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Phytochemical and Physicochemical Characterization of Starches from Non-Edible Sources: *IcacinatrichanthaOliv* and *AnchomanesdifformisBlume*.

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Abstract: *Two non-edible tubers IcacinatrichanthaOliv (ITO, family: Icacinaceae) and Anchomanesdifformisblume (ADB, family: Araceae) and their starches were characterized for the phytochemical, proximate and physicochemical properties. The phytochemical screening of the ethanolic extract of the tubers showed the presence of phenols, tannins and saponin in only ADB. The analysis also indicted the presence of carbohydrate, flavonoids and alkaloids and absence of steroids and glycosides in both biomass. The proximate analysis (%) indicated that ITO tuber contains (moisture -9.20, ash -3.50, protein -6.51, fat -5.60, carbohydrate -71.43, fiber -3.71) and ITO starch contain (moisture -10.40, ash -0.80, protein -4.38, fat -2.80, carbohydrate -80.79, fiber -0.64) while ADB tuber contains (moisture-5.20, ash-2.40, protein-5.25, fat-4.10, carbohydrate-78.57, fiber-4.48) and ADB starch contains (moisture -8.10, ash -0.30, protein -4.30, fat -1.50, carbohydrate -85.27, fiber -0.56). The starch isolated from the tubers using 1% sodium metabisulphite gave a yield of 64.9% and 27.3% for ITO and ADB respectively. The physicochemical properties show the following; ITO had gelatinization temperature (78^{o}C), pH (6.2±0.1), amylose content (11.2±0.01%)water absorption capacity (71±0.2%) and swelling power of 14.5g/g at 90^{o}C while ADB had gelatinization temperature (73^{o}C), pH (5.8±0.1), amylose content (15.02±0.01%) water absorption capacity (83±0.2%) and swelling power of 12.1g/g at 90^{o}C. These properties when compared to other conventional starches shows that both ITO and ADB are potential sources of industrial starch.*

Keywords: *AnchomanesdifformisBlume, IcacinatrichanthaOliv, Non-edible starch, Phytochemical, Proximate, Physicochemical Properties*

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I. Introduction

Starch, a polysaccharide consisting mainly of D-glucose as its monomer unit is a valuable raw material for many industrial processes such as food, paper, textile and ethanol industries[1,2]. Many cereals and tubers such as corn, cassava, wheat, rice has been found to have starch content of more than 60% and as such, used as edible food source for many tropical and sub-tropical countries [2,3]. Starch consists of mainly two polysaccharide type: Amylose and Amylopectin. Amylose which occupies approximately 15-30% of starch is a linear molecule with α-1,4 glucan linkage while amylopectin the major component of starch with 70-85% is a highly branched molecule with α-1,6 glucan linkage [1,4]. Studies have shown that amylose and amylopectin content of starch influences many industrial processes such as susceptibility to enzymes and acids especially in biorefinning[1, 5, 6]. Their proportion depends on the starch source or variety [2, 7].

Most starches used for industrial productions are obtained from edible crops like corn, cassava, sweet potato etc, which have an overall effect on the price of commodities in the market. In 2012, the cost of starches and their derivatives stood at US$ 51.2 billion and was further projected to reach US$ 77.4 billion by 2018 [4, 8, 9, 10].In petroleum industry, chemically modified starches are used in water based drilling mud to enhance fluid loss and viscosity in the formation during drilling.In the quest to reduce the effect of global warming and climate change through the use of biofuels, over 80% of the global bioethanol production are from these edible crops: corn (U.S.A), cereals (Europe), cassava (China and Nigeria)[11,12]. The use of these crops has serious effect on the food security as millions of people in the world are without sufficient food. Secondly, the use of these edible crops as raw materials account for 60-75% of the total ethanol production cost making the process expensive[13].These have led to an increased search for non-food biomass and technologies that would eliminate or mitigate these problems.

IcacinatrichanthaOliv (ITO) from the family of Icacinacea is a small perennial shrub that grow up to 2-3m above the ground. The plant is indigenous to central and west Africa and is predicted to have high resistance to drought where it can survive at least four years without rain[14]. It produces a large underground tuber which can weigh up to 3kg in southern part of Nigeria [15]. The tuber is not edible; the plant is reported to grow as weed in Edo state, Nigeria [14,16]. ITO is known by different tribes in the southern part of Nigeria, it is called gbe-gbe which translate into "throw-away" by the "Yorubas" in south-west Nigeria, Urumbia, ofo-ala and Eriagbo (which means eat and vomit) by the "Igbo" speaking tribe in eastern Nigeria. ITO tuber is not a conventional source of starch, but studies has shown that it contain a high carbohydrate content in the range of 71.13-91.93% [15, 17].

AnchomanesdifformisBlume (ADB) is a wild herbaceous plant that belongs to the family of Araceae. It is characterized with prickly stem with divided leaves and spathe that sprought from a horizontal tuber. The plant grows about 2meters from the ground surface with its tuber measuring about 80cm long and 20cm wide. The tuber is slightly off white in colour and has high carbohydrate content of 88.9% [18]. The tuber is not edible in Nigeria especially in southern region where it grows as a wild plant because it causes irritation to the mouth, tongue and throat if eaten. The plant is known as abrisoko by the "Yorubas" in south west Nigeria [19].

This study is aimed to characterize and compare the phytochemical parameters and physicochemical properties of ITO and ADB starch and the importance of these starches in bioethanol production.

II. Materials And Methods

Fresh tubers of ITO was harvested from AmankutaMbieri in Mbaitoli L.G.A, of Imo state, while the tubers of ADB was harvested from Alakahia, in Obio-Akpor, L.G.A Rivers state, both in Nigeria. The tubers were peeled, washed, sliced into pieces and oven dried at $60^{O}C$ before milling to powder

Proximate Analysis:

The proximate (carbohydrate, fat, protein, fiber and moisture) content of the tubers were determined using the standard method of analysis[20]. The oven dried method at 105 °C was used to determine the moisture content. The protein content was determined by calculating the nitrogen content ($N \times 6.25$) usingKjeldahl method, carbohydrate by Anthraone method, the crude fat was determined by continuous soxhlet extraction with petroleum spirit.The ash content was evaluated by furnace method at temperature 600 °C for 3h followed by weighing after cooling to room temperature in a desiccator,while the crude fiber content was determined by calculating the percentage difference of all the parameters obtained above in accordance with method A.O.A.C [20].

Therefore %fibre = 100-(% moisture +% protein + % fat + crude carbohydrate + % ash).

Phytochemical analysis:

The ethanol extract of each biomass obtained from 100g of the dry samples were used for the analysis. The phytochemical parameter of each extract was determined according to the methods described byVimalkumar[21].

Starch isolation:

The starch extraction from the tuber of each biomass was done as described by Omojola[22] with slight modification. 200g each of the dried milled tubers were soaked in 500ml of 1% sodium metabisulfite at $28^{O}C$. The mixtures were allowed to stand for 24hours before they were filtered through a muslin cloth in a large volume of water. The starch of ITO settled after 24hours and was collected using a whatman (No1, 90nm) filter paper, whereas that of ADB which did not settle and was centrifuged (Sorvall Instrument GLC-4, Dupont) at 3500rpm for 10mins. The supernant was carefully decanted and the starch scrapped off. The resultant starch was air dried for 24hours and was further dried to constant weight at $60^{o}C$ in an oven. Each starch was weighed and stored in an air tight container.

Physicochemical characterization.

The gelatinization temperature and swelling power of ITO and ADB starches were determined according to the method of Omojola[22]. The method described by Ogunwa[15] was used to determine the pH of each starch. 2ml of distilled water was added in a flask containing 0.5g of each starch and the mixture was agitated for 3mins, the suspension was filtered through a filter paper (whatman No1, 90nm). The filtrate was then tested for its acidity or alkalinity with the use of pH meter (Hanna instrument model).The amylose content of each starch was evaluated by calorimetric iodine affinity procedure developed by Juliano[23]. Each starch sample(100mg) was weighed into a 100ml flask, 1ml of 95% ethanol and 9ml of 1MNaOH was added to the flask. The flask was covered with a foil and the mixture was heated in a water bath for 10mins to gelatinize the starch. Subsequently, the solution was made up to 100ml with distilled water and content completely homogenized. 5ml of the solution was pipette into another 100ml volumetric flask, 1ml of 1N acetic acid followed by 2ml of iodine solution was added and the solution made up to mark with distilled water. The solution was thoroughly shaken and allowed to stand for 20mins. The absorbance was determined at 620µm using UV-Visible spectrophotometer (Jenway 6305 spectrophotometer).

III. Result And Discussion

The result of the proximate analysis(Table 1) of the starch and tubers shows that ITOhasa moisture content of 9.20g/100g and 10.40g/100g for its tuber and starch while ADB have 5.20g/100g and 8.10g/100g for its tuber and starch respectively. The moisture content of the starch of both biomass compares well withthose of corn (8.1g/100g), potato (8.5 g/100g), rice (8.3 g/100g) and cocoyam (6.92 g/100g)[24, 25]. Research has shown that flour with moisture content lower than 11% have better shelf life which is an important parameter in the storage of flour, with levels greater than 12% susceptible to microbial attack [25].

Table 1: Proximate composition of raw tubers of ITO and ADB

Components (g/100g)	*Icacinatrichantha*		*Anchomanesdifformis*	
	Flour	Starch	Flour	Starch
Moisture	9.20	10.40	5.20	8.10
Ash	3.50	0.80	2.40	0.30
Protein	6.51	4.38	5.25	4.30
Fat	5.60	2.80	4.10	1.50
Carbohydrate	71.43	80.89	78.57	85.27
Fibre	3.71	0.64	4.48	0.56

Most edible crops have high carbohydrate content such as rice 89.78 g/100g , corn 90.48 g/100g , potato 90.75 g/100g , cocoyam 80.94g/100g and cassava 79.38-83.10 g/100g and found use in pharmaceutical, cosmetics, food and bio-refining industries[24,25,26]. The present study shows a carbohydrate content of71.43 g/100g and 80.89g/100g for ITO tuber and starch while ADB tuber-78.57 g/100g and starch-85.27g/100g compares well with other crops. Abe and Lajide[27] obtained (73.09-88.87) for ADB while Omojola[22] and Ogunwa[15] obtained 88.91g/100g and 71.13g/100g for ITO starch and tuber respectively. The result also shows that ITO tuber has a fibre content of 3.71g/100g which is lower than the fibre content of other crops as reported [15]. This may be attributed to the plant source or the age of the tuber before harvesting as research has shown that the tuber have high resistance to drought and can survive up to 2-3 planting seasons [14].

Table 2, shows the result of the phytochemical screening of the ethanol extract of the tubers. This was done to determine the presence or absence of each phytochemical, because report has it that some metabolites like polyphenols has inhibitory effects on microorganisms during the fermentation processes.[28, 29].

The extract of bothbiomass showed the presence of carbohydrates from the Molish test but negative to Fehling solution test which indicate the absence of reducing sugars. The result reveals the strong presence of phenols, tannins and saponinin ADB extract while they are conspicuously absent in the extract of ITO, this agree with the work of Shagal[30]. The presence of these metabolites in ADB may indicate a strong antioxidant property as well as greater inhibition on microorganisms than in ITO. In addition, the extract of ADB exhibits a stronger presence of alkaloids than those of ITO in both Mayer and Dragendroff's test whereas ITO shows a negative test to Wagner test. However, both extracts invariably showed absence of steroids and glycosides. Studies have shown that antioxidants such as phenols and flavonoids rich compounds have inhibition effect on microorganisms[31, 32] and potential in disease prevention and therapy [33]. Hence the present study reveals the stronger antioxidant and antimicrobial ability of ADB than ITO. This confirms the report of Oyetayo[19] that extracts obtained from ADB tuber have more pronounced antibacterial effect.

Table 2: Phytochemical screening of the ethanol extract of ITOand ADB tubers.

Phytochemicals	Tests	*Icacinatrichantha*	*Anchomanesdifformis*
Phenols/ Tanins	$FeCl_3$ test	-	++
	Acetic Acid test	-	++
Flavonoids	Alkali test	+	++
	Shinoda	+	++
Carbohydrate	Fehling solution test	-	-
	Molisch test	++	++
Alkaloids	Wagner test	-	+
	Mayer test	+	++
	Dragendroff's test	++	++
Saponin	Water	-	++
Steroids	Liebermann-Burchard	-	-
Glycosides	Liebermann test	-	-

Legend: (-) absent, (+) low or reduced (++) Abundant

Table 3: Physicochemical properties of ITO and ADB starches

PARAMETERS	ITO	ADB
Appearance/Colour	Off white	Off white
Odour	Odourless	Odourless
Starch Yield (%)	64.9 ±0.2	27.3±0.2
Ph	6.2±0.1	5.8±0.1
Gelatinization Temperature (°C)	78	73
Amylose Content (%)	11.2±0.01	15.0±0.02
Water Absorption Capacity	71.0±0.2	83.3±0.2
Swelling Power (SP)		
50 (°C)	4.6	7.4
60 (°C)	5.3	8.9
70 (°C)	6.8	8.0
80 (°C)	13.4	11.9
90 (°C)	14.5	12.1

The result of the physicochemical properties of ITO and ADB starches are shown in Table 3. The physicochemical properties of a starch clearly define its industrial application and these properties depends on the biological origin or variety [2, 7, 18, 24]. The starches obtained from the tubers are slightly off white in colour and the results are reported in average value of triplicate measurement.

The starch yield obtained from ITO (64.9%) is very high when compared to that of ADB (27.3%), this may be attributed to the easy of extraction from ITO than ADB. The high starch yield of ITO may be related to the bulk density and starch granule size as reported by Ogunwa[15] who obtained a starch yield of 61% from his work with ITO. Starch yield and its easy of extraction is a key factor to consider when choosing a starch source as raw material for industrial application [15]. This therefore offers ITO an edge over other conventional crops in terms of starch yield. The amylose content of ADB (15.0±0.02) is higher than that of ITO (11.2±0.01), both results are higher than ones reported by [15, 18, 22]. The amylose content of both starches are lower than those of cassava (22.11-27.96) and corn (35.77-40.68) as reported by Adejumo[1].

Studies have shown that starches with high amylose content such as corn and cassava form gels with good mechanical property and thus show high resistance to chemical and enzymatic degradation [24]. This agrees with the work of Aprianita[3] where taro (cocoyam) starch with lower amylose content have higher enzymatic digestibility than yam with higher amylose content. Thus the lower amylose content of both biomass especially ITO is an indication that there will be lower resistance to chemical and enzymatic digestibility.The gelatinization temperature of ITO and ADB were observed at 78°C and 73°C respectively. The gelatinization of ITO and ADB falls within the range observed by other starches such as cocoyam (79.75), sweet potato (73.57) and yam (78.70) [3]. ADB have higher water absorption capacity when compared with that of ITO. The ITO result is in line with the work of Ogunwa[15] but lower than that reported by Omojola[22] while that of ADB is higher than that reported by Afolayan[18].

There is a marked increase in the swelling power of ITO with temperature as shown in the swelling power profile in figure 1: this is not the case with ADB which shows a slight decrease in its swelling power between 60-70°C before further increase from 70-90°C. The slight decrease with the swelling power of ADB was attributed to two sets of internal bonding forces that relax at different temperature [18]. An increase in the swelling power of a starch is an indication of its suitability for industrial application especially in pharmaceutical and petroleum industries[34].

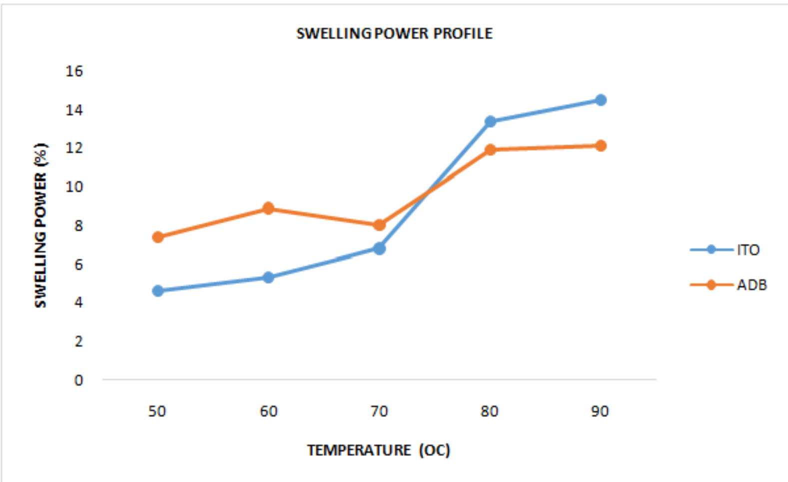

Figure 1: Swelling Power profile of ITO and ADB.

IV. Conclusion

The physicochemical properties such as carbohydrate content, amylose content and starch yield among others have shown that ITO and ADB compare favorably with other conventional crops and thus are good source of starch for industrial applications. The high starch yield of ITO shows that it can be used as an alternative source of starch for industrial applications. The high swelling power of the starch implies that they can be effectively used petroleum and pharmaceutical industries. The use of starch from ITO and ADB for industrial applications will greatly reduce the burden on other crops like corn and cassava, it will also ensure food security and reduce the cost of commodities in the market.

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