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Profiling the *In vitro* starch contents of some underutilized tubers flours as affected by some pre-processing operations.

Rachel Nnenna Orji *

Department of Food Technology, Akanu Ibiam Federal Polytechnic Unwana, Afikpo, Ebonyi State, Nigeria, P.O. Box 1007.

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Abstract

Aerial yam(*Dioscorea bulbifera*),cocoyam(*Xanthosoma robustum*) and water yam (*Dioscorea alata*) were analyzed for their starch contents using in vitro method after subjecting them to four pre-processing operations of boiling, sprouting, frying, roasting and a set was left untreated. Boiled Cocoyam (*Xanthosoma robustum*) was significantly($p<0.05$)different in total starch contents(58.50%) and rapidly digestible starch (55.88%) when compared to all the samples while among the processing operations, boiling as a treatment was significantly ($p<0.05$) different from sprouting, frying, roasting as well as the untreated.

Keywords: Yam *species*; Starch contents; Digestible starch; Pre-processing operations; *Dioscorea bulbifera; Xanthosoma robustum; Dioscorea alata;* Untreated yam flour.

1. Introduction

Recent interest in slow digesting starches due to their beneficial role in human health which includes moderate glycemic and insulin responses has led to so much research and discoveries on starch especially tuber starches. Starch can be defined as an essential source of energy for human diet. It is the major reserve in plant tubers and seed endosperm where it is found as granules[1](Ogori and Taofeek,2022).In food processing, starch plays a crucial role in the quality and nutritional value of many foods which is attributed to its multifunctional properties such as swelling, gelatinization, thickening and gelling [2](R Jia *et al*,2023).It has two major components; amylose and amylopectin. Amylose is the linear polymer of alpha-D glucose linked by alpha 1,4 glycosidic linkages while amylopectin is a branched polymer of alpha D glucose units linked by alpha 1,4 and alpha 1,6 glycosidic linkages[3](Dhull, *et al*,2021). Amylose have different effects on the glycemic index of a food in that they are harder to digest than amylopectin. This is because numerous branches in amylopectin molecules provide areas for digestive enzymes activity than amylose which is composed of straight chain molecules. Starches with higher amylose content have higher tendency to retrograde in the digestive track [4](Li *et al*,2015).

Starches can be further classified into three according to their digestibility: Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS), and Resistant Starch (RS). Rapidly Digestible Starch (RDS) are starches that can be rapidly converted to glucose within 20 minutes while Slowly Digestible Starch (SDS) delivers a slow and sustained release of blood glucose along with benefits resulting from low glycemic and insulinemic response,[5]((Jyothsna and Hymavathi,2017). The Resistant Starch (RS) is the portion of starch that is not digested in the small intestine but is fermented in the colon by micro organisms, resulting in the formation of long chain fatty acids, which may be associated with some metabolic effects, [6](Adrianna *et al*,2022).It is concluded that the RDS has a large impact in the glycemic response in humans, They improve carbohydrate, lipid metabolism and body weight. Resistant starch serves as

* Corresponding author: Rachel Nnenna Orji; Email: rachelorji123.ro@gmail.com

probiotic and improves intestinal function. The consumption of Resistant starch has positive effects on human health,[6](Adrianna *et al*,2022).

2. Material and methods

2.1. Study Site

The study was carried out in the Department of Food Technology, Akanu Ibiam Federal Polytechnic Unwana, Afikpo in Ebonyi State, and the analyses were done at the Department of Food Technology, Akanu Ibiam Federal Polytechnic Unwana, Laboratory of Food Science and Technology in the University of Agriculture, Umudike and Biochemical Department of the National Root Crops Research Institute (NRCRI) Umudike in Umuahia, Abia state.

The sample collection, preparation and investigations were conducted within three (3) months.

2.2. Raw Materials

Aerial yam (*Dioscorea bulbifera*), cocoyam (*Xanthosoma robustum*)and water yam (*Dioscorea alata*) were purchased in large quantity from Eke market in Afikpo in Ebonyi State.

2.3. Sample Preparation

Samples were prepared at the processing laboratory of the department of Food Technology, Akanu Ibiam Federal Polytechnic Unwana, Afikpo in Ebonyi State and the analytical grade chemicals and Enzymes used in the bench work for the analysis of this research were from the Laboratory of Food Technology, Akanu Ibiam Federal Polytechnic Unwana, Laboratory of Food Science and Technology Department, Michael Okpara University of Agriculture, Umudike, Umuahia and Biochemical Department of the National Root Crops Research Institute (NRCRI) Umudike in Umuahia, Abia state.

2.4. Raw materials preparations

The selected tuber crops; water yam, aerial yam and cocoyam were sorted (to remove bad or spoilt ones), washed with the back, peeled and washed again. They were then sliced into 2-3cm thickness, labeled and subjected to four different treatments: boiling, frying, sprouting and roasting.500g of each of the samples were boiled at 100^0C for 15 minutes for cocoyam and water yam and 100^oC for 20 minutes (for aerial yam).500g of each of the samples were deep fried at 160^oC and oil was drained off. Another 500g of each of the samples were sprouting for 7-21 days at 36^0C/room temperature. 500g of each of the samples were oven roasted at 180^0C for 20 minutes for all and 30 minutes for aerial yam .A set (500g) was left untreated which served as the control samples The samples were oven dried until a constant weight was achieved for each of the samples showing maximum drying.. Glucose was used as the reference food for the glycemic index evaluation.

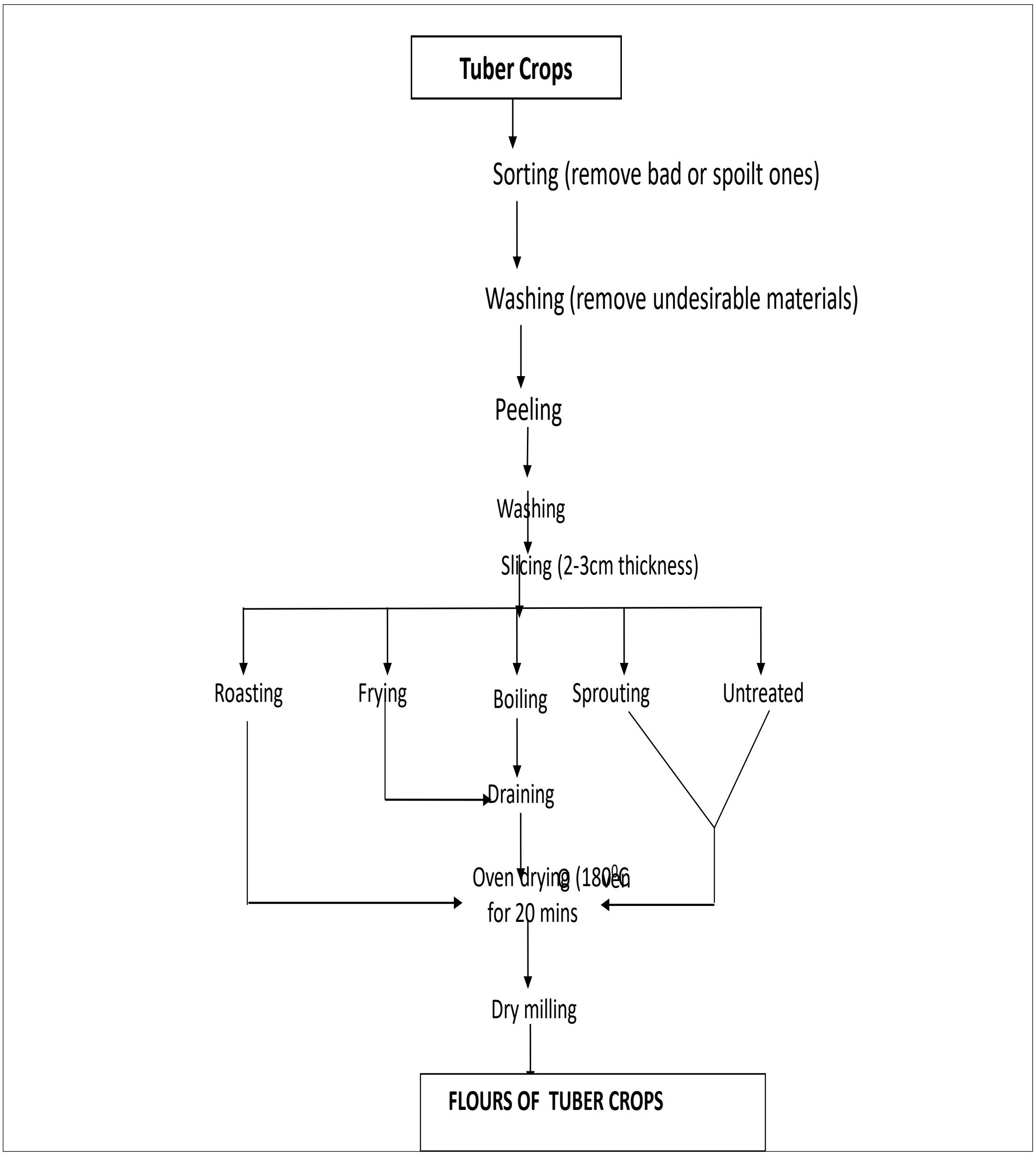

Figure 1 The production of the tuber flours with the different pre processing methods.

Source: [7]Olarenwaju *et al*,(2022)with some modifications.

Figure 2 Water yam(*Dioscorea alata*)

Figure 3 Sprouted Wateryam

	
Figure 4 Aerial yam(*Dioscorea bulbifera*)	**Figure 5** Sprouted Aerial yam
	
Figure 6 Cocoyam (*Xanthosoma robustum*)	**Figure 7** Sprouted cocoyam

2.5. Total starch (TS) determination

Total starch (Ts) analysis according to the modified in vitro method of [8]Goni *et al.* (1997) was used. The flour sample, fifty(50) mg was weighed and dispersed in six(6) mL of 2M KOH solution. It was incubated in a shaking water bath for 1 to 2 h at room temperature until total starch dissolved. Solubilized starch was then hydrolyzed by adding 0.83µL of amyloglusidase from *Aspergilus. niger* and incubating at 60 ^{o}C for 45 min in a shaking water bath. It was centrifuged at 4500g for 15min.The supernatant was collected. The glucose content in the supernatant was measured using a glucose oxidase-peroxidase kit and the total starch was calculated as below:

$$\text{Total Starch Ts (\%)} \quad \frac{0.9 \text{ x } CG}{100}$$

Where CG = Glucose concentration (µg/ml)

0.9 = Stoichiometric constant of glucose content conversion into starch

Ts (%) = Total starch expressed as percentage in dry matter

2.6. Resistant starch (RS) determination

Resistant starch (Rs) analysis according in vitro method of [9]Goni *et al.*(1996) was used. The flour sample, hundred(100) mg was incubated with a solution containing 20 mg of pepsin enzyme to remove any protein. Tris – maleate buffer was mixed with 40 mg pancreatic amylase and incubated at 37^{o}C for 16 h to hydrolyse digestible starch. The hydrolysates were centrifuged at 4500 g for 15 min and the residues were solubulized and incubated with 0.83µL of amyloglusidase from *Aspergilus. niger* at 60 ^{o}C for 45 min to hydrolyze the Resistant starch(Rs).The glucose content was measured using a glucose oxidase-peroxidase kit and Rs was calculated as mg of glucose x 0.9

2.7. Rapidly Digestible Starch (RDS)

This was calculated as the difference between the Total starch (TS) and Resistant Starch (RS) expressed as a percentage of the sample weight. Which is: RDS=TS- RS [10](Onwuka,2018).

Where: RDS= Digestible Starch

TS=Total Starch

RS=Resistant Starch

3. Result and Discussion

3.1. Total Starch (%)

The range of the samples' total starch were from (47.44—58.50%) and these were close to what [11]Afolabi *et al* (2016) reported on raw water yam flour (56.00%) and cocoyam flour (70.67%) but a little higher compared to what [12]Adegunwa *et al.,*(2011) reported on freshly sundried white yam flour (72.41%), freshly sundried water yam flour (62.94%) and freshly sundried cocoyam flour (65.33%),boiled and oven dried water yam flour (73.74%) boiled and oven dried cocoyam flour (68.37%), roasted and oven dried water yam flour (68.90%) and roasted and oven dried cocoyam flour (77.27%). Starchy roots and tuber crops play a pivotal role in the human diet. They add variety to the diet in addition to offering numerous desirable nutritional and health benefits such as antioxidative, hypoglycaemic, hypocholesteromic, antimicrobial and immunomodulatory activities,[13](Anoma and Thamilini,2016). Boiled cocoyam flour sample was significantly ($p<0.05$) different than all the other samples with total starch of (58.50%). This was in agreement with [14]Onyango (2016) report on starch content of boiled foods. it reported that individual starch granules absorb liquid and swell through a process called gelatinization which happens at different temperatures for different types of starch and can cause increase in starch contents. Sprouted cocoyam followed closely with total starch content of (53.50%).Fried aerial yam with starch content of (47.44%) was significantly lower than all the samples while among the species, cocoyam was significantly ($p<0.05$) different from all the other species.

Starch content of a food influences its suitability for formulations into different products including sweetners and ethanol,[3](Dhull *et al*,2021) .Thus high starch content such as exhibited by these samples is an indication that they posses good nutritional status and could be recommended as source of energy for both young and old people.

Table 1 Profiling the *invitro* Total Starch (TS) % contents of the selected underutilized tuber flours as affected by some pre-processing methods.

Processing Methods	Species		
	Aerial yam (*Dioscorea bulbifera*)	**Cocoyam (*Xanthosoma robustum)***	**Water yam (*Dioscorea alata*)**
Untreated	$50.20^{c}\pm0.02$	$52.53^{a}\pm0.02$	$51.13^{b}\pm0.02$
Boiled	$49.94^{c}\pm0.01$	$58.50^{a}\pm0.02$	$53.82^{b}\pm0.01$
Roasted	$49.14^{b}\pm0.02$	$54.24^{a}\pm0.01$	$50.99^{a}\pm0.01$
Fried	$47.44^{c}\pm0.01$	$51.28^{a}\pm0.01$	$49.62^{b}\pm0.01$
Sprouted	$50.04^{b}\pm0.01$	$53.50^{a}\pm0.01$	$51.04^{b}\pm0.02$

Values are mean ± standard deviation of duplicate determinations. Mean on the same column with the same superscript are not significantly different at $p<0.05$%.

Table 2 Profiling the *invitro* Resistant Starch (RS) % contents of the selected underutilized tuber flours as affected by some pre-processing methods

Processing Methods	Species		
	Aerial yam (*Dioscorea bulbifera*)	**Cocoyam *(Xanthosoma robustum)***	**Water yam (*Dioscorea alata*)**
Untreated	2.13^{a}±0.01	1.18^{b}±0.01	1.21^{c}±0.01
Boiled	2.96^{a}±0.01	2.62^{b}±0.01	1.68^{c}±0.01
Roasted	2.26^{a}±0.01	1.44^{c}±0.01	1.52^{b}±0.01
Fried	2.10^{a}±0.01	1.78^{b}±0.01	1.24^{c}±0.01
Sprouted	2.12^{a}±0.01	1.51^{b}±0.01	1.50^{b}±0.01

Values are mean ± standard deviation of duplicate determinations. Mean on the same column with the same superscript are not significantly different at p<0.05%.

3.2. Resistant Starch (%)

The range of resistant starch content of the samples were from (1.50—2.96%). These were higher to what [11]Afolabi *et al* (2016) reported after an *in vitro* starch digestion on cocoyam (4.88%) and water yam (15.23%). The resistant starch content of boiled aerial yam (2.96%) was significantly (p<0.05) different than all the other samples. This was followed closely by roasted cocoyam(2.44%) and sprouted aerial yam (2.12%) respectively . Resistant starch (RS) is defined as the sum of the starch and the products of starch degradation that are not absorbed in the small intestine of a healthy individual [5](Jyothsna and Hymavathi, 2017). However, boiling was significantly different (p<0.05) from all the processing methods in terms of resistant starch. This supported what [15]Inan-Eroglu and Buyuktuncer,2017) reported on boiling. They reported that boiling, frying microwave cooking and extrusion have the potential of increasing the amount of resistant starch in foods. Aerial yam samples showed high resistant starch content; (2.10-2.96%) and this is related to its health importance. [16]Bojarckzuk *et al*,(2022) highlighted that starchy foods with high resistant starch content have been shown to exhibit lots of health benefits including the prevention of colorectal cancer, slow rate of digestibility and provision of reduced calorific value.

Table 3 Profiling the invitro Rapidly Digestible Starch (RDS) % content of the selected underutilized tuber flours as affected by some pre-processing methods.

Processing Method	Species		
	Aerial yam (*Dioscorea bulbifera*)	**Cocoyam *(Xanthosoma robustum)***	**Water yam (*Dioscorea alata*)**
Untreated	48.07^{c}±0.01	51.35^{a}±0.01	50.92^{b}±0.01
Boiled	46.98^{c}±0.00	55.88^{a}±0.01	52.14^{b}±0.00
Roasted	46.88^{b}±0.01	52.80^{a}±0.01	49.47^{a}±0.01
Fried	45.34^{c}±0.00	49.50^{a}±0.00	48.38^{b}±0.01
Sprouted	47.92^{c}±0.00	51.99^{a}±0.00	49.54^{b}±0.01

Values are mean ± standard deviation of duplicate determinations. Mean on the same column with the same superscript are not significantly different at p<0.05%.

The samples showcased RDS content in the range of (45.34—55.88%).These were within the range of what [11]Afolabi *et al*.(2016) reported on water yam (40.77%) and cocoyam (65.79%) after an *in vitro* starch digestion. Boiled cocoyam with rapidly digestible starch of (55.88%) was significantly (p<0.05) different than the other samples. This was followed closely by roasted cocoyam (52.80%) and boiled water yam (52.14%). Fried aerial yam with RDS of (45.34%) was significantly (p<0.05) lower than all the other samples.

Rapidly digestible starch (RDS) has been used as an alternative method to evaluate starch digestion. RDS is the starch fraction digested *in vitro* within 20 minutes of hydrolysis. When the RDS is high, the starch is considered rapidly digestible,[5](Jyothsna and Hymavathi,2017). The samples were exposed to processing operations such as boiling, roasting and frying which can induce gelatinization in food materials containing starch thereby permanently disrupting the amylose –amylopectin structure of the starch complex thus making it accessible for digestive enzymes,[4](Li *et al*,2015).This may be one of the reasons why the samples showed a moderate proportion of RDS.

4. Conclusion

This work was done to profile the *in vitro* starch contents of some underutilised tuber flours: aerial yam(*Dioscorea bulbifera*), cocoyam(*Xanthosoma robustum)* and water yam(*Dioscorea alata*) subjected to some pre-processing operations of boiling, sprouting, frying an roasting and a set left untreated. Boiled cocoyam was outstanding in its total starch content(58.50%), followed closely by sprouted cocoyam(53.50%). Boiled aerial yam was different from the other samples in resistant starch content(2.96%) and roasted cocoyam was next(2.44%).The rapidly digestible starch content of boiled cocoyam stood out among others (55.88%) while fried aerial yam had the lowest value(45.34%) as regards this.

Boiling, as a treatment brought to limelight the starch contents of mostly cocoyam among the other treatments.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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