

EXAMINATION OF VITAMIN AND AMINO ACID PROFILES OF GMELINA (GMELINA ARBOREA) FRUIT AND FRUIT PULP

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ABSTRACT

The study examined the vitamin and amino acid profiles of Unripe Gmelina Whole Fruit (UGWF), Ripe Gmelina Whole Fruit (RGWF) and Ripe Gmelina Fruit Pulp (RGFP). The combined riboflavin, thiamin, niacin, ascorbic acid, vitamins A and E of all the samples ranged from 0.09-0.30 mg/100g, 0.22-0.88 mg/100g, 0.12-0.64 mg/100g, 5.75-22.88 mg/100g, 4.60-13.60 IU/100g and 0.0-6.09 mg/100g, respectively. They were poor in all the vitamins except the vitamin E content of RGWF. The total essential amino acid (TEAA), percentage total non essential amino acid (%TEAA), total semi essential amino acid (TSEAA), percentage total semi essential amino acid (%TSEAA), total non essential amino acid (TNEAA) and percentage total non essential amino acid(%TNEAA) ranged from 13.28-23.77 g/100g protein, 31.43-40.75%, 3.79-5.11 g/100g protein, 8.92-8.97%, 25.18-29.45 g/100g protein and 50.49-59.60%, respectively. The ingredients were high in %TEAA, valine (1.63-3.66 g/100g protein), isoleucine (1.90-3.76 g/100g protein) but low in TEAA, TSEAA, %TSEAA, TNEAA and %TNEAA, phenylalanine (1.61-2.70 g/100g protein), lysine(2.09-2.63 g/100g protein), leucine (2.03-4.72 g/100g protein), threonine (1.72-2.50 g/100g protein), methionine (0.36-0.70 g/100g protein), cystine (0.66-0.80 g/100g protein), tyrosine (1.28-2.30 g/100g protein), histidine (1.07-1.61 g/100g protein), arginine (2.72-3.50 g/100g protein), aspartic acid (3.65-7.80 g/100g protein), serine (2.05-3.10 g/100g protein), glutamic acid (10.16-13.63 g/100g protein), proline (1.91-2.34 g/100g protein), glycine (2.14-3.25 g/100g protein) and alanine (1.80-2.80 g/100g protein). RGFP had the highest ($p < 0.05$) value for all the amino acids except glutamic acid. The use of gmelina fruit in monogastric feeding may require supplementation with other sources rich in the deficient vitamins and essential amino acids.

Key words: Amino acid, vitamin, gmelina, fruit, pulp, livestock, feed.

INTRODUCTION

Agriculture is a major contributor of greenhouse gases which exacerbate climate change and climate change critically affects agriculture and the environment (Spore, 2008). One source of

green house gases from agriculture is decomposing agricultural wastes (Anonymous, 2001). Suggestions have been made on how to eliminate the wastes and arrest environmental pollution. Among the proffered solutions, recycling of agricultural wastes seems the best option (Sheehan, 2000). During the fruiting season, tons of gmelina fruits litter villages and towns in Nigeria (Onyekwelu *et al.*, 2009). The littering and non-utilization constitute environmental pollution. This study examined the vitamin and amino acid profile of gmelina fruit in order to understand the possibility of its usage in livestock feed. This will enable the use of gmelina fruit regarded as a waste, to solve the problems of animal feed scarcity, high cost of feed and environmental pollution. The proximate, mineral and anti-nutritional composition of gmelina fruit or its pulp have been examined (Ingweye and Okon, 2012) but an assessment of the vitamin and amino acid composition of gmelina fruit is yet to be carried out. Hence, the study was aimed at fulfilling this need, thus enriching the information on the use of gmelina fruit in livestock feeding to mitigate climate change and other environmental consequences from the accumulating of fruits.

MATERIALS AND METHODS

Source and preparation of samples

The gmelina fruits were obtained from the gmelina plantation in Akamkpa, near Calabar, Nigeria. The ripe fruits were gathered from the floor of the plantation, while the unripe green fruits were harvested from the trees. Contaminants like leaves and rotten fruits were removed. The ripe fruits were pulped by removing the woody seed. The fruit, devoid of the seed constituted the pulp. Fresh fruits (ripe and unripe) and the ripe fruit pulp was oven dried at 55°C in a paper envelope for 24 hours to a constant weight for the calculation of percentage dry matter (Abuye *et al.*, 2003). The dry materials were milled to powder using a laboratory grinder model no. 01-HDDM and sieved using a 1.0 mm mesh sieve for the analyses. Each analysis was done in triplicates and the mean taken as the reading.

Vitamin analyses

Vitamin C was determined according to the method of Adebooye (2008). Ascorbic acid was measured by titration with phenolindo-2, 6-dichlorophenol (DPIP). The powder (0.2 g) was mixed with 4 ml/L of a buffer solution made up of 1.0 g/L oxalic acid and 4.0 g/L sodium acetate anhydrous. This was titrated against a solution containing 295 mg/L DPIP and 100 mg/L sodium bicarbonate. The results were expressed as mg/100g DM. Vitamin A was determined using the method described by Zhang and Hamazu (2004) where 15g of powdered sample was homogenized with 10ml acetone at -20 °C. The homogenate was filtered through four layers of cheesecloth. The residue was treated with acetone at -20 °C for three successive extractions until the green colour could no longer be visually detected in the extract and residue. The filtrate was combined and centrifuged at 4000 rpm for 10 min. The supernatant was collected and filtered

through a 0.45 µm Advantec filter pore for High Performance Liquid Chromatography (HPLC) analysis. Samples were separated on a Luna 5µ C18 column at 40 °C by an HPLC. The mobile phase consisted of a combination of acetonitrile with water and ethyl acetate in a ratio of 9:1:90. The flow rate was 1.0 ml/min. Samples were detected at 450 nm. The carotenoids were expressed in IU. Vitamins B₁, B₂, B₃ and E were determined spectrophotometrically as described by AOAC (1999).

Amino acid analyses

The amino acid profile of the sample was determined using methods described by Shahidi *et al.* (1999) and Adeyeye and Afolabi (2004). The sample was dried to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator and loaded into the Technicon Sequential Multi-sample Amino Acid Analyzer (TSM) using ion-exchange chromatography (Technicon Instruments Corporation, Dublin, Ireland).

RESULTS AND DISCUSSION

Vitamins composition

The result of vitamin composition is presented in Table 1.

Table 1: Vitamin composition of Unripe Gmelina Whole Fruit (UGWF), Ripe Gmelina Whole Fruit (RGWF) and Ripe Gmelina Fruit Pulp (RGFP)

Vitamin (mg/100g)*	Sample type			
	UGWF	RGWF	RGFP	±SEM
Riboflavin	0.30 ^a	0.25 ^b	0.09 ^c	0.06
Thiamin	0.64 ^b	0.88 ^a	0.22 ^c	0.19
Niacin	0.64 ^a	0.22 ^b	0.12 ^c	0.16
Ascorbic acid	22.88 ^a	5.72 ^c	14.52 ^b	4.96
Vitamin A (IU/100g)	ND**	4.60 ^b	13.60 ^a	4.51
Vitamin E	ND**	ND**	6.09	0.00

*Values are means of three replicates; **Not detected; ^{NS} Not significantly different (p>0.05); ^{a-c} means in the same row with different superscripts are significantly different (p<0.05)

The riboflavin content ranged from 0.09 (RGFP) to 0.30 (UGWF) mg/100g. There was a difference (p<0.05) among the values. The higher riboflavin content of UGWF (which was green) could be attributed to its unripe nature. According to McDonald *et al.* (1995), green parts of plants are rich in riboflavin. Compared to other sources like *Amaranthus hybridus* (Akubugwo *et al.*, 2007), banana and apple (Chatterjea and Shinde, 2007), the riboflavin contents of UGWF, RGWF and RGFP were poor.

The thiamin content ranged from 0.22 (RGFP) to 0.88 (RGWF) mg/100g. There was a difference ($p < 0.05$) among the values. Compared to the content of thiamin in apple and banana (Chatterjea and Shinde, 2007), the observed values were low. This agrees with reports by Banerjee (2004) that though widely distributed in feedstuffs, thiamin is rich in only a few. Mammals cannot synthesize thiamin and carbohydrates are deficient in it; hence, the use of gmelina fruit or its pulp in diets for monogastrics as the main energy source would need supplementation with other rich sources of thiamin like brewers' dried yeast and aleurone layer of cereals.

The niacin content of the feedstuffs ranged from 0.12 (RGFP) to 0.64 (UGWF) mg/100g. Niacin contents among the samples differ ($p < 0.05$). Compared to the values reported for *A. hybridus* (Akubugwo et al., 2007), the observed values were low thus agreeing with Chatterjea and Shinde (2007) that fruits are poor in niacin. High levels of cereal grains in diets hike the requirement for niacin. Therefore, supplementation with tryptophan and niacin rich sources like rice polishing, peanut meal and legumes will alleviate the effects of niacin deficiency which include pellagra and skin problems (Vasudevan and Sreekumari, 2007).

The ascorbic acid content ranged from 5.72-22.88 mg/100 g. RGWF had the least ($p < 0.05$) content while UGWF recorded the highest ($P < 0.05$). This range indicated poor vitamin C content in the samples compared to values observed for guava, Indian gooseberry, snake tomato, *Carica papaya* (Vasudevan and Sreekumari, 2007; Adebooye, 2008; Unnithan, 2008). Fresh fruits, leafy vegetables, green chilli, tomatoes and citrus are rich in vitamin C. This could be the reason why vitamin C content was higher ($p < 0.05$) in UGWF than others. Vitamin C is essential as an antioxidant. Its deficiency in gmelina fruit based diets could be alleviated by supplementation with other sources rich in it. However, some animals may convert glucose to vitamin C.

The vitamin A contents of RGWF and RGFP were 4.60 IU/100g and 13.60 IU/100g, respectively. It was below detectable limit in UGWF. The vitamin A content of RGFP was higher ($P < 0.05$) than that of RGWF. This could be explained by the higher concentration in the pulp after removal of the seed and the presence of carotenoids (precursors of vitamin A) shown by yellow colour of fruit (Banerjee, 2004).

The vitamin E content of RGFP was 6.09 mg/100g. Vitamin E was not detected in UGWF and RGWF. Though widely distributed in plants, vitamin E is concentrated in the germ layer of cereals and green herbage. Compared to values reported for *A. hybridus* and brown rice, the values for RGFP in this study were high.

Essential amino acids composition

The results of essential amino acid composition are presented in Table 2.

Table 2: Essential Amino Acid Composition of Unripe Gmelina Whole Fruit (UGWF), Ripe Gmelina Whole Fruit (RGWF) and Ripe Gmelina Fruit Pulp (RGFP)

Amino acid	Concentration (g/100g protein)*			±SEM
	UGWF	RGWF	RGFP	
Phenylalanine	2.11 ^b	1.61 ^c	2.70 ^a	0.32
Lysine	2.34 ^b	2.09 ^c	2.63 ^a	0.16
Valine	3.14 ^b	1.63 ^c	3.66 ^a	0.61
Leucine	2.80 ^b	2.03 ^c	4.72 ^a	0.80
Isoleucine	3.14 ^b	1.90 ^c	3.76 ^a	0.55
Threonine	2.05 ^b	1.72 ^c	2.50 ^a	0.23
Methionine	0.42 ^b	0.36 ^c	0.70 ^a	0.10
Cystine	0.66 ^b	0.66 ^c	0.80 ^a	0.05
Tyrosine	1.80 ^b	1.28 ^c	2.30 ^a	0.29
TEAA	18.46 ^{ab}	13.28 ^c	23.77 ^a	-
%TEAA	37.77 ^b	31.43 ^c	40.75 ^a	-

*Values are means of three replicates; ^{a-c} means in the same row with different superscripts are significantly different ($p < 0.05$); UGWF: Unripe gmelina whole fruit; RGWF: Ripe gmelina whole fruit; RGFP: Ripe gmelina fruit pulp.

The phenylalanine (Phe) content ranged from 1.61 to 2.70 g/100g protein. RGFP had the highest ($p < 0.05$) value while RGWF had the least ($p < 0.05$). Compared to the standard reference value (6.3mg/100g) FAO/WHO (1990) and the Phe content of *Cassia floribunda* (Vadivel and Janardhanan, 2001) the values were low. Therefore, feeding animals especially monogastrics with gmelina fruit based diets may need supplementation with other sources rich in Phe.

The lysine content ranged from 2.09 (RGFP) to 2.63 (RGWF) g/100g protein. The values differ ($p < 0.05$) from each other. Compared to the standard reference value (FAO/WHO, 1990) and the value reported for Desi chick pea (Zia-Ul-Haq et al., 2007) gmelina fruits and fruit pulp were poor in lysine thus, it is necessary to supplement with synthetic lysine when feeding them to monogastrics.

The valine, leucine, isoleucine, threonine, methionine, cystine and tyrosine contents in g/100g protein ranged from 3.66-1.63, 4.72-2.03, 3.76-1.90, 2.50-1.72, 0.70-0.36, 0.80-0.66 and 2.30-1.28, respectively. Also, the total essential amino acid (TEAA) and percentage total essential amino acid (%TEAA) values ranged from 23.77-13.28 and 40.75-31.43, respectively.

RGFP had the highest ($p < 0.05$) valine content while RGWF had the least ($P < 0.05$). Compared to the FAO/WHO (1991) reference values, the valine content of RGFP was high while that of UGWF

and RGWF were low. The higher ($p < 0.05$) content of valine in the pulp indicates that it is potentially a good source of protein for ruminants because the deamination of valine in the rumen produces isobutyric acid which is utilized by rumen microbes to produce microbial protein (Banerjee, 2004).

Ripe gmelina fruit pulp had the largest ($p < 0.05$) leucine value while RGWF had the least ($p < 0.05$). All the samples were poor in leucine compared the FAO/WHO (1991) reference value, hence the need for supplementation.

The isoleucine content of RGFP was the highest ($p < 0.05$) while that of RGWF was the least ($p < 0.05$). All the values of the three samples except RGWF were higher than the FAO/WHO (1991) reference values, thus indicating that RGFP and UGWF were rich in isoleucine, hence, could be recommended as alternative isoleucine source.

RGFP and RGWF recorded the highest ($p < 0.05$) and lowest ($p < 0.05$) threonine values, respectively. The three samples were low in threonine compared to values reported for coffee fruit pulp and reference values (Bressani, 2009; FAO/WHO, 1991). This implies that supplementation with threonine rich sources in livestock diets is recommended in order to obviate the suppression of phosphor-protein formation which is the consequence of threonine deficiency (Murray *et al.*, 2006). The methionine content was highest ($p < 0.05$) for RGFP and lowest ($p < 0.05$) for RGWF. The range of methionine was within that reported for coffee fruit pulp (Bressani, 2009) but lower than standard reference values (FAO/WHO, 1991). Considering the role of methionine in carbohydrate metabolism in animals (McDonald *et al.*, 1995), low methionine value and its deficiency in gmelina fruits based diets may require supplementation with synthetic sources so as to achieve maximum productivity.

The cystine content of RGFP was the highest ($p < 0.05$) while that of the RGWF was the least ($P < 0.05$) though not different ($p > 0.05$) from the value recorded for UGWF. Cysteine (a combination of two molecules of cystine) functions in amino acid transport, maintenance of red blood cells integrity, oxygen transport and detoxification of several compounds (Vasudevan and Sreekumari, 2007; Chatterjea and Shinde, 2007). Compared to the values reported for coffee fruit pulp and reference values (Bressani, 2009; FAO/WHO, 1991), the cystine content was low. In order to improve the cystine content of gmelina fruit pulp rich diet, there may be need for supplementation with other sources.

With respect to tyrosine, RGFP had the highest ($p < 0.05$) value while that of RGWF was the least ($p < 0.05$). All the values except that of RGFP were below the range reported for *C. floribunda* seeds and coffee fruit pulp (Vadivel and Janardhanan, 2001; Bressani, 2009). Tyrosine is essential for the formation of the thyroid hormone thyroxin, skin pigment melanin and also plays a role in neurotransmission. Deficiency of tyrosine could be potentially observed in a gmelina fruit based diet and can be avoided if the diet is rich in phenylalanine but gmelina fruit is also deficient

in phenylalanine. Therefore, supplementation with other rich sources is necessary in livestock diets. As regards the total essential amino acid (TEAA) content, RGFP had the highest ($p < 0.05$) value, though not different ($p > 0.05$) from that of UGWF. RGWF recorded the least ($p < 0.05$) value. This could be a reflection of the amino acid values of the individual amino acids. The percentage total essential amino acid value was highest ($p < 0.05$) for RGFP and lowest ($p < 0.05$) for the RGWF. Compared to the FAO/WHO (1991) reference value, the observed range was high. However, because of the low level of sulphur containing amino acids in the fruit and fruit pulp, there may be need for supplementation especially in monogastric diets.

Semi-essential and non-essential amino acid composition

Table 3 presents the results of semi essential and non essential amino acid composition.

Table 3: Semi-essential and Non-essential Amino Acid Composition of Unripe Gmelina Whole Fruit (UGWF), Ripe Gmelina Whole Fruit (RGWF) and Ripe Gmelina Fruit Pulp (RGFP)

Amino acid	Concentration (g/100g protein)*			±SEM
	UGWF	RGWF	RGFP	
Histidine	1.30 ^b	1.07 ^c	1.61 ^a	0.16
Arginine	3.06 ^b	2.72 ^c	3.50 ^a	0.22
TSEAA	4.36	3.79	5.11	-
%TSEAA	8.92	8.97	8.76	-
Aspartic acid	5.11 ^b	3.65 ^c	7.80 ^a	1.22
Serine	2.80 ^b	2.05 ^c	3.10 ^a	0.31
Glutamic acid	11.20 ^b	13.63 ^a	10.16 ^c	1.03
Proline	2.12 ^b	1.91 ^c	2.34 ^a	0.12
Glycine	2.50 ^b	2.14 ^c	3.25 ^a	0.33
Alanine	2.32 ^b	1.80 ^c	2.80 ^a	0.29
TNEAA	26.05 ^b	25.18 ^c	29.45 ^a	-
%TNEAA	53.30 ^b	59.60 ^a	50.49 ^c	-

*Values are means of three replicates; ^{a-c} means in the same row with different superscripts are significantly different ($p < 0.05$); UGWF: Unripe gmelina whole fruit; RGWF: Ripe gmelina whole fruit; RGFP: Ripe gmelina fruit pulp.

The histidine, arginine, total semi essential amino acid (TSEAA) and percentage total semi essential amino acids (%TSEAA) contents ranged from 1.61(RGFP)-1.07(RGWF) g/100g protein, 3.50(RGFP)-2.72(RGWF) g/100g protein, 5.11(RGFP)-3.79(RGWF) g/100g protein and 8.97(RGWF)-8.92(RGFP)%, respectively. There were significant differences ($p < 0.05$) among the values except TSEAA and %TSEAA. Compared to values for coffee fruit pulp and Desi chickpea (Bressani, 2009; Zia-UI-Haq et al., 2007) the observed values were low. Since histidine is a

dietary essential for young growing animals (Chatterjea and Shinde, 2007) feeding gmelina fruit pulp based diets to young animals may require supplementation with other dietary sources.

The aspartic acid, serine, glutamic acid, proline, glycine, alanine, total non essential amino acid (TNEAA) and percentage total non essential amino acid (%TNEAA) profile in g/100g ranged from 7.80(RGFP)-3.65(RGWF), 3.10(RGFP)-2.05(RGWF), 13.63(RGWF)-10.16(RGFP), 2.34(RGFP)-1.91(RGWF), 3.25(RGFP)-2.14(RGWF), 2.80(RGFP)-1.80(RGWF), 29.45(RGFP)-25.18(RGWF) and 59.60(RGWF)-50.49(RGFP), respectively. All the non essential amino acids values showed significant ($p < 0.05$) differences among the treatment groups with RGFP having the highest ($P < 0.05$) values while RGWF had the least ($p < 0.05$) except glutamic acid and %TNEAA where the reverse order was the case. Compared to reported values for Desi chickpeas (Zia-Ul-Haq et al., 2007), all the three feed ingredients were poor in the non essential amino acids. Their high concentration in pulp could be due to the role of most of the amino acids (e.g. proline and glycine) in fibrous proteins and collagen that are abundant in fruits (McDonald et al., 1995; Champe et al., 2008).

CONCLUSION

The study examined the vitamin and amino acid composition of UGWF, RGWF and RGFP as potential livestock feed ingredients to mitigate climate change. The results indicate that fruit samples were high in %TEAA, valine, isoleucine and vitamin E content of RGFP but poor in riboflavin, niacin, ascorbic acid, vitamin A, TEAA, TSEAA, %TSEAA, TNEAA, %TNEAA, phenylalanine, lysine, leucine, threonine, methionine, cystine, tyrosine, histidine, arginine, aspartic acid, serine, glutamic acid, proline, glycine and alanine. Except the values for glutamic acid, RGFP had the highest ($p < 0.05$) value for all the amino acids. The use of gmelina fruit in livestock feeding as climate change mitigation tool may require supplementation with other sources rich in the deficient essential amino acids.

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