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Total amino acid profiles of heat-processed fresh *Elaeis guineensis* and *Raphia hookeri* wines

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ABSTRACT

Total amino acid (AA) profiles of heat-processed fresh *Elaeis guineensis* and *Raphia hookeri* wines were studied. Heating their fresh wines to 85 °C, cooling and diluting to original volumes distilled off ethanol, but did not change their moisture and nitrogen contents. *R. hookeri* wine contained more ($p < 0.05$) Phe, Val, Ala, Gly, Pro, Asp, Asn, His and Lys than *E. guineensis* wine which contained more ($p < 0.05$) Met, Cys, Glu, Gln, Ser and Arg. Tyrosine, Leu, Ile and Thr contents did not vary ($p > 0.05$). Glycine and Pro contents were low suggesting high globular protein concentrations. $\sum$basic AA/$\sum$acidic AA ratios were >1 suggesting high basic protein contents. The *E. guineensis* and *R. hookeri* wines contained 58.25 ± 0.56% and 56.79 ± 0.4% essential AAs, respectively. Essential AA scores suggested Leu as their limiting AA. In conclusion, the wines can adequately meet daily nitrogen and essential AA needs when a 70 kg adult drinks 1425.45 ml.

1. Introduction

Amino Acids (AAs) are the building blocks of proteins. The twenty AAs that make up proteins are generally classified as essential or indispensible AAs and non-essential or dispensable AAs; meaning that they are provided in diets and synthesised by the body, respectively (Cooper, 2000; Nelson & Cox, 2000). Non-Essential AAs (NEAAs) like Cys and Tyr have also been classified as semi-essential, which means that they must be synthesised from Essential AAs (EAAs) if insufficient amounts are eaten. Glutamine can also become essential at times of injury and surgery (Wardlaw & Kessel, 2002). Glutamine is an important fuel for enterocytes, lymphocytes and macrophages which divide rapidly and also require it for the synthesis of purines and pyrimidines (Harris & Crabb, 2006). Although Arg is classified as a NEAA, growing children must obtain additional Arg from their diets (Cooper, 2000). The content and balance of amino acids, particularly the ratio of EAA to NEAA, is what determines the body and health building value of a food. In addition to being influenced by the carbohydrates, fats and total calories associated with it, protein quality is related to the amount of specific AAs within both the EAA and NEAA categories. While the amounts of EAAs are generally of greater importance, the NEAAs are also significant because they may be synthesised too slowly to support maximum growth (Finnin & Peters, 1996). For example, the amount of Gln and Branched Chain AAs (BCAAs) like Leu, Ile and Val (Finnin & Peters, 1996). The BCAAs have been reported to act as ergogenic aids and are metabolised in the muscles, kidney, adipose and brain tissues instead of the liver (Nelson & Cox, 2000; Wardlaw & Kessel, 2002) because these extrahepatic tissues contain an aminotransferase that is absent in the liver (Nelson & Cox, 2008). They are added to meal replacement supplements given to hospitalised patients as well as fluid replacement formulae marketed to athletes (Wardlaw & Kessel, 2002).

Amino acids are also classified as glucogenic and ketogenic. The glucogenic AAs give rise to intermediates like oxaloacetate, pyruvate and α-ketoglutarate that can be used to synthesise glucose via gluconeogenesis while the ketogenic AAs give rise to acetyl-CoA and are used for the synthesis of fatty acids and ketone bodies (Nelson & Cox, 2008). While some of the AAs are strictly glucogenic, some are both glucogenic and ketogenic and the rest are strictly ketogenic (Nelson & Cox, 2000; Wardlaw & Kessel, 2002).

The palm wines made by fermenting saps of tropical palm tree (*Elaeis guineensis* Jacq.) and *Raphia hookeri* G. Mann and H. Wendl tree, are mainly used as social and ergogenic drinks. The wine made from the sap from *E. guineensis* is called *Nkwu* in Igbo while that from *R. hookeri* is called *Ngwo* in Igbo. Omigbodun and Babalola (2004) reported that palm sap is given widely to children possibly as a result of its nutritional values and the general belief about the absence or negligible alcohol content. It is a good source of vitamins B_1 (thiamin) and C (ascorbic acid). It also offers supplemental nutrition to a meal. The drink is a rich nutrient medium containing sugars, proteins, amino acids, alcohols and minerals (Ukhun, Okolie, & Oyerinde, 2005). Palm wine yeast, *Saccharomyces*

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cerevisiae, is able to concentrate large quantities of thiamin, nicotinic acid and biotin and thus form enriched products. West African palm wine is rich in vitamins B_{12}, which is very important for people with low meat intake and those who subsist primarily on vegetarian diets (Ezeagu & Fafunso, 2003; FAO, 1998).

Low-income households form the bulk of the population in developing countries and have limited access to high protein or protein-rich foods (Igwe, Ojiako, Anugweje, Nwaogu, & Ujowundu, 2012; Oshodi, Olaofe, & Hall, 1992). The use of *E. guineensis* and *R. hookeri* wines as alternate sources of quality protein to combat protein malnutrition has not been exploited. One major problem faced by the consuming public is preservation, which limits their distributions. Ibegbulem (2012) however reported that heat-treatment is a preservation technique for *Ngwo*. The study examines the total AA profiles of heat-processed fresh *E. guineensis* and *R. hookeri* wines.

2. Materials and methods

2.1. Materials

2.1.1. Procurement of samples

The fresh palm wine samples used were tapped overnight from *E. guineensis* Jacq. and *R. hookeri* G. Mann and H. Wendl trees (10 of each) by palm wine tappers at Orodo, Mbaitoli Local Government Area of Imo State, Nigeria.

2.2. Methods

2.2.1. Processing of samples

A quantity (100 ml) of the 10 wine samples apiece was heated to 85 °C to distill off ethanol (boiling point, 78 °C) and halt fermentation, then cooled, made up to their initial volume (100 ml) with distilled water and stored in a refrigerator at 4 °C until required for analyses.

2.2.2. Determination of ethanol, moisture and nitrogen contents of samples

The ethanol contents of the pre- and post-heated wines were estimated using the methods of Haddad, Sterns, and Warclaw (1978). Their moisture and nitrogen contents were determined using the methods of AOAC (1990).

2.2.3. Determination of total amino acid contents of samples

The total AA compositions of the processed wines (which included the free and protein-based AAs) were quantified using the ion-exchange chromatography-based Technicon Sequential Multi-sample (TSM) AA analyser (Technicon Instruments Corporation, New York) method described by Spackman, Stein, and Moore (1958). Exactly 300 ml portions of each wine were first defatted thrice with equal volumes of chloroform/methanol mixture (2:1). The layers were allowed to separate and the chloroform (lower) layer bearing the fat discarded. The moisture in each defatted wine was then evaporated using a rotary evaporator at 50 °C. This was repeated several times to achieve a reasonable quantity of the defatted wine sample. Known weight (500 mg) of the wine's dry matter was put into a glass ampoule. Hydrolysis was achieved by pipetting 7 ml of 6 N HCl into the ampoule and oxygen expelled by passing nitrogen into the ampoule. This was to avoid possible oxidation of some AAs during hydrolysis. The glass ampoule was then sealed with a Bunsen burner flame and put in an oven, preset at 105 ± 5 °C, for 22 h. The glass ampoule was allowed to cool, broken open at the tip and the content filtered to remove humus. Each filtrate was then evaporated to dryness at 40 °C *in vacuo* in a rotary evaporator. The residue was dissolved in 5 ml acetate buffer (pH 2.0) and stored at −4 °C in a plastic specimen bottle, until required for analysis. Known volume (10 μL) of each sample was dispensed into the cartridge of the TSM AA analyser and analysed. The amounts of the various AAs were calculated using the chromatogram generated as described by Spackman et al. (1958) and expressed as g/100 g protein.

2.2.4. Determination of Glu from Glx and Asp from Asx contents

The ratio of 6.3/4.2 for Glu and Gln, respectively, and 5.3/4.3 for Asp and Asn, respectively, for the average percentage occurrences of these AAs were used to resolve their contents from Glx and Asx contents. The values of 6.3 for Glu, 4.2 for Gln, 5.3 for Asp and 4.3 for Asn are their average percentage occurrences in 1150 proteins of known amino acid sequences as reported by Nelson and Cox (2008). The amide side-chain in Asn and Gln are often hydrolysed in the chemical procedure used for the determination of the AAs of proteins and their Asp and Glu residues add to the Asp and Glu contents (Nelson & Cox, 2000; Schultz, 2011).

2.2.5. Estimation of total amino acid profile

The total amino acid profiles of the processed wines (which included the free and protein-based AAs) were estimated by calculating the Total Essential AA (TEAA), excluding Trp content; by summing the Arg, His, Ile, Leu, Lys, Met, Phe, Thr and Val contents; Total Non-Essential AA (TNEAA) by summing the Ala, Asp, Asn, Cys, Glu, Gln, Gly, Pro, Ser and Tyr contents; Total Neutral AA (TNAA) by summing all the AAs excluding the TAAA, TBAA and Trp; Total Acidic AA (TAAA) by summing the Asp and Glu contents; Total Basic AA (TBAA) by summing the Arg, Lys and His contents; Total Sulphur-containing AA (TSAA) by summing up the Met and Cys contents; Total Branched-Chain AA (TBCAA) by summing the Leu, Ile and Val contents; Total Aromatic AA (TArAA), excluding Trp content, by summing the Phe and Tyr contents; percentage occurrences of these groupings and some AAs, ratios of some AAs and these groupings.

2.2.6. Estimation of total essential amino acid scores

The total EAA scores of the processed wines were calculated as the ratio of the actual amount (mg) of each EAA per g of the protein to the required amount (mg) of that EAA per g of a reference protein as described by FAO/WHO (1973) and Wardlaw and Kessel (2002) using the FAO/WHO/UNU (1981) provisional scoring pattern.

2.2.7. Statistical analysis

Data were analysed using the Student's *t*-distribution test of significance. Values were declared significant at $p < 0.05$.

3. Results and discussion

Heat-processing of the wines distilled off their ethanol contents (Table 1). Such heat-processing method eliminated ethanol from *R.*

Table 1
Ethanol, moisture and nitrogen contents (%) of pre- and post heat-processed wines.*

Parameter	Condition	Sample	
		R. hookeri	*E. guineensis*
Ethanol content	Before heating	1.11 ± 0.07[a]	1.15 ± 0.11[a]
	After heating	0.00 ± 0.00[b]	0.00 ± 0.00[b]
Moisture content‡		95.05 ± 0.01[a]	95.07 ± 0.03[a]
Nitrogen content‡		0.44 ± 0.04[a]	0.44 ± 0.03[a]

Values on the same row with the same superscript letter are not significantly different ($p > 0.05$).
* Values are mean ± SD of 10 determinations.
‡ Same for before and after heating.