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(Research Article)

Microorganisms, proximate analysis, nutritional contents and amino acids profile of naturally opened ackee apple fruits (*Blighia sapida*)

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Abstract

Fruits are important foods that contain nutrients which contribute to the growth and mental health. A total of 100 naturally opened Ackee apple (*Blighia sapida*) fruits were collected in Ado-Ekiti. The samples were analyzed using standard microbiological techniques examination. The proximate analysis, nutritionally viable minerals contents, and functional properties of the amino acid profile were determined. Fungal isolated were *Asperrgillus niger* (23 %), *Mucor* species (11 %), *Rhizopus* species (15 %) and *Aspergillus flavus* (35 %) while Bacterial isolates were *Bacillus laterusporus* (7 %), *Staphylococcus aureus* (5 %) and *Pasteurella* sp. (4 %). The sources of microorganisms that contaminated these fruits may include air, birds, insects, rabbits, rats and other animals. The proximate analysis of Ackee Apple Fruits were Ash(3.62 %), Moisture content (40.23%), Crude Protein (5.5 %), Fat (11.55 %), Fibre (3.49 %) and Carbohydrate (35.63%); Mineral contents were Sodium (201.49 mg/100 g), Potassium (652.91 mg/100g), Calcium (8.24 mg/100g), Zinc (258.68 mg/100 g), Magnesium (20.86 mg/100 g), Manganese (0.05 mg/100 g), Iron (7.87 mg/100g) and Phosphorus (48.6 mg/100g); Functional properties were oil absorption (144.5 W/V), Water absorption (378.06 W/V), Foaming capacity (14.50 W/V) and Least gelation concentration (6 W/V) while the amino acid contents ranged from 16 to 132 g/100 g. Crude protein Glutamic acid was the highest Amino acid while Tyrosine was the least. Ackee apple fruits provide nutritive values for health since it contains essential minerals. The health implications of isolated microorganisms are that they can cause mycotoxin and cause food-borne diseases. It is imperative for the consumers of Ackee apple fruits should ensure proper washing and maintain a high standard of hygiene.

Keywords: Microorganisms; Proximate Analysis; Mineral Contents; Amino Acids and Naturally Ripened opened Ackee Apple (*Blighia sapida*) fruits

1. Introduction

Animals and plants have been fascinating sources of food with health advantages [1],[2]. Additionally, plant foods have a major role in the efficient hormonal balancing and nutritional health of men. Plant-based foods are essential parts of diets designed for both adults and newborns. This is because when plant-based foods are consumed, digested, and ingested, nearly all needed minerals and organic nutrients for humans are either directly or indirectly absorbed into the body [3]. In terms of biology, plants are autotrophic and capable of manufacturing their food [4]. According to research, the majority of Ackee apple fruit samples found in the wild contain some vital nutrients that play a viable role in human health such as minerals, water-soluble vitamins, different carotenoids, and carbs that the body needs. Additionally, these fruits. These fruits also contain phytochemicals with notable pharmacological properties [5],[6]. These phytochemicals include glucosinolates, flavonoids, flavones, phytosterols, phenols, and polyphenols. Low or inadequate intake of these nutrients results in malnutrition [7]. Malnutrition is caused by insufficient nutritional consumption, which puts people at risk for illnesses, impairments, and different hormonal dysfunctions [8], [9]. The nutritional analysis of the Ackee's

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apple (*Blighia sapida*) fruit as a source of corrective vitamin supplements to combat malnutrition has received little attention throughout the years. A fruit tree native to the Guinean woods of West Africa, the ackee's apple tree is prized for both its therapeutic and aesthetic qualities.

The ackee fruit aril, in particular, is edible and can be consumed fresh, dried, fried, boiled, roasted, or prepared into sauce or soup. The dried ackee's arils and ackee's soap, formed from the ash of fruit pods and seeds, are marketed in local marketplaces in some parts of Nigeria, and they provide significant income to growers and dealers, who are primarily women [10]. This paper aims to investigate the microorganisms associated with these underused fruit samples in the wild, their diverse nutritional composition, and the anti-nutrient elements to ensure an appropriate dietary intake of all key nutrients in food formulation.

2. Material and methods

2.1. Sample Collection and Preparation

Fresh Ackee apple fruit samples were obtained from ten different Ado Ekiti, Ekiti State, Nigeria. The Department of Plant and Biotechnology at Ekiti State University in Ado-Ekiti, Nigeria, recognised the fruit sample that was gathered. The fruit sample was separated, finely cut using a stainless steel knife, ground, and kept in the refrigerator at 4°C until further investigation. To determine the composition of the nutrients in the pulverised samples, a portion of the samples was sun-dried for a week and kept in the refrigerator.

2.2. Methods

2.2.1. Isolation of Microorganisms

2.3. One gramme of the fresh Ackee apple fruit was crushed, weighed, and added to ten millilitres of sterile distilled water in a transparent test tube. After giving it a good shake, 1 millilitre was put into 9 millilitres of sterile, distilled water in a transparent test tube. After that, 1 millilitre was taken and transferred to the next test tube, and so on, until there were 10^6 test tubes serially diluted. The 10^{-3} and 10^{-4} fold dilutions of 1 mL each were aseptically pipetted into sterile Petri plates of Nutrient Agar and Potato Dextrose Agar respectively in duplicates. To ensure uniform dispersion of the inoculum, they were gently shaken for five seconds, Nutrient Agar plates (bacteria) and Potato Dextrose Agar plates (fungi) were inverted and incubated for 24 hours at 37 ^{0}C and 72 hours at room temperature respectively. The microorganisms were sub-cultured from the initial cultured plates to obtain pure isolates. The morphological identifications were done by checking for colour, elevation, surface, size, and spore count are among the features that were recorded. Microscopic examination and biochemical reactions were carried out.

2.4. Nutritional composition

2.4.1. Proximate Analysis

The crude protein, moisture, crude fibre, and fat contents of the samples were determined using AOAC (Association of Official Analytical Chemists) methods [11]. These analyses were done in three copies. Each parameter's approximate values were given as a percentage. Ash concentration was assessed by ashing at 550 °C for three hours. However, the Kjeldahl technique was used to determine the crude protein (AOAC [11]. The calculated nitrogen value from the experiment and math was multiplied by a conversion factor (6.25). The samples' crude fibre content was assessed using a digestion technique, and their lipid content was assessed using a Soxhlet extraction technique (AOAC [11]. The difference in the sum of the total soluble carbohydrate measurements was used to calculate.

2.4.2. Mineral Analysis

According to the procedures outlined by the Association of Official Analytical Chemists (AOAC) [12], the mineral contents of monkey kola and ackee's apple were measured using an atomic absorption spectrophotometer (AAS-Buck 205). These minerals included Ca, Mg, K, Na, Fe, Zn, Mn, and Cu. According to AOAC [12], phosphorus was determined calorimetrically. Sodium, iron, zinc, phosphorus, manganese, and copper readings were stated in parts per million, whereas calcium, magnesium, and potassium values were provided as percentages (ppm).

3. Results and discussion

3.1. Microorganisms, Proximate, Vitamin and Mineral Analysis

Fresh produce consumption has increased mostly as a result of greater public knowledge of the advantages of a healthy diet and the results of benefits of fresh fruits [13]. Due to this, consumers are now demanding a wider variety of products, including minimally processed, prepackaged, ready-to-eat fruits and vegetables [14], as well as food that is available out of season. Produce that has been prepared, such as packaged salads, may offer the right environment for human diseases to grow and survive. In this research, the bacteria isolated from the Ackee apple fruits include *Pasteurella* sp., *Microcococcus* sp., *Pasteurella peumotropica,* and *Bacillus laterosporus*, while the fungus included *Aspergillus niger, Aspergillus flavus, Mucor* sp., and *Rhizopus* sp. All of the discovered bacteria may be considered to be likely inhabitants of naturally ripe Ackee apple fruits. It is important to keep in mind that this is an assumption that may not be accurate since some of them may contain airborne contaminants as they must naturally open before being harvested. Although apple juice (known as cider in the USA) is a fascinating carrier because of the product's acidity, which is thought to impede bacterial growth, leafy vegetables are more frequently associated with *E. coli* infection. Since ruminant faeces frequently contain *Escherichia coli* O157:H7, grazing livestock in orchards may contaminate fallen apples with faeces. Because *E. coli* O157:H7 can thrive in damaged apple tissue [15], this could lead to the contamination of unpasteurized fruit juices and/or ciders. Fruit flies were shown by [16] to be a significant vector for the pre-harvest and postharvest *E. coli* O157:H7 contamination of apples. Microorganisms are present everywhere, including in water, soil, faeces, and on plants [17]. A variety of fresh food, such as sprouting seeds, asparagus, broccoli, cauliflower, carrot, celery, cherry tomatoes, courgette, cucumber, lettuce, mushroom, pepper, turnip, and watercress, have been shown to have *Aeromonas* [18]. According to epidemiological data, salad vegetables, including lettuce, sweet potatoes, cucumber, melon, and strawberries, are the second-highest risk factor for *Campylobacter* infection after poultry [19]. From spinach, fenugreek, lettuce, radish, parsley, green onions, potatoes, and mushrooms, [20] isolated *C. jejuni.* Although comprehensive investigations of raw, organic, and prepackaged salad veggies have been conducted, [21] found *Campylobacter* spp. on 1–6-3% of the vegetables they studied.

The pathogens *E. coli* O157:H7 and *Listeria monocytogenes* have been demonstrated to cling to the cut surfaces of lettuce leaves and penetrate the internal tissue, showing protection against chemical sanitisers. Cut surfaces also leak nutrients that can be used for growth [22]. However, examinations of packed salads often find few enteropathogens. [23] analysed the illness risks associated with various dietary categories and found that salad vegetables had a low-risk ratio, even though fresh produce was said to sometimes or significantly affect the incidence of disease in a community. The rise in imports to satisfy customer expectations for a wide selection of exotic fruits and vegetables year-round is another issue that has a direct influence on microbiological quality.

The risk for product contamination may be raised, and consumers may be exposed to large numbers and/or various strains of pathogens since hygiene standards at harvest, during storage, and in irrigation water might range greatly between nations. However, some outbreaks have been connected to the imported product, such as lettuce imported into the UK from Spain in 2005. Imported produce from Mexico has been proven to be of equal microbiological quality to domestic samples in the USA [24]. The majority of fresh produce-related infections are caused by *Salmonella* spp., which was found in 48% of cases in the USA [25] and 41% of cases between 1992 and 2000.

Two significant tomato-related Salmonella outbreaks in the USA during 2006 were responsible for 23% of all Salmonella infections that were recorded [26]. A variety of fresh fruit and vegetable items, most frequently lettuce, sprouting seeds, melon, and tomatoes, have been linked to Salmonella illness. Mushrooms, lettuce, cauliflower, sprouts, mustard cress, endive, and spinach are among the vegetable samples frequently found to have *Salmonella* spp. [27]. The infective dosage of enteric viruses is minimal, and they may survive exposure to low pH (3) and high or low temperatures. For instance, freezing increases viral persistence [28]. Therefore, regardless of processing circumstances, contaminated food is expected to continue to be infectious, regardless of processing conditions, although [28] suggested that exposure to plant metabolites such as phenolics may cause loss of activity. Hepatitis A epidemics connected to spring onion and lettuce eating were related to sewage-contaminated water [29]. Food can be contaminated by contact with an infected food handler, and as viruses can survive on inanimate objects and surfaces, inadequate hygiene procedures can result in food contamination. Several outbreaks have been linked to workers who prepare or pick food who are infected with the disease [30]. The manner a product is produced or kept might provide some microbial groups with a selective advantage for development, therefore the total number of bacteria is not the only factor in determining quality. In addition, fungal isolates from this study such as *Aspergillus niger*, *Aspergillus flavus, Rhizopus stonifer,* and *Mucor* species may be contaminated by the environment. In particular, *Rhizopus stonifer,* an opportunistic fungus, handling, or airborne microorganisms may cause *Rhizopus stonifer* to contaminate Ackee apple fruits. Fruit moisture content might facilitate microbial infiltration as well. Aflatoxin, a toxin secreted by *Aspergillus* species, is toxic to both humans and animals. When the fruits of the Ackee apple are ingested together with a toxin or infection, this may result.

Table 1 Proximate Analysis of Fresh Ackee Apple Fruits

S/N	% Ash	% Moisture content	% Crude protein	% Fat	% Fibre	% Carbohydrate
1.	2.60	40.21	5.67	11.56	3.50	35.46
2.	3.63	40.24	5.32	11.54	3.48	35.79
Average	3.62	40.23	5.50	11.55	3.49	35.63

Table 2 Mineral content of Ackee Apple Fruits (mg/100 g)

Mineral Content	parts per million (ppm)
Weight used	1.085g
Sodium	201.49 mg/100g
Potassium	652.91 mg/100g
Calcium	8.24 mg/100g
Zinc	258.68 mg/100g
Magnesium	20.86 mg/100g
Manganese	0.05 mg/100g
Copper	0.005
Cobalt	-
Selenium	-
Iron	48.6 mg/100g
Phosphorus	7.87 mg/100g

Table 3 Functional Property of Ackee Apple Fruits in Percent (%)

Functional property	%
Oil Absorption	144.5
Water absorption	378.06
Foaming capacity	14.5
Foaming stability	4.0
Emulsion capacity	8.5
Least gelation concentration	6 % w/v

The proximate analysis of *Blighia sapida* fruit samples was determined as shown in Table 1. The mineral analysis of the two fruit samples under investigation is shown in Table 2. According to [31], the Ackee apple fruit sample has a moisture content of 40.23 %, which is lower than 64.35 %. Based on their percentage of moisture, it can be assumed that the fruit samples are perishable due to microbial and biochemical processes [32]. The least amount of nutrients found in Ackee apple fruit was found to be 11.55 % fat, 3.62 % ash, and 3.49 % fibre. This study found that Ackee apples had a carbohydrate level of 35.63 %, which is greater than the findings of [33], who claimed that the fruit had a low carbohydrate content. The functional property of Ackee apple fruits was 44.5 oil absorption, 378.06 water absorption, 14.5 Foaming capacity, 4.0 Foaming stability, 8.5 Emulsion capacity and 6 % w/v Least gelation concentration.

Table 4 Amino acid Profile of Ackee Apple fruit

Amino acid	1 NH (mm)	2 NH/2 (mm)	3 W (mm)	4 NH x W	5 NE	6 Sstd	(Y) 1 x3x6xC g/100gp	(Y) x 100 mg/100p	Y/100 gcp	mg/100 gcp	mg/100 gcp	Mg/gcp
Lysine 'c'	79.0	39.5	5	395	2.76	12.6	3.339	3339	33.39	3.382	3382	33.842
Histidine'c'	74.0	37	5	270	3.10	16.25	4.034	4034	40.34	4.06	4060	40.60
Arginine 'c'	40.0	20	13	520	2.26	9.84	3.433	3433	34.33	3.076	3076	30.76
Asparagine	28.0	64	4	512	3.1	10.32	7.08	7080	70.8	4.016	4016	40.16
Theonine 'c'	58.0	29	5	290	2.25	6.7	2.63	2603	26.3	1.767	1767	17.67
Serine	50.0	25	4	200	2.53	6.65	1.78	1780	17.8	5.89	5890	58.90
Glutamic acid	132.0	66	5	660	2.88	0.59	9.365	9365	9365	0.563	563	5.63
Proline	100	50	4	400	5.65	16.26	8.71	8715	87.1	8.068	8068	80.68
Glycine	77	38.5	5	385	2.99	5.61	2.89	2890	28.9	2.899	2899	28.99
Alanine	10	5	6	60	2.92	6.5	0.52	520	5.2	0.523	523	5.23
Cysteine	55	27.5	2.5	137.5	1.94	11.65	2.15	2150	21.5	2.128	2128	21.28
Valine	27	13.5	2.5	67.5	3.29	9.64	0.87	870	8.7	0.699	699	6.99
Methionine 'c'	40	20	3.5	120	3.15	11.75	1.89	1890	18.9	1.881	1881	18.81
Isoleucine	43	21.5	4	172	1.92	6.29	1.45	1450	14.5	1.41	141	14.1
Leucine	88	44	4	352	1.99	6.53	3.08	3080	30.8	3.064	3064	30.64
Tyrosine	16	8.0	8	128	2.98	13.49	2.31	2310	23.1	2.36	2360	23.60
Phenyalanine 'c'	20	10	11	220	2.64	10.9	3.21	3210	32.1	3.357	337	33.57

Key: E represents Essential amino acid; N represents non-essential amino acid; Cp represents crude protein; Sstd represents standard deviation; and W represents weight

Furthermore, compared to the results reported by [33] (9.38+0.01), Ackee's apple's crude protein percentage of 5.5% is low. In comparison to Jackson's nutritional study, the crude protein content yields better findings [33]. According to [34], protein is a natural substance that contains nitrogen and is crucial for both human and animal existence and health. Due to its ability to aid in the development and repair of both adult and newborn tissues, the high protein content of both fruit samples is crucial in food composition [4]. In addition to the proximate value, Table 2 showed the mineral composition of the Ackee apple fruit samples revealing that the fruit has a high concentration of body-building metals that support healthy bone growth and the growth, development, and maintenance of body tissues. According to a paper by [32] that examined the nutritional composition, and mineral component of Ackee apple fruits, potassium (6529.1 ppm) had the greatest mineral content for the fruit (4970.18 ppm). Following potassium, sodium (2014.9), phosphorus (486), magnesium (208.68), zinc (2586.8), and copper (208.68) were the elements with the lowest values. Selenium and cobalt were not found. As a result, fruit samples with this substantial mineral content improve blood flow, prevent paralysis, and build the body.

Other important minerals that are noticeable in Ackee's apple are magnesium, calcium, and sodium, with values of 498.01, 478.56, and 398.80. These minerals are necessary for managing the contraction and relaxation of the skeletal and muscular systems, and a high calcium intake promotes the formation of bones and teeth [35]. Due to their crucial roles in enzymatic activities, minerals like cobalt (which is necessary for the biosynthesis of vitamin B12 co-enzymes), copper (which includes cytochrome c oxidase), and iron are needed in trace amounts (RDA 200 mg/day). They are plant polyphenols that are water soluble and precipitate proteins [36].

4. Conclusion

The research on Ackee's apple fruit specifically reveals that contaminations may occur if not well handled, and washed before consumption. The majority of people consume these fruits raw without processing them. In due course, they can be carriers of pathogenic microorganisms. It is advisable to ensure thorough washing and proper hygienic practice to rid of contaminations. It was observed that the Ackee apple fruits are perishable owing to their high moisture content but are also easily absorbed by the body. Because both fruit samples contain a considerable quantity of protein, it also suggests a body-building role. Their mineral content, particularly magnesium, potassium, and phosphorus, has a curative effect on malnutrition, which is caused by a deficiency in essential minerals in the body. The inclusion of these fruit samples in meal formulations will significantly assist in providing the bodies with the necessary minerals for optimal enzymatic performance.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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