

Compositional Characterization of Vitamins, Fatty Acids, and Amino Acids in Functional Smoothies Formulated with Selected Fruit and Vegetables

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Abstract

Smoothies produced from a blend of coconut extract, apples, carrots, cucumbers, beetroot juice, and bitter gourd leaves powder were evaluated for vitamins, fatty acids, and amino acids profiles. Four samples, AAA, BBB, CCC, and DDD, were formulated with varying ratios of bitter gourd leaves powder, beetroot juice, to the base (equal combination of carrot, cucumber, apple, and coconut extract). The Smoothie sample BBB had the highest concentration of vitamin A (730.50 ± 1.57 mg/100 g) and vitamin C (4.53 ± 0.02 mg/100 g), while sample CCC had the lowest vitamin B₂ (0.06 ± 0.01 mg/100 g) and Vitamin D (0.01 ± 0.01 mg/100g). Fatty acid analysis revealed, Lauric acid range of 25.23 to 29.08 g/100g. Oleic acid was most abundant in all samples (25.52 to 29.97g/100g), with a balanced profile of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) ratio. In particular, the smoothie sample AAA had the greatest MUFA/PUFA ratio (2.55), which could help to lower the risk of heart disease. The essential amino acids (EAA), dominate smoothie samples. Sample AAA had the highest concentration (2.87 g/100 g) of all, while sample DDD was the lowest (2.19 g/100 g). There were significant concentrations of conditional essential amino acids (CEAA) and non-essential amino acids (NEAA) in all samples. The study emphasizes the nutritional content of these functional smoothies, which comprise both macronutrients and important micronutrients, confirming smoothie importance as a beneficial dietary supplement.

Keywords: Functional foods, Amino and fatty acids profile, Fruit and Vegetable Smoothie, Essential vitamins, Nutrient-dense

Introduction

Global awareness of functional foods, which are defined by their capacity to offer additional health benefits beyond basic nutrition, is increasing due to their numerous health benefits. Fruit and vegetable smoothies are one example; they are rich in micronutrients, phytochemicals, and dietary fiber to promote health and well-being (Li et al., 2022). Smoothies combine a range of fruits, vegetables, and other natural extracts to provide a balanced combination of essential nutrients, including vitamins, minerals, antioxidants, fatty acids, and amino acids (Ructon, 2008; Watz, 2008). Due to diverse phytochemical profiles, fruits and vegetables are combined in no distinct format or proportion, but, for this research, bitter gourd leaves (*Momordica charantia*), beetroot (*Beta vulgaris*), cucumber (*Cucumis sativus*), apple (*Malus domestica*), carrot (*Daucus carota*), and coconut extract (*Cocos nucifera*) are combined to produce a functional smoothie.

Apples are rich in dietary fiber, vitamin C, and polyphenols, which have cardiovascular and antioxidant benefits. Antioxidants inhibit or delay biologically relevant molecules (Dayrit, 2014). Beta-carotene, a precursor to vitamin A with significant antioxidant capabilities, is found in considerable concentrations in carrots, while cucumbers hydrate the body system due to their large volume of water content (Okwori *et al.*, 2024). Beetroot juice contains vitamins A and B₆. Bioactive compounds such as charantin and vicine present in bitter gourd leaves possess antidiabetic, anti-inflammatory, and lipid-lowering qualities, and are particularly valued traditionally (Gayathry and John, 2022). Coconut extract contains medium-chain fatty acids (MCFAs), like lauric acid, which have antimicrobial and energy-boosting properties, in addition to essential amino acids (Dayrit, 2014). These elements, when combined, could create a nutrient-dense smoothie. The composition of the selected fruit and vegetables provides a balanced profile of essential vitamins, minerals, fatty and amino acids, particularly monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), which are crucial for brain functioning and reducing the risk of cardiovascular diseases (Wainwright, 2022). As the building blocks of proteins, amino acids are necessary for immune system function, muscle regeneration, and the synthesis of enzymes (Wainwright, 2022). Leucine, isoleucine, and valine are essential amino acids that are necessary for protein synthesis and muscle maintenance, which could be found in the smoothie. To the best of our knowledge, the specific vitamin, fatty acid, and amino acid makeup of smoothies made with a combination of apple, carrot, cucumber, beetroot juice, bitter gourd leaves, and coconut extract is limited; therefore, this study is designed to fill this gap and further the understanding of smoothies' nutritional value and potential health benefits.

Study area

The research was carried out in the Department of Food Technology, Auchi Polytechnic, Auchi (7.0667N and 6.27330E), Edo State, Nigeria.

Materials and methods

Apple (*Malus domestica*), cucumber (*Cucumis sativus*), carrot (*Daucus carota*), coconut (*Cocos nucifera*), and beetroot (*Beta vulgaris*) were all purchased from a local market in Auchi, while bitter gourd (*Momordica charantia*) was harvested from a farm within the premises of Auchi Polytechnic, Auchi, Etsako West Local Government Area, Edo State, Nigeria. All the materials were authenticated at the Department of Crop Production Management, Auchi Polytechnic, Auchi, Edo State, Nigeria

Preparation of bitter gourd powder

Freshly harvested bitter gourd leaves were sorted, thoroughly cleaned under running water, and drained before dicing. The diced leaves were then dried in a Genlab drying cabinet (Model DC 125) at 40°C for 48 hours. A Kenwood Blender (Model BL335) was used to blend the dried leaves into a fine powder (Michael *et al.* 2024).

Production of Coconut extract and Beetroot juice

Coconut shells were carefully broken to prevent the coconut water from spilling. The coconut water was then collected in a clean container with a lid and stored safely for future use. The coconut's endosperm (or meat) was carefully separated from the shells, washed, and weighed before grating to ease the blending operation. The grated coconut was then combined with coconut water and additional 1.5cl of clean water to ease extraction in a Kenwood Blender (Model BL335) and blended. A white, clean muslin cloth was used for the extraction. The extract was then stored in a refrigerator before smoothies. The beetroots were sorted, cleaned under running water, peeled, and grated before juice was extracted using a very clean muslin cloth (Adelu *et al.*, 2024).

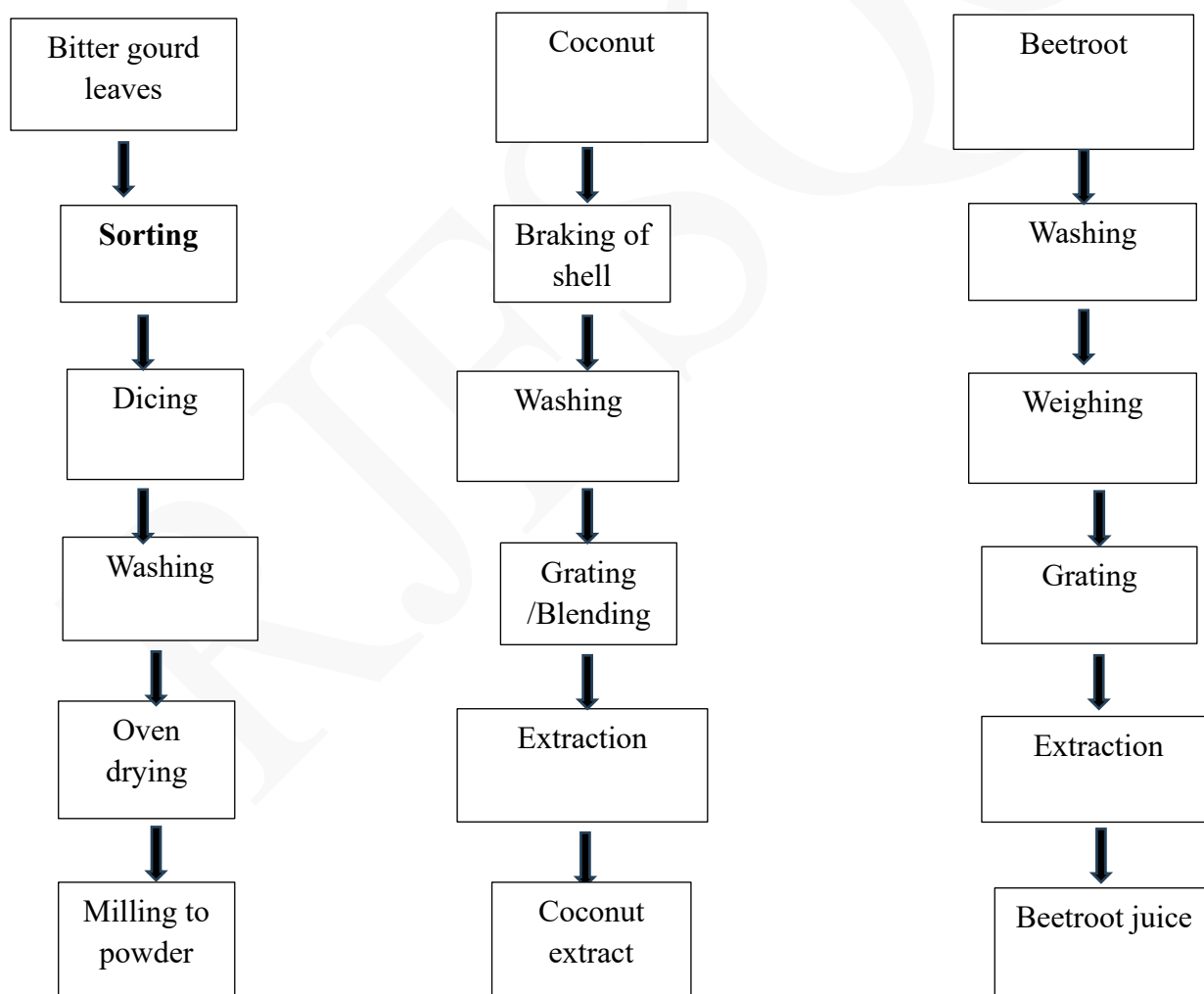


Fig 1: Flow chart for production of Bitter gourd leaves powder, Coconut extract, and Beetroot juice.

Smoothie production

Carrot, cucumber, and apple were weighed equally using an automated weighing balance before being blended with the addition of coconut extract using a Kenwood BL335 blender, until a semi-viscous and smooth was formed. The smoothies was then grouped into four different samples with varying ratio of beetroot juice and bitter gourd leaves powder to base (blend of equal weight of apple, coconut, cucumber and coconut extract) (Table 1). Each group was freeze-dried and kept in a refrigerator for analysis.

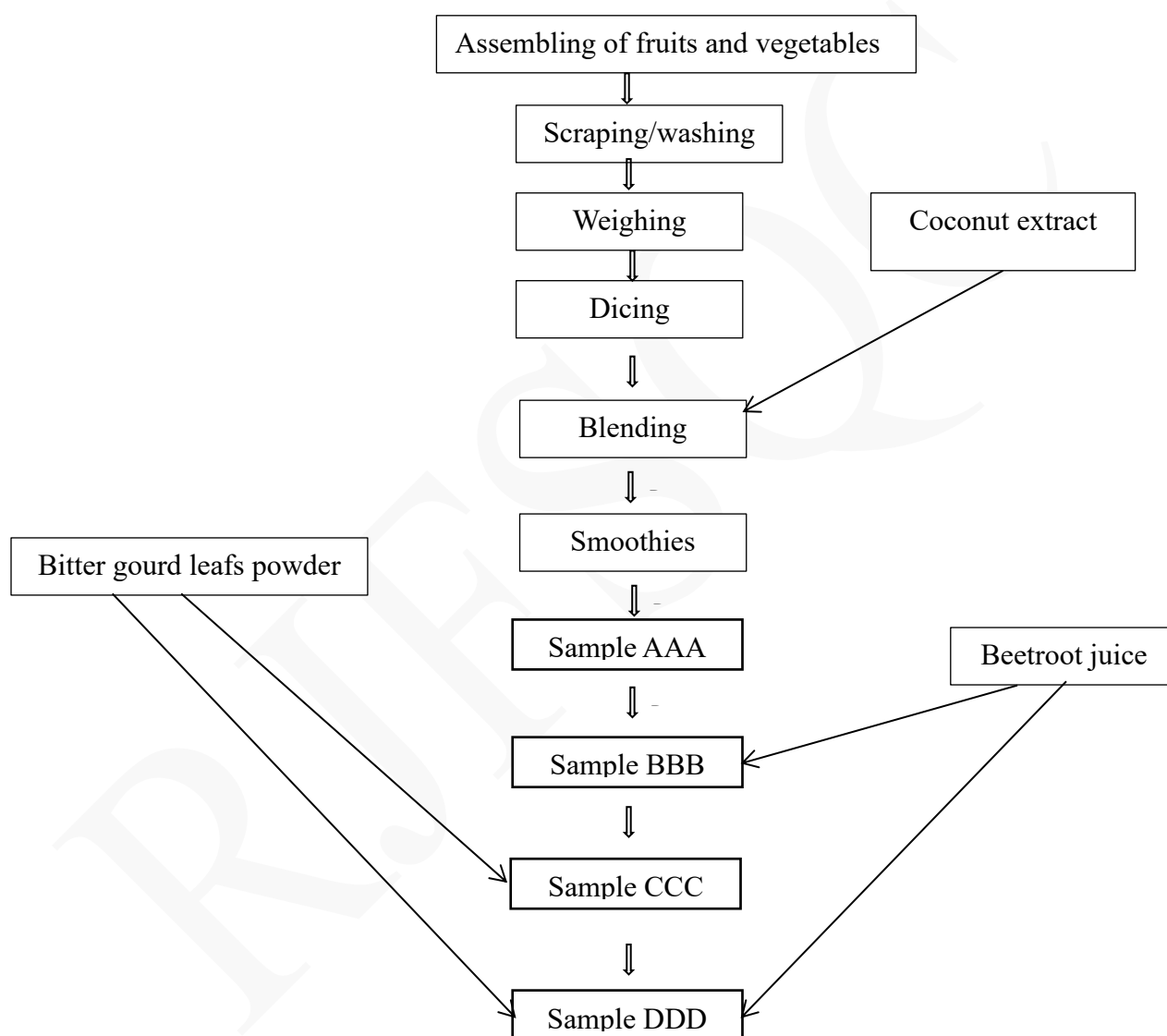


Fig 2: Flow chat for the production of smoothie's samples.

Table 1: Smoothie group

Sample	Base (%)	Beetroot Extract	Bitter gourd powder
AAA	100	Nil	Nil
BBB	98	2	Nil
CCC	98	Nil	2
DDD	98	1	1

KEY: The base is an equal blend of carrot, cucumber, apple, and coconut extract

Fatty Acids, Amino Acids Profile, and Vitamins Analysis of Smoothie Samples

The vitamins, fatty acids, and amino acid profiles of smoothie samples were analyzed according to AOAC (2016) methods. Fatty acids were measured using calibration curves from standard FAME solutions and peak regions from the chromatogram. The data were expressed as percentages of total fatty acids, while calibration curves with recognized standards were used for the measurement of amino acids, and the results were recorded in milligrams of amino acid per 100 grams of the smoothie samples. Vitamins A, C, E, and B in the smoothie samples were determined using High-performance liquid chromatography (HPLC). The vitamins were separated and measured according to their distinct absorption at various wavelengths after the samples had been solvent extracted. Caution was exercised during the extraction of fat-soluble vitamins A and D to guarantee successful isolation and correct quantification.

Statistical analysis

All analyses were carried out in triplicate, and SPSS version 21.0 for Windows was used to analyze the data. One-way analysis of variance (ANOVA) was used to determine the statistical difference between the means, and the findings were displayed as mean ($\pm$ SD). Duncan's Multiple Range Test (DMRT) was used to separate the means at a significance level of 0.05.

Result and Discussion

Vitamins Composition of Smoothie Samples

The importance of vitamins cannot be overemphasized. Healthy growth, immune system support, metabolic process regulation, and oxidative stress defense are just a few examples of known benefits (Calder *et al.*, 2020). The vitamin composition of smoothie samples is displayed in Table 2.

Table 2: Vitamins (g/100 g) compositions of smoothie samples

Vitamins	AAA	BBB	CCC	DDD
A	280.11 $\pm$ 1.57 ^c	730.50 $\pm$ 1.57 ^a	247.46 $\pm$ 1.57 ^d	373.69 $\pm$ 1.57 ^b
B1	1.82 $\pm$ 0.01 ^b	2.32 $\pm$ 0.02 ^a	1.36 $\pm$ 0.01 ^c	2.32 $\pm$ 0.02 ^a
B2	0.08 $\pm$ 0.01 ^c	0.10 $\pm$ 0.02 ^b	0.06 $\pm$ 0.01 ^d	0.48 $\pm$ 0.02 ^a
B3	0.11 $\pm$ 0.02 ^b	0.12 $\pm$ 0.02 ^a	0.02 $\pm$ 0.01 ^c	0.11 $\pm$ 0.02 ^b
C	2.56 $\pm$ 0.79 ^d	4.53 $\pm$ 0.20 ^a	2.87 $\pm$ 0.64 ^c	2.96 $\pm$ 0.28 ^b
D	0.03 $\pm$ 0.01 ^c	0.07 $\pm$ 0.01 ^a	0.01 $\pm$ 0.01 ^d	0.04 $\pm$ 0.03 ^b
E	1.47 $\pm$ 0.01 ^a	1.17 $\pm$ 0.01 ^b	1.16 $\pm$ 0.01 ^c	1.02 $\pm$ 0.01 ^d
K	0.00	0.00	0.00	0.00

Values are Mean $\pm$ SEM. Where: AAA = 100% base (Blend of carrot, cucumber, apple, and coconut extract). BBB 98% BASE; 2%beetroot extract. CCC = 99% base; 1%bitter gourd powder. DDD 98% base; 1% beetroot extract; 1% bitter gourd powder. Values with different alphabets across the row differ significantly at $p < 0.05$.

Vitamin A was the most abundant of all vitamins analyzed. Sample BBB had the highest vitamin A concentration ($730.50 \pm 1.57 \mu\text{g}/100 \text{ g}$), while sample CCC had the lowest ($247.46 \pm 1.57 \mu\text{g}/100 \text{ g}$). The largest concentrations of thiamine were found in BBB and DDD ($2.32 \pm 0.02 \mu\text{g}/100 \text{ g}$), followed by AAA ($1.82 \pm 0.01 \mu\text{g}/100 \text{ g}$) and CCC ($1.36 \pm 0.01 \mu\text{g}/100 \text{ g}$), respectively. The higher vitamin A and thiamine levels observed in smoothie samples BBB and DDD could be attributed to the beetroot juice composition. While beetroot isn't typically thought of as a major source of vitamins, it does have modest levels (Kale *et al.* 2018). The concentration of riboflavin was highest in smoothie sample DDD ($0.48 \pm 0.02 \mu\text{g}/100 \text{ g}$) and lowest in sample CCC (0.06 ± 0.01). As a coenzyme in redox processes, riboflavin (Vitamin B₂) is essential for cellular activity and energy production (Yauheni and Wolf, 2024). Although bitter melon and carrots are not inherently high in riboflavin, they could provide a significant amount of the vitamin when mixed in smoothies, according to USDA Food Data Central (USDA, 2023). Green leafy vegetables and root crops are moderate sources of riboflavin, and preparing them into fresh juice or smoothies without heat application preserves their water-soluble vitamin content (Lima *et al.* 2020). Samples' BBB and AAA had the highest values of niacin ($0.11 \pm 0.02 \mu\text{g}/100 \text{ g}$), and CCC had the lowest ($0.02 \pm 0.01 \mu\text{g}/100 \text{ g}$). Vitamin B₃, often known as niacin, is essential for metabolism, DNA repair, and healthy skin and digestive systems. The contribution of coconut, carrot, and beetroot, all well-known sources of niacin in a plant-based diet, could be the reasons for the trend. DDD ($2.96 \pm 0.28 \mu\text{g}/100 \text{ g}$), CCC ($2.87 \pm 0.64 \mu\text{g}/100 \text{ g}$), AAA ($2.56 \pm 0.79 \mu\text{g}/100 \text{ g}$), and BBB ($4.53 \pm 0.20 \mu\text{g}/100 \text{ g}$) are the vitamin C concentrations in each sample. The relatively high vitamin C concentration in all samples is proof that the raw processing method (such as blending or juicing without heating) maintains nutrients. Vitamin C is a powerful antioxidant that is necessary for the production of collagen and the health of the immune system (Carr and Maggini, 2017). Vitamin D contents of the samples were AAA ($0.03 \pm 0.01 \mu\text{g}/100 \text{ g}$), BBB ($0.07 \pm 0.01 \mu\text{g}/100 \text{ g}$), CCC ($0.01 \pm 0.01 \mu\text{g}/100 \text{ g}$), and DDD ($0.04 \pm 0.03 \mu\text{g}/100 \text{ g}$), respectively. Vitamin E protects cellular membranes from oxidative damage since it is an antioxidant (Howard *et al.* 2011). Sample BBB ($1.17 \pm 0.01 \mu\text{g}/100 \text{ g}$), CCC ($1.16 \pm 0.01 \mu\text{g}/100 \text{ g}$), and DDD ($1.02 \pm 0.01 \mu\text{g}/100 \text{ g}$) exhibited somewhat lower levels of vitamin E than AAA ($1.47 \pm 0.01 \mu\text{g}/100 \text{ g}$). Oliveira *et al.* (2021) found similar results in carrot-coconut blends and also noted improved vitamin E stability when consumed in emulsified smoothies.

Fatty acid composition of the smoothies

Table 3: Fatty acid (g/100 g) compositions of smoothies

Fatty acids	Formular	AAA	BBB	CCC	DDD
Caproic	C6:0	2.13 ± 0.02^b	2.93 ± 0.01^a	2.12 ± 0.03^b	1.04 ± 0.01^c
Capric	C8:0	3.92 ± 0.04^a	3.54 ± 0.01^b	2.82 ± 0.01^c	2.02 ± 0.01^d
Lauric	C12:0	29.08 ± 0.01^a	28.73 ± 0.01^b	25.23 ± 0.01^d	27.34 ± 0.01^c
Mysristic	C14:0	5.50 ± 0.01^b	5.46 ± 0.01^b	4.34 ± 0.01^c	11.15 ± 0.01^a
Palmitic	C16:0	12.57 ± 0.01^b	13.93 ± 0.01^a	12.46 ± 0.07^c	11.17 ± 0.01^d
Palmitoleic	C16:1	2.86 ± 0.01^a	2.25 ± 0.01^b	1.17 ± 0.01^d	1.36 ± 0.01^c
*Stearic	C18:0	0.54 ± 0.01^c	0.48 ± 0.01^d	3.43 ± 0.01^a	$2.13 \pm 0.01^=$
Patroselinic	C18:1	0.31 ± 0.01^d	0.55 ± 0.01^a	0.46 ± 0.01^b	0.41 ± 0.01^c
#Oleic C18:1		29.97 ± 0.01^a	27.60 ± 0.07^c	25.52 ± 0.02^d	28.15 ± 0.01^b
Linoleic	C18:2	7.79 ± 0.01^d	8.32 ± 0.02^c	11.06 ± 0.01^a	9.82 ± 0.03^b
Linolenic	C18:3	2.34 ± 0.01^d	3.62 ± 0.03^a	2.66 ± 0.01^c	2.86 ± 0.01^b
α -eleosteric	C18:3	2.87 ± 0.01^c	2.57 ± 0.01^d	8.16 ± 0.01^a	7.56 ± 0.01^b

*Arachidic acid C20:0	0.00±0.00 ^c	0.00±0.01 ^c	0.60±0.01 ^b	1.43±0.01 ^a
SFA	53.74	55.07	50.40	54.83
MUFA	33.14	30.40	27.15	29.92
PUFA	13.00	14.51	22.42	21.67
MUFA/PUFA	2.55	2.09	1.21	1.38

Values are Mean ± SEM. Where: AAA = 100% base (Blend of carrot, cucumber, apple and coconut extract). BBB = 98% BASE; 2% beetroot extract. CCC = 99% base; 1% bitter gourd powder. DDD = 98% base; 1% beetroot extract; 1% bitter gourd powder. Values with different alphabets across the row differ significantly at $p < 0.05$.

The fatty acid composition of smoothie samples is displayed in Table 3. Lauric acid was the predominant saturated fatty acid (SFA) in all samples, reaching its highest in AAA (29.08 g/100 g) and its lowest in CCC (25.23 g/100 g). This is mostly due to coconut extract composition, a known medium-chain SFAs, particularly lauric acid, source (Dayrit, 2014). Sample BBB had the highest concentration of palmitic acid (13.93 g/100 g). The most prevalent monounsaturated fatty acid (MUFA) in all samples was oleic acid. It range from 27.60 to 29.97 g/100 g though, sample AAA had the highest (29.97 g/100 g). According to Swingshackl and Hoffmann (2014), oleic acid is well known for its heart-protective qualities, which include lowering oxidative stress and LDL cholesterol. Linoleic acid and α -linolenic acid were the two most abundant polyunsaturated fatty acids found in all smoothies' samples. PUFAs, particularly omega-3 and omega-6 fatty acids, are essential for brain function, cell membrane integrity, and anti-inflammatory reactions (Wainwright, 2022). . The combination of SFA, MUFA, and PUFA in the samples shows how smoothie recipes can be changed to create certain lipid profiles that may be in accordance with dietary needs or health goals.

Amino acid composition of the smoothie samples

The amino acids profile of functional smoothies produced from beetroot juice, bitter gourd leaves powder, cucumber, and coconut extract is shown in Table 4.

Table 4: Amino acid (g/100 g) compositions of smoothie samples

Amino Acids	AAA	BBB	CCC	DDD
Essential amino acids (EAA)				
Histidine	2.00±0.02 ^a	2.00±0.02 ^a	1.91±0.02 ^b	1.84±0.02 ^c
Isoleucine	0.39±0.45 ^a	0.08±0.02 ^b	0.05±0.01 ^c	0.04±0.01 ^d
Leucine	0.10±0.01 ^b	0.12±0.01 ^a	0.70±0.02 ^c	0.05±0.02 ^c
Lysine	0.04±0.00 ^b	0.05±0.02 ^a	0.03±0.01 ^c	0.02±0.01 ^d
Methionine	0.07±0.02 ^b	0.08±0.02 ^a	0.07±0.01 ^b	0.06±0.01 ^c
Phenylalanine	0.06±0.02 ^a	0.06±0.02 ^a	0.05±0.01 ^b	0.05±0.01 ^b
Threonine	0.07±0.02 ^a	0.07±0.02 ^a	0.05±0.01 ^b	0.04±0.01 ^c
Tryptophan	0.06±0.02 ^a	0.06±0.02 ^a	0.05±0.01 ^b	0.04±0.01 ^c
Valine	0.08±0.01 ^b	0.10±0.02 ^a	0.07±0.01 ^b	0.05±0.01 ^c
Total essential amino acid	2.87 ^a	2.62 ^b	2.35 ^c	2.19 ^d
Non-essential amino acids (TNEAA)				
Alanine	0.67±0.04 ^a	0.69±0.01 ^a	0.52±0.04 ^b	0.49±0.02 ^b
Aspartic acid	0.05±0.01 ^a	0.07±0.02 ^a	0.04±0.01 ^b	0.03±0.01 ^c
Serine	0.07±0.02 ^a	0.07±0.02 ^a	0.05±0.02 ^b	0.04±0.02 ^c
Glutamic acid	0.63±0.02 ^a	0.64±0.03 ^a	0.53±0.01 ^b	0.50±0.01 ^b
Conditional essential amino acid (TCEAA)				

Arginine	0.12±0.01 ^a	0.12±0.02 ^a	0.10±0.02 ^{ab}	0.08±0.01 ^b
Cysteine	0.13±0.02 ^a	0.10±0.01 ^b	0.90±0.01 ^b	0.80±0.02 ^b
Proline	0.69±0.01 ^b	0.67±0.01 ^b	0.60±0.01 ^c	0.84±0.01 ^a
Asparagine	0.06±0.01 ^{ab}	0.05±0.01 ^{ab}	0.04±0.01 ^b	0.06±0.01 ^a
Glycine	2.08±0.04 ^a	2.12±0.01 ^a	1.80±0.01 ^b	1.73±0.04 ^b
Tyrosine	0.10±0.01 ^b	0.12±0.01 ^a	0.10±0.01 ^b	0.08±0.01 ^c
Predicted nutritional qualities				
Total non-essential AA	4.60	4.65	4.68	3.93
TAA	7.47	7.27	7.03	6.12
%TEAA	28.61	26.12	23.43	21.83
%TNEAA	25.75	26.03	26.20	22.00
TEAA/TAA	0.38	0.36	0.33	0.36
TNAA/TAA	0.62	0.64	0.67	0.64
Ratio of TEAA: TNEAA	1.11	1.00	0.89	0.99

Values are Mean ± SEM. Where: AAA = 100% base (Blend of carrot, cucumber, apple, and coconut extract). BBB 98% BASE; 2%beetroot extract. CCC = 99% base; 1%bitter gourd powder. DDD 98% base; 1% beetroot extract; 1% bitter gourd powder. Values with different alphabets across the row differ significantly at p <0.05. AC=Amino acid.

Histidine predominates in all samples (2.00 g/100 g in both AAA and BBB) and 1.91g/100g, 1.84g/100 g respectively in sample CCC and DDD. BBB had the highest concentration of leucine (0.12g/100 g), an important branched-chain amino acid (BCAA) that supports muscle protein synthesis, while DDD had the lowest concentration at 0.05 g/100 g. Sample BBB had the highest levels of lysine (0.05 g/100 g), which is necessary for collagen production, calcium absorption, and growth. These levels, however, are below the FAO/WHO/UNU (2013) recommendation of 30 mg/g protein, indicating lysine insufficiency, a known disadvantage of any plant-based preparation. Glutamic acid and alanine were the most prevalent non-essential amino acids (NEAAs). With the highest concentration in sample BBB (0.64 g/100 g and 0.69 g/100 g). Glutamic acid is essential as a precursor to γ -aminobutyric acid (GABA), neurotransmitter, which is important for mood and stress management (Nasiru *et al.*, 2020). BBB's had highest alanine content (0.69 g/100 g). Alani is essential for the metabolism of glucose, particularly during periods of fasting or energy demand (Petersen *et al.*, 2019). This observation is in accordance with Wu (2013), who found that fruits and vegetables frequently have greater levels of glutamate and alanine, which are necessary for their flavor and metabolic profiles. The most prevalent conditional essential amino acid (CEAA) was glycine, particularly in sample AAA (2.08 g/100 g) and BBB (2.12 g/100 g), while DDD had the highest levels of proline (0.84g/100g) and tyrosine is highest in the BBB (0.12 g/100 g). Glycine supports proper function and detoxification process of liver, contribute to collagen synthesis, and essential for the synthesis of glutathione and other essential biomolecules, while proline is important for wound healing and maintenance of skin structure (Kishore Kumar and Vikrant Jain, 2024).

Conclusion

This study examined the vitamin, fatty acid, and amino acid contents of four plant-based smoothie formulations (AAA, BBB, CCC, and DDD), all samples had distinct advantages: The highest concentrations of vitamin A and C, as well as glutamic acid, alanine, and glycine, components crucial for metabolism and immunological function, were found in the BBB, while monounsaturated fatty acids (MUFA), especially oleic acid, which promotes cardiovascular

health, and total essential amino acids (TEAA), were found in sample AAA. Although it had lower concentrations of vital vitamins and amino acids, CCC had the highest levels of polyunsaturated fatty acids (PUFA), particularly linoleic acid, suggesting possible anti-inflammatory properties. .

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Ethical Approval

Not applicable. There were no human subjects or animal experiments in this study.

Competing Interests

The author declares that no conflicting financial interests or personal ties influenced the work described in this paper

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