 3 mg/g (Table 1). The tryptophan contents of *Vigna sp.* (2.6 mg/g) and *Phaseolus sp*. (1.8 mg/g) on one hand, showed significant ($p<0.05$) differences. Similarly, the tryptophan content of the pair- *Vigna sp* and *Phaseolus sp.* showed significant differences ($p<0.05$) relative to the rest of the NULs (*Mucuna sp., Cajanus sp. and Canavalia sp*.). Generally, the tryptophan content of the NULs studied, were very limiting and the values recorded were significantly ($p<0.05$) very low compared to the recommended thresholds for infants (17 mg/g), children (8.5 mg/g) and adults (6.6 mg/g). It has however, been reported (Yao *et al.* 2015) levels of 6.0 mg/g in *Vigna sp.* which was comparable to the recommended threshold of 6.6 mg/g for adults but not for infants (17 mg/g) and children (8.5 mg/g). However, there are reports of (Adebowale *et al.* 2007; Monteiro *et al.* 2014) high levels of tryptophan (22.3 - 34.6 mg/g) in *Mucuna sp.* and in in *Lupinus sp.* (19.56 and 26.39 mg/g) respectively.

The highest quantity of valine was obtained in *Vigna sp.* (23.5 mg/g) and the lowest in *Mucuna sp.* (3.7 mg/g) (Table 1). Relative to the recommended thresholds for valine in the diets of infants (55 mg/g), children (43 mg/g) and adults (40 mg/g), the values obtained in this study was very limiting. Reports from other studies gave values of 8.7 mg/g in *Phaseolus vulgaris* (Nestares *et al.* 2001) and 14.46-18.27 mg/g in *Lupinus sp*. (Monteiro *et al.* 2014). This indicates that the values of valine obtained in this study (23.5 mg/g) were relatively better than in other studies. In spite of this, the highest level of valine (*Vigna sp.*, 23.5 mg/g) obtained in this study was still inadequate relative to the recommended thresholds for infants, children and adults. Indeed, compared to valine concentration of 62.4 mg/g in *Vigna sp.* (Yao *et al.* 2015), or a ranging valine value of between 54 mg/g and 58 mg/g in *Phaseolus sp.* (Kathirvel & Kumudha 2011), the levels of valine in the NULs obtained in this study are low.

4. Conclusion

Two of the NULs: *Vigna sp.* and *Phaseolus sp.* out of the five studied, showed a potential to supply essential amino acids required for infant, children and adult recommended amino acid thresholds. *Vigna sp.* showed adequate quantities of histidine for infants, children and adult requirements. However, *Vigna sp.* provided only adequate support for isoleucine and aromatic amino acids for children and adults. *Phaseolus sp.* on the otherhand could support lysine and threonine requirements for only adults. The remaining essential amino acids; leucine, methionine + cysteine, tryptophan and valine were not adequately supplied. It is possible that by compositing these two NULs, together with other sources of protein, a potential supplement capable of supplying the required essential amino acids for some consumers could be achieved.

Appendix 1: The limit of detection and quantification based on the reverse phase C18 silica column (4.6 x 150 mm with a particle size of 5 µm) HPLC system (Cecil Adept with Shimazu 10 Axl, UV detector (λ=338 nm) at 40 ^{o}C

Essential amino acids	Limit of detection(LOD) mg/g	Limit of quantification(LOQ) mg/g
His	0.1	0.3
Thr	0.1	0.4
Tyr	0.1	0.4
Cys	0.2	0.5
Val	0.1	0.3
Met	0.1	0.4
Trp	0.1	0.3
Phe	0.2	0.7
Ile	0.1	0.3
Leu	0.2	0.6
Lys	0.1	0.3

Reference

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