

Evaluation of Essential Amino Acids in Unclarified and Limestone-Clarified Cashew Apple Juices and Their Concentrates from Kogi East, Nigeria

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ABSTRACT: Cashew apple is an underutilized pseudofruit with appreciable nutritional value, but its rapid post-harvest deterioration and characteristic astringency limit direct consumption and wider industrial utilization. This study evaluated the essential amino acid composition of unclarified and limestone-clarified red, yellow and mixed red-yellow cashew apple juices and their corresponding concentrates produced from fruits obtained in Anyigba and environs, Kogi East, Nigeria. Twelve samples were examined in triplicate: RT, CR, D and A for the red group; YT, CY, E and B for the yellow group; and MT, CM, F and C for the mixed group. Juice was extracted by blending and filtration, clarified with limestone (CaCO_3) using an equal juice-volume-to-limestone-weight ratio for 1 h 30 min, and concentrated at 50 °C for 5 h. Amino acids were determined after 6 N HCl hydrolysis using HPLC with post-column derivatization and spectrometric detection at 570 nm. Nine essential amino acids were identified in all samples: isoleucine, valine, histidine, threonine, phenylalanine, lysine, leucine, methionine and tryptophan. Tryptophan was the most abundant essential amino acid across the products, ranging from 18.00 ± 0.00 mg/kg in clarified red juice to 44.20 ± 0.00 mg/kg in both unclarified yellow and mixed concentrates. Among the juices, clarified mixed juice contained the highest isoleucine (5.27 ± 0.00 mg/kg) and valine (5.45 ± 0.00 mg/kg), while yellow unclarified juice contained the highest phenylalanine (4.40 ± 0.00 mg/kg), leucine (6.30 ± 0.00 mg/kg) and methionine (4.50 ± 0.00 mg/kg). Concentration produced the most pronounced enrichment in tryptophan, while the effects of limestone clarification on individual essential amino acids varied with varietal group. The findings show that cashew apple juice and concentrate retain a broad essential amino acid spectrum and support their further utilization as nutrient-containing food ingredients.

KEYWORDS: essential amino acids; cashew apple juice; limestone clarification; concentrate; tryptophan; *Anacardium occidentale*; Kogi East

1. INTRODUCTION

Cashew (*Anacardium occidentale* L.) is widely cultivated in tropical regions for its nut, while the fleshy cashew apple that constitutes the larger proportion of the fruit is frequently underutilized. The apple is highly juicy and contains sugars, vitamins, minerals, phytochemicals and amino acids, but its rapid perishability and astringent taste contribute to substantial post-harvest losses in many producing regions (Nwosu et al., 2016; Das, 2017; Akyereko et al., 2023). In Kogi East, where cashew cultivation is economically important, processing the apple into clarified juice and concentrate offers a practical route for reducing waste and broadening its food applications.

Amino acids are central to protein synthesis, nitrogen metabolism and numerous physiological processes. Nine amino acids are generally regarded as essential in human nutrition because they cannot be synthesized in quantities sufficient to meet physiological needs and must therefore be obtained from the diet: histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (FAO/WHO/UNU, 2007; Trumbo et al., 2002). Their nutritional relevance extends beyond protein synthesis. Branched-chain amino acids such as leucine, isoleucine and valine participate in muscle and energy metabolism; lysine contributes to protein synthesis and tissue development; methionine serves as a sulfur-containing metabolic precursor; phenylalanine participates in aromatic amino acid metabolism; histidine supports protein and haem-related functions; threonine contributes to structural proteins; and tryptophan is a precursor in serotonin and related metabolic pathways (Dietzen, 2018).

Cashew apple has previously been reported to contain several free amino acids. Oliveira et al. (2002) demonstrated that the free amino acid composition of cashew apple juice varies during the harvest season, while Sivagurunathan et al. (2010) also documented amino acids as part of the nutritional profile of cashew apples. Studies on cashew products have further shown that amino acid

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composition can vary with sample matrix, cultivar, origin and processing conditions (Fagbemi, 2009; Adou et al., 2012). These observations make varietal and processing comparisons important when cashew apple juice is being considered for food-product development.

Clarification and concentration can modify the measurable nutrient profile of fruit juices. In cashew apple juice, clarification is particularly important because tannins contribute to astringency and may interact with proteins and other nitrogenous components. Limestone (CaCO_3) provides a locally accessible clarification option, while subsequent low-temperature concentration reduces water content and increases the density of soluble components. The combined effects of these operations on the essential amino acid profile of red, yellow and mixed cashew apple juices from Kogi East have received little attention.

This study therefore evaluated the essential amino acids in unclarified and limestone-clarified cashew apple juices and their concentrates from Kogi East, Nigeria. The objectives were to determine the amino acid profile of the three colour-defined groups, compare the essential amino acids before and after limestone clarification, assess changes associated with concentration, and identify the products with the most prominent essential amino acid characteristics.

2. MATERIALS AND METHODS

2.1 Study area and sample collection

Fresh cashew apples were collected from Anyigba and its environs in Dekina Local Government Area of Kogi State, Nigeria, within the central Guinea savanna belt. A total of 50 kg of fresh red and yellow cashew apples was collected. Medium-to-large, crisp, firm, fully colour-developed ripe fruits were selected, sorted and washed with water before processing.

2.2 Experimental design and sample coding

The study followed an experimental design involving red, yellow and mixed red-yellow cashew apple groups. Twelve sample types were analysed in triplicate. The unclarified juices were RT (red variety with tannin), YT (yellow variety with tannin) and MT (mixed red-yellow variety with tannin). The limestone-clarified juices were CR (clarified red), CY (clarified yellow) and CM (clarified mixture). The corresponding unclarified concentrates were D (concentrated red with tannin), E (concentrated yellow with tannin) and F (concentrated mixed variety with tannin), while the clarified concentrates were A (concentrated clarified red), B (concentrated clarified yellow) and C (concentrated clarified mixture).

2.3 Juice preparation

Following sorting and washing, the cashew nuts were removed and the apples were shredded and blended. The resulting pulp was filtered using a high-vacuum pump, with repeated extraction to recover juice retained in the pulp. The juice was collected in a clean container and refrigerated at 4 °C before clarification and concentration.

2.4 Limestone clarification

Clarification was carried out using limestone (CaCO_3). An equal volume of juice to weight of limestone (v/w) was mixed and allowed to stand for 1 h 30 min. The mixture was then filtered through filter paper in a funnel to obtain the clarified juice.

2.5 Preparation of cashew apple juice concentrates

The clarified and unclarified juices were concentrated by heating at 50 °C for 5 h until thick, syrup-like concentrates were obtained through evaporation of water. The concentrates were transferred into tightly sealed containers and stored at ambient temperature (25 °C) away from light.

2.6 Amino acid profile determination

Two millilitres of each sample were prepared for amino acid determination by acid hydrolysis with 6 N HCl for 24 h at 110 °C in a vial under the stated vacuum and 2-normal-atmosphere conditions. The hydrolysed sample was evaporated and dissolved in sodium citrate buffer at pH 2.2. The hydrolysate was analysed by a post-column derivatization method using high-performance liquid chromatography (HPLC) combined with a Pickering derivatizer and an ion-exchange column (3.0 × 250 mm). Amino acid identification was performed spectrometrically using a UV-752 spectrophotometer at 570 nm. The analysis covered 21 amino acids, including the nine essential amino acids evaluated in this manuscript.

2.7 Statistical analysis

Data were analysed using SPSS version 16.0. Mean values and standard error of the mean (SEM) were calculated for triplicate analyses, and analysis of variance (ANOVA) was performed for the amino acid composition data.

3. RESULTS AND DISCUSSION

3.1 Essential amino acid profile of red cashew apple products

The amino acid composition of the red cashew apple products is presented in Table 1. All nine essential amino acids were detected in RT, CR, D and A. Tryptophan was the most abundant essential amino acid, with values of 18.88 ± 0.00 mg/kg in RT, $18.00 \pm$

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0.00 mg/kg in CR, 42.21 ± 0.00 mg/kg in D and 38.50 ± 0.00 mg/kg in A. Lysine ranged from 6.30 ± 0.00 to 6.80 ± 0.00 mg/kg, while leucine ranged from 4.40 ± 0.00 to 5.54 ± 0.00 mg/kg. Isoleucine, valine, histidine, threonine, phenylalanine and methionine were also present in all four red samples.

Limestone clarification produced a selective rather than uniform response in the fresh red juice. Isoleucine increased from 2.00 ± 0.00 mg/kg in RT to 2.12 ± 0.00 mg/kg in CR, threonine from 2.60 ± 0.00 to 3.50 ± 0.00 mg/kg, leucine from 4.50 ± 0.00 to 5.54 ± 0.00 mg/kg, and methionine from 3.25 ± 0.00 to 3.50 ± 0.00 mg/kg. In contrast, valine, histidine, phenylalanine, lysine and tryptophan showed modest numerical reductions after clarification. This pattern indicates that limestone clarification preserved the complete essential amino acid spectrum while altering the distribution of individual amino acids.

Concentration had its strongest effect on tryptophan. The unclarified red concentrate D contained 42.21 ± 0.00 mg/kg compared with 18.88 ± 0.00 mg/kg in RT, while clarified concentrate A contained 38.50 ± 0.00 mg/kg compared with 18.00 ± 0.00 mg/kg in CR. The increases are consistent with the removal of water during concentration, which raises the measured amount of retained solutes per unit mass of concentrate. Other essential amino acids showed smaller and more variable changes, indicating that the compositional response to concentration depended on the individual amino acid.

3.2 Essential amino acid profile of yellow cashew apple products

The yellow cashew apple products also contained all nine essential amino acids (Table 2). Tryptophan ranged from 19.00 ± 0.00 mg/kg in YT to 44.20 ± 0.00 mg/kg in E. Among the fresh juices, YT contained relatively high values of isoleucine (3.42 ± 0.00 mg/kg), valine (5.24 ± 0.00 mg/kg), phenylalanine (4.40 ± 0.00 mg/kg), leucine (6.30 ± 0.00 mg/kg) and methionine (4.50 ± 0.00 mg/kg). Lysine was 6.50 ± 0.00 mg/kg in both YT and CY, while the unclarified concentrate E recorded the highest lysine value within the yellow group at 9.30 ± 0.00 mg/kg.

Following limestone clarification, tryptophan increased from 19.00 ± 0.00 mg/kg in YT to 20.00 ± 0.00 mg/kg in CY, whereas isoleucine, valine, histidine, threonine, phenylalanine, leucine and methionine were numerically lower in CY. The unchanged lysine concentration indicates that the clarification step did not modify all essential amino acids in the same direction. Similar selective effects have been observed in processed plant foods, where amino acid behaviour depends on the chemical form of the amino acid, its association with soluble or particulate protein fractions and the nature of the processing treatment (Fagbemi, 2009).

The concentrate data again showed pronounced enrichment of tryptophan. E contained 44.20 ± 0.00 mg/kg and B contained 36.00 ± 0.00 mg/kg. The high lysine concentration in E (9.30 ± 0.00 mg/kg) was also notable. The clarified concentrate B retained all nine essential amino acids, with isoleucine at 3.14 ± 0.00 mg/kg, valine 4.50 ± 0.00 mg/kg, histidine 2.30 ± 0.00 mg/kg, threonine 2.47 ± 0.00 mg/kg, phenylalanine 2.46 ± 0.00 mg/kg, lysine 6.53 ± 0.00 mg/kg, leucine 5.60 ± 0.00 mg/kg and methionine 3.43 ± 0.00 mg/kg.

3.3 Essential amino acid profile of mixed red-yellow cashew apple products

The mixed red-yellow group displayed a distinct response to limestone clarification (Table 3). In MT, isoleucine and valine were each 1.80 ± 0.00 mg/kg, histidine 2.40 ± 0.00 mg/kg, threonine 1.50 ± 0.00 mg/kg, phenylalanine 1.50 ± 0.00 mg/kg, lysine 9.70 ± 0.00 mg/kg, leucine 2.60 ± 0.00 mg/kg, methionine 1.40 ± 0.00 mg/kg and tryptophan 19.00 ± 0.00 mg/kg. After clarification, CM showed higher values for isoleucine (5.27 ± 0.00 mg/kg), valine (5.45 ± 0.00 mg/kg), histidine (2.60 ± 0.00 mg/kg), threonine (2.60 ± 0.00 mg/kg), phenylalanine (4.12 ± 0.00 mg/kg), leucine (4.50 ± 0.00 mg/kg) and methionine (3.43 ± 0.00 mg/kg). Lysine decreased to 6.40 ± 0.00 mg/kg and tryptophan to 18.20 ± 0.00 mg/kg.

The clarified mixed juice therefore produced the highest measured isoleucine and valine concentrations among all six fresh and clarified juices. This feature may be useful in product selection where branched-chain amino acid content is of interest. The substantial numerical increases in several amino acids after clarification may reflect changes in the soluble matrix following removal of tannin-rich and suspended fractions, as well as differences in the mixed fruit matrix. Cashew apple composition is known to vary with fruit type, origin and harvest conditions, and varietal differences have previously been reported for other nutritional constituents (Adou et al., 2012; Akyereko et al., 2023).

In the concentrates, tryptophan increased to 44.20 ± 0.00 mg/kg in F and 40.20 ± 0.00 mg/kg in C. Leucine increased to 5.30 ± 0.00 mg/kg in F and 5.52 ± 0.00 mg/kg in C, while methionine increased to 3.15 ± 0.00 and 3.50 ± 0.00 mg/kg, respectively. Histidine and threonine were highest in clarified concentrate C within the mixed group, at 3.21 ± 0.00 and 3.49 ± 0.00 mg/kg, respectively. These results show that both clarified and unclarified mixed concentrates retained a broad essential amino acid profile.

3.4 Comparative effects of limestone clarification and concentration

Across the three varietal groups, limestone clarification did not eliminate any of the nine essential amino acids. Instead, it changed their relative concentrations in a variety-dependent manner. The red juice showed increases in threonine, leucine and methionine after clarification; the yellow juice showed a modest increase in tryptophan with lysine maintained at the same value; and the mixed juice showed marked increases in isoleucine, valine, phenylalanine, leucine and methionine. These differences indicate that the interaction between limestone clarification and the amino acid-containing matrix depended on fruit composition.

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The behaviour is consistent with the chemistry of cashew apple juice. Tannins are abundant in unclarified juice and can interact with proteins and other nitrogen-containing constituents. Removal of tannin-rich material during clarification can therefore modify the partitioning of amino-acid-containing fractions. At the same time, removal of suspended solids and changes in the liquid matrix can alter the concentration of soluble constituents in the clarified fraction. The present data show that these effects were selective and did not produce a single uniform pattern for all essential amino acids.

Concentration exerted a clearer overall effect on tryptophan than on the other essential amino acids. The measured tryptophan concentration approximately doubled in all three unclarified juice-to-concentrate comparisons and increased strongly in the clarified products. This is consistent with the reduction in water content during the 50 °C concentration step. The other essential amino acids showed smaller increases, little change or moderate decreases depending on the sample. Thus, concentration increased the density of the essential amino acid profile primarily through strong enrichment of selected constituents rather than through identical increases in every amino acid.

3.5 Nutritional relevance of the essential amino acid profile

The detection of all nine essential amino acids in every juice and concentrate demonstrates that cashew apple contributes a wider amino acid spectrum than is often considered when the fruit is viewed only as a source of sugars and vitamin C. This observation complements earlier reports that cashew apple juice contains nutritionally relevant free amino acids and that the profile can change with harvest period and processing (Oliveira et al., 2002; Sivagurunathan et al., 2010). The present results extend that evidence to red, yellow and mixed cashew apple products from Kogi East and show that the essential amino acid spectrum is retained after limestone clarification and concentration.

Tryptophan was consistently the dominant essential amino acid in the present products. This differs from some cashew kernel studies in which tryptophan has been reported among the lower amino acid fractions, a distinction that can be attributed to differences between the fleshy apple juice matrix and the protein-rich kernel, as well as variety, geographical origin and processing method (Fagbemi, 2009). Cashew apple juice itself has a distinct free-amino-acid pool, and Oliveira et al. (2002) demonstrated seasonal behaviour of major free amino acids in the juice. The high tryptophan values observed here therefore contribute to the distinctive nutritional profile of the Kogi East samples.

The FAO/WHO reference values included in Tables 1–3 provide a nutritional benchmark for the nine essential amino acids. Tryptophan showed the highest numerical relationship to the listed reference value, whereas the other essential amino acids occurred at lower concentrations. Because the analytical results are expressed as milligrams per kilogram of food and the reference requirements are expressed on a body-weight-per-day basis, the values are most appropriately used to describe amino acid composition and product selection rather than to assign daily dietary sufficiency without a defined serving size. From a food-development perspective, the results support the use of cashew apple concentrate as a nutrient-containing ingredient and show that fruit group and clarification treatment can be selected according to the amino acid characteristic desired in the final product.

4. CONCLUSION

Unclarified and limestone-clarified red, yellow and mixed cashew apple juices and their concentrates from Kogi East contained all nine essential amino acids evaluated: isoleucine, valine, histidine, threonine, phenylalanine, lysine, leucine, methionine and tryptophan. Tryptophan was the dominant essential amino acid in every sample, ranging from 18.00 ± 0.00 mg/kg in clarified red juice to 44.20 ± 0.00 mg/kg in the unclarified yellow and mixed concentrates. Among the juices, clarified mixed juice recorded the highest isoleucine and valine values, yellow unclarified juice recorded the highest phenylalanine, leucine and methionine values, and mixed unclarified juice contained the highest lysine concentration. Limestone clarification retained the complete essential amino acid spectrum while producing variety-dependent shifts in individual amino acids. Concentration particularly increased the measured tryptophan concentration and preserved a broad essential amino acid profile in all six concentrates. These findings demonstrate that cashew apple juice from Kogi East is not only a source of sugars, vitamins and phytochemicals but also contains a diverse essential amino acid fraction that can contribute to the nutritional value of clarified juices, concentrates and related food products.

DECLARATIONS

Conflict of interest: The authors declare no conflict of interest.

Ethics statement: Not applicable. The study involved laboratory analysis of plant-derived food samples and did not involve human or animal subjects.

Data availability: All data supporting the findings of this study are presented within the article.

Acknowledgements: The authors acknowledge the technical assistance provided by staff of the Biochemistry Department, Prince Abubakar Audu University, Anyigba, and the assistance provided during statistical analysis.

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TABLES

Table 1. Essential amino acid analysis – clarified red/red with tannin and their concentrates

Essential amino acid (mg/kg)	RT	CR	D	A	Recommended Dietary Allowance (RDA), FAO/WHO (mg/kg/day)
Isoleucine	2.00 ± 0.00	2.12 ± 0.00	2.21 ± 0.00	2.10 ± 0.00	20
Valine	4.60 ± 0.00	4.50 ± 0.00	4.42 ± 0.00	4.42 ± 0.00	16
Histidine	3.50 ± 0.00	3.40 ± 0.00	2.50 ± 0.00	2.52 ± 0.00	10
Threonine	2.60 ± 0.00	3.50 ± 0.00	2.57 ± 0.00	2.55 ± 0.00	15
Phenylalanine	2.20 ± 0.00	1.55 ± 0.00	2.12 ± 0.00	2.25 ± 0.00	25
Lysine	6.80 ± 0.00	6.44 ± 0.00	6.70 ± 0.00	6.30 ± 0.00	30
Leucine	4.50 ± 0.00	5.54 ± 0.00	4.45 ± 0.00	4.40 ± 0.00	40
Methionine	3.25 ± 0.00	3.50 ± 0.00	3.23 ± 0.00	3.40 ± 0.00	15
Tryptophan	18.88 ± 0.00	18.00 ± 0.00	42.21 ± 0.00	38.50 ± 0.00	4

Note: Values are mean ± SEM of triplicate analyses. RT = red variety with tannin; CR = clarified red; D = concentrated red variety with tannin; A = concentrated clarified red.

Table 2. Essential amino acid analysis – clarified yellow/yellow with tannin and their concentrates

Essential amino acid (mg/kg)	YT	CY	E	B	Recommended Dietary Allowance (RDA), FAO/WHO (mg/kg/day)
Isoleucine	3.42 ± 0.00	2.15 ± 0.00	2.40 ± 0.00	3.14 ± 0.00	20
Valine	5.24 ± 0.00	4.20 ± 0.00	4.80 ± 0.00	4.50 ± 0.00	16
Histidine	2.60 ± 0.00	2.44 ± 0.00	2.30 ± 0.00	2.30 ± 0.00	10
Threonine	2.50 ± 0.00	1.89 ± 0.00	1.50 ± 0.00	2.47 ± 0.00	15
Phenylalanine	4.40 ± 0.00	2.40 ± 0.00	1.50 ± 0.00	2.46 ± 0.00	25
Lysine	6.50 ± 0.00	6.50 ± 0.00	9.30 ± 0.00	6.53 ± 0.00	30
Leucine	6.30 ± 0.00	5.50 ± 0.00	5.63 ± 0.00	5.60 ± 0.00	40
Methionine	4.50 ± 0.00	3.20 ± 0.00	3.20 ± 0.00	3.43 ± 0.00	15
Tryptophan	19.00 ± 0.00	20.00 ± 0.00	44.20 ± 0.00	36.00 ± 0.00	4

Note: Values are mean ± SEM of triplicate analyses. YT = yellow variety with tannin; CY = clarified yellow; E = concentrated yellow variety with tannin; B = concentrated clarified yellow.

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Table 3. Essential amino acid analysis – mixed varieties and their concentrates

Essential amino acid (mg/kg)	MT	CM	F	C	Recommended Dietary Allowance (RDA), FAO/WHO (mg/kg/day)
Isoleucine	1.80 ± 0.00	5.27 ± 0.00	3.43 ± 0.00	2.10 ± 0.00	20
Valine	1.80 ± 0.00	5.45 ± 0.00	4.53 ± 0.00	4.53 ± 0.00	16
Histidine	2.40 ± 0.00	2.60 ± 0.00	2.60 ± 0.00	3.21 ± 0.00	10
Threonine	1.50 ± 0.00	2.60 ± 0.00	2.44 ± 0.00	3.49 ± 0.00	15
Phenylalanine	1.50 ± 0.00	4.12 ± 0.00	2.15 ± 0.00	1.50 ± 0.00	25
Lysine	9.70 ± 0.00	6.40 ± 0.00	6.60 ± 0.00	6.24 ± 0.00	30
Leucine	2.60 ± 0.00	4.50 ± 0.00	5.30 ± 0.00	5.52 ± 0.00	40
Methionine	1.40 ± 0.00	3.43 ± 0.00	3.15 ± 0.00	3.50 ± 0.00	15
Tryptophan	19.00 ± 0.00	18.20 ± 0.00	44.20 ± 0.00	40.20 ± 0.00	4

Note: Values are mean ± SEM of triplicate analyses. MT = mixed red-yellow variety with tannin; CM = clarified mixture; F = concentrated mixture with tannin; C = concentrated clarified mixture.