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The effect of chemical modifications on the alpha amylase digestibility of some varieties of yam flour in Nigeria

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ABSTRACT

Yam (Dioscorea *spp*), a staple Nigerian food has been studied to be low in amylose; low amylose starches are easily hydrolyze by alpha amylase and elicit higher insulin demand. This research was aimed at the chemical modifications of some yam varieties in Nigeria for possible attenuation in the alpha amylase digestibility. Two accessions of white yam 'Aro' and 'Efuru', Yellow yam and water yam were purchased from a yam market in Osun state, Nigeria. The tubers were made into flour and subjected to chemical modifications by acetylation, phosphorylation and citric acid esterification. The results obtained from the alpha amylase digestibility showed that acetylation significantly increased the digestibility of the modified samples in relative to native samples from 48.24±0.50g/100g to 54.44±0.45g/100g ('Aro' flour), 50.74±1.66g/100g to 51.51±0.77 g/100g ('Efuru' flour), 54.86±0.82g/100g to 56.95±0.97g/100g (Yellow yam flour) and 46.92 ±1.22g/100g to 54.72 ±0.91g/100g (Water yam flour). Phosphorylation with sodium tripolyphosphate significantly reduced the digestibility of 'Efuru' flour to 48.80±0.89g/100g) and yellow yam flour to 51.65±0.60g/100g while phosphorylation with sodium trimetaphosphate significantly increased the digestibility of all the modified yam flours. Very high attenuation in alpha amylase digestibility (74.54% - 90.85% digestibility reduction) were obtained from all the citric acid esterified yam flour samples.

Keywords: Dioscorea *spp,* acetylation, phosphorylation, esterification, alpha amylase, digestibility

INTRODUCTION

Yam is a multi-variant crop, comprising different species, varieties, and accessions. Nigeria accounts for 65.5% of global production [1]. White yam (*D. rotundata poir*), yellow yam (*D.*

cayennesis) and water yam (*D. alata)* are the mostly cultivated varieties in Nigeria. Yam is a major and preferred staple food for over 300 million people in West Africa [2, 3]. It is a source of digestible carbohydrate and its major nutritional component is starch, accounting for approximately 70-90% w/w of the tuber. Due to the high starch content, extensive research have been done which have revealed intra-varietal variations in their physicochemical and functional properties, among different cultivars [4-8]. The digestibility of starch is related to its source, particle size, ratio of amylose to amylopectin, amylose-lipid complex, amylose content, amylopectin chain length distribution, crystallinity, crystal type and other factors [9]. Studies have also shown that the differences in amylose content may elicit variations in characteristics such as digestibility, crystallinity, physical properties, functionality and glycemic indices. Such variations have been found to impact on the resulting metabolic effects and susceptibility of the native starch to α-amylase digestion [5, 10, 11]. Yam belong to tropical root tubers high in amylopectin and low in amylose (11% -30%) [5, 8, 10, 12, 13]. Low amylose starches are easily hydrolyzed by alpha amylase [14-16]. Hence, post prandial hyperglycaemia in regions where yams are major staples is expected [17-19]. Starch digestibility is of primary significance to health conscious, diabetic and hyperlipidemic individuals, as starches that are highly degraded tend to elicit higher insulin demand than those that are less digestible [20, 21].

Resistant starches (RS) is a fraction of starch that can escape digestion leading to its fermentation by several bacteria in the colon. The fermentation products (short chain fatty acids, e.g. butyric acid) are then absorbed into the colon and pass into the bloodstream where they play an important role in reducing blood cholesterol [22-25]. Another health benefit of RS is that as they are not digested, they can serve as part of the diet of obese and diabetic individuals [26]. For these reasons, these types of starches can be considered as functional starches [25]. RS4 (Resistant Starch type 4) is type of resistant that is obtained by chemical modification.

Chemical modified starch is a starch whose hydroxyl group has been converted by a chemical reaction [27]. The repeating glucose units in starch molecules provide abundant hydroxyl groups (positions of C2, C3 and C6), which are excellent sites for the incorporation of functional groups [28]. Chemical modification resulting in RS4 can manipulate the digestibility of starch by forming a steric barrier at the site of enzymatic action thus, inhibiting the digestive process and producing small amount of energy [29-31].

The types of chemically modified starches that are useful in food processing are the converted, dextrinized, esterified, cross-linked, stabilized and oxidized starches [28]. Cross-linking is generally performed by treatment of granular starch with multifunctional reagents capable of forming either ether or ester inter-molecular linkages between hydroxyl groups on starch molecules. Sodium trimetaphosphate (STMP), monosodium phosphate (SOP), sodium tripolyphosphate (STPP), epichlorohydrin (EPI), phosphoryl chloride ($POCL_3$), a mixture of adipic acid and acetic anhydride, and vinyl chloride are the main agents used to cross-link food grade starches. Starch ester is a kind of modified starches in which some hydroxyl groups have been replaced by ester groups. Citric, tartaric, maleate, and polylactic acids have all been used for starch esterification. [32-37].

The majority of research to date on chemical modification of starch for alpha amylase digestion resistivity have been based on starch isolated from the various food matrices [36, 37,41-46,48-57]. During starch isolation, substances that are of great nutritional value, such as minerals, proteins, fiber, and fats are removed. Moreover, the vast majority of starch consumption is based on flour. Therefore, the need to look at alpha amylase resistivity in the context of the food matrix. In this research work it is hypothesized that chemical modification of some varieties of yam flour in Nigeria, by acetylation, phosphorylation and citric acid esterification could synthesize resistant starch RS4 and thus attenuate the alpha amylase digestibility in the modified yam flours.

MATERIALS AND METHODS

Materials

Two accessions of white guinea yam (Dioscorea *rotundata*) 'Aro' and 'Efuru', Yellow yam (*D. cayanensis*) and water yam (*D.alata*) were purchased from a local market in Osun state, Nigeria. Pancreatic a-amylase type VI-B from porcine pancreas (EC 3.2.1.1, A3176) was obtained from Sigma Canada, Citric acid was obtained from Tianjin Kermel Chemicals, Acetic anhydride was obtained from Jiodine Chemicals Qingdao, while sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP) are the products of Molychem and Lobal Chemie respectively.

Yam flour preparation

The yam tubers were processed into flours using the method of Flores-Silva *et al.* [38]. Briefly, yam tuber was peeled, cut into 1 cm slices and immediately rinsed. Slices were dried at 45 °C in a convection oven and ground using commercial grinder to pass a US 50 sieve (300 µm) and stored at 25 °C in sealed plastic containers until further analyses.

Modification by acetylation

Acetylation was carried out according to the method of 'Rincón *et al.,* [39] using acetic anhydride [$(CH_3CO)_2O$] as a modifying agent. The flour sample (50 g) was dispersed in 250 ml of distilled water and stirred magnetically for 20 minutes. The slurry was heated to and maintained at 45^{o}C on a magnetic stirrer while the pH was adjusted to 10 with 2.5% of NaOH solution. Acetic anhydride (10 g; 20 g) was added drop wise to the slurry from a burette while maintaining the pH at 10 and the temperature at 45 ^{o}C. The reaction was allowed to proceed for 3 hours. After 3 hours, the pH was lowered to 7 with 2.5% HCl solution. The slurry was then washed three times by suspension in distilled water, centrifuged at 1500 r/min for 15 min. and dried in an air oven for 24 h at 45 °C. The dried modified flour was milled and passed through a 50-mesh sieve and packed for further analysis.

Modification by phosphorylation

Phosphorylation was carried out with sodium trimetaphosphate (STMP) and sodium tripolyphosphate (STPP) as modifier agents using the method described by Gutiérrez *et al.* [24]. Yam flour sample plus sodium sulphate (5% w/w) suspended in water was adjusted to pH 10 with 2.5% NaOH. The slurry was heated to 45 ^{o}C, 3% w/w STMP or STPP with respect to the weight of the flour was added. The pH was maintained at 10 and the temperature at 45 ^{o}C with continuous stirring on a magnetic stirrer, the reaction was allowed to proceed for 3hours. After 3 hours, the pH was lowered to 7 with 2.5% HCl solution. The slurry was then washed three times by suspension in distilled water, centrifuged at 1500 r/min for 15 min. and dried in an air oven for 24 h at 45 °C. The dried modified flour was milled and passed through a 50-mesh sieve and packed for further analysis.

Modification by citric acid esterification

Citric acid esterification of yam flours was prepared according to the method of Wepner *et al.,* [40] with some modifications [35, 41]. Citric acid (10%, 20%) per 100 g w/w flour on a dry basis was prepared by dissolving (10 g, 20 g) in 50 mL of distilled water with constant stirring. The pH was adjusted to 3.5 with 10 M sodium hydroxide solution, and the resulting solution was brought to a final volume of 100 mL by adding water. The citric acid solution was mixed with 100 g of yam flour in stainless steel trays and conditioned for 16 h at room temperature. The mixture was dried at 40 °C for 24 h until a moisture level of 5.0 – 10.0% w/w was reached. The samples were placed in a forced-air oven at 140^{o}C for 5hours. The dry products were cooled at room temperature for 30 min, and the unreacted citric acid was removed by washing the products repeatedly with distilled water. The washed product were dried at 45°C, milled and passed through a 50-mesh sieve and packed for further analysis.

Determination of alpha amylase starch digestibility

Exactly1% flour solutions of native and modified samples were prepared with 0.02 mol/L (PBS) sodium phosphate buffer saline (pH 6.9 with 0.006 mol/L sodium chloride) in appropriate beaker, covered with aluminum foil and then heated to boiling on heating mantle. About 1 ml of the 1% sample preparation was pipetted into test tube with the addition of 0.5 mg/ml porcine pancreas α-amylase in PBS solution. The reaction mixture was then incubated for 30 minutes at 37 °C for the digestion of the starch. The reducing sugar in the digested sample was determined using the dinitrosalycylic acid (DNSA) assay. 1.0 mL of DNSA color reagent (1 g of 3, 5 dinitrosalycylic acid with 20 ml of 2 M NaOH and 30 g sodium potassium tartrate made up to 100 mL with distilled water) was added to the test tube. The test tube was placed in a boiling water bath for 5 minutes to develop the colour and then cooled in an ice bath. After addition of 40 ml water, the absorbance was measured at 540 nm using uv/visible spectrophotometer. A standard curve was prepared using maltose (0.0 to 10.0 mg/ml) and a linear regression analysis was used to determine the total reducing sugar present as mg maltose equivalents

Statistical analysis

The data reported were the means of triplicate measurements. Statistical analysis were carried out with Duncan’s multiple test ($P \leq 0.05$) using IBM SPSS statistics version 23 software.

RESULTS AND DISCUSSION

Alpha amylase digestibility results of the native samples and the acetylated samples are as shown in Table 1

Table 1: α- Amylase digestibility of acetylated yam flour (g/100g DWB)

Modification	α- Amylase Digestibility [g/100g]			
	white yam 'Aro' flour	white yam 'Efuru' flour	Yellow yam Flour	Water yam flour
NS	48.24^{a}±0.50	50.74^{a}±1.66	54.86^{a}±0.82	$46.92\ ^{a}$±1.22
AC_0	53.74^{b}±0.85	50.29^{a}±0.84	55.69^{a}±0.62	$47.40\ ^{a}$±0.74
AC_{10}	54.02^{b}±0.66	51.65^{b}±0.56	56.60^{b}±0.64	$46.95\ ^{a}$±0.42
AC_{20}	54.44^{b}±0.45	51.51^{b}±0.77	56.95^{b}±0.97	$54.72\ ^{b}$±0.91

NS = Native samples AC_0 = No addition of modifier agent (control), AC_{10} = 10 g acetic anhydride /100g flour, AC_{20} = 20 g acetic anhydride/100g flour. Alphabet in superscript = gram of acetic anhydride per 100g flour sample. DWB = Dry weight basis

*Mean values in each column with the same alphabets do not vary significantly at $p \leq 0.05$

The results of alpha amylase digestibility of the acetylated yam flour as depicted in Table 1, showed significant increase ($p \leq 0.05$) in the α- amylase digestibility in all the acetylated yam flour samples 'Aro' flour (48.24^{a}±0.50 to 54.44^{b}±0.45g/100g), 'Efuru' flour (50.74^{a}±1.66 to 51.51^{b}±0.77 45g/100g), Yellow yam (54.86^{a}±0.82 to 56.95^{b}±0.97g/100g) and Water yam flour ($46.92\ ^{a}$±1.22 to $54.72\ ^{b}$±0.91). Variation in the quantity of acetic anhydride used did not show significant difference in the digestibility of the flour samples except in acetylated water yam flour at 20g acetic anhydride/100g flour that showed significantly higher digestibility than its other variants. Increased α- amylase digestibility of acetylated yam flour showed that modification through acetylation did not reduce the alpha amylase digestibility of the native yam flours. Similar result was reported by Gutiérrez, [27] for acetylated plantain flour and Liu et al.,

[42] for acetylated chestnut starch. The increase in digestibility could be attributed to the destruction of the integrity of the granules and the degradation of the ordered chain structure during modification, which can increase the susceptibility of starch to enzymatic attack. However, Simsek *et al.,* [43] reported increased resistant starch of 36.2% to 44.3%, and 41.6% to 43.0%, for black bean and pinto bean starch respectively. Lin et al., [44] also reported increased resistant starch for acetylated corn starch noodles. These variations could also be attributed to differences in the botanical sources of sample and the effect of food matrices in the acetylated yam flour.

The alpha-Amylase digestibility results of the native and the phosphorylated yam flours are shown in Table 2.

Table 2: α- Amylase digestibility of native and phosphorylated yam flours (g/100g DWB)

Modification	α- Amylase Digestibility [g/100g]			
	white yam 'Aro' flour	white yam 'Efuru' flour	Yellow yam Flour	Water yam flour
NS	$48.24^{a}\pm0.50$	$50.74^{ab}\pm1.66$	$54.86^{b}\pm0.82$	$46.92^{a}\pm1.22$
$Phos_0$	$49.70^{b}\pm1.04$	$50.88^{ab}\pm0.80$	$55.90^{bc}\pm0.87$	$49.28^{b}\pm0.80$
$Phos_{STMP}$	$51.09^{b}\pm0.77$	$51.86^{b}\pm0.68$	$56.39^{c}\pm0.21$	$49.70^{b}\pm0.43$
$Phos_{STPP}$	$57.85^{c}\pm0.67$	$48.80^{a}\pm0.89$	$51.65^{a}\pm0.60$	$50.68^{b}\pm0.74$

NS = Native samples, $Phos_0$ = No addition modifier agents (control), $Phos_{MP}$ = STMP modified samples, $Phos_{PP}$ = STPP modified samples. DWB = Dry weight basis

*Mean values in each column with the same alphabets do not vary significantly at $p \leq 0.05$

According to the results of the of the phosphorylated yam flours as depicted in Table 2, varietal differences existed in the α- amylase digestibility of the phosphorylated samples. The two modifier reagents also impacted differently on each of the yam flour samples. The digestibility results of white yam 'Aro' flour significantly increased from $48.24^{a}\pm0.50$g/100g (native sample) to 57.85 ^{c}±0.67g/100g in the STPP modified sample while that of STMP (51.09 ^{b}±0.77 g/100g] differed not significantly from the control (49.70 ^{b}±1.04 g/100g). On the other hands, STPP modified "Efuru" flour (50.74^{ab}±1.66 to 48.80^{a}±0.89) and yellow yam flour (54.86^{b}±0.82 to 51.65^{a}±0.60 g/100g) recorded a significant decrease in their α- amylase digestibility. The STMP and STPP modified water yam increased significantly from 46.92^{a}±1.22 g/100g to 49.70^{b}±0.43 g/100g and 50.68^{b}±0.74 g/100g respectively. The reduced digestibility obtained for STPP modified "Efuru" flour and yellow yam flour suggest an increase in the resistant starch due to the synthesis of RS4 by crosslinking of the phosphate group with glucose unit of the modified flour which created a hindrance to the action of the hydrolyzing enzyme. Reduction in digestibility as a result of phosphorylation were previously reported [45, 46], for wheat, potato, rice and mandua starches. The increase in α- Amylase digestibility in phosphorylated AF and WF could be attributed to several variable factors such as botanical source, food matrix effect, reaction time, pH, temperature, presence and nature of the catalyst, type and concentration of the reactants [47]. Liu et al., [42] also reported that phosphorylation and acetylation of chestnut starch showed significant decreases in slowly digested starch relative to native starch.

The alpha - amylase digestibility results of the native yam flour and citric acid esterified yam flour samples are presented in Table 3.

Table 3: α- Amylase Digestibility of Citric acid esterified yam flours (g/100g DWB)

Modification	α- Amylase Digestibility [g/100g]			
	white yam 'Aro' flour	white yam 'Efuru' flour	Yellow yam Flour	Water yam flour
NS	48.24^{d}±0.56	50.74^{d}±1.66	54.86^{d}±0.82	46.92^{d}±1.22
CAE_0	47.19^{c}±0.49	49.00^{c}±0.68	51.16^{c}±0.56	42.52^{c}±0.56
CAE_{10}	12.35^{b}±0.49	9.56^{b}±0.68	9.84^{b}±0.80	8.77^{d}±0.73
CAE_{20}	6.70 ^{a}±0.92	7.12^{a}±0.56	4.96^{a}±0.75	5.82^{a}±0.46

NS = Native samples, CAE_0 = No addition of modifier agent (control), CAE_{10} = citric acid esterified sample at 10%w/v/100g flour, CAE_{20} = citric acid esterified sample at 20%w/v/100g flour. DWB = Dry weight basis. *Mean values in each column with the same alphabets in the superscript do not vary significantly at $p \leq 0.05$

From the results as shown in Table 3, it could be seen that very high reduction in α- amylase digestibility were obtained in all the esterified samples and the digestibility reduced as the concentration of citric acid used for the esterification, increased from 10% to 20%; 12.35±0.49 g/100 g and 6.70 ±0.92 g/100 g ('Aro' flour), 9.56±0.68 g/100 g and 7.12±0.56 g/100 g ('Efuru' flour), 9.84±0.80 g/100 g and 4.96±0.75 g/100g (Yellow yam flour), 8.77±0.73 g/100 g and 5.82±0.46 g/100 g (Water yam flour) at 10%w/v and 20%w/v citric acid concentration respectively. These results showed that the higher the percentage of citric used, the greater the resistivity of the esterified yam flour to digestion. This is due to the occurrence of esterification of starch with citric acid, which forms a cross-linking structure that inhibited α- amylase to digest starch [48-51]. Similar results were obtained for citrate modified cassava starch in a study by Mei et al., [52]. As the concentration of citric acid increased, hydroxyl groups in starch molecules replaced by citrate group increased, which leads to the formation of cross-linked citrate starch which resist the hydrolysis of digestive enzymes and increases the resistant starch content. Related studies mostly on isolated starches have found that citric acid modification leads to resistance of hydrolyzing enzymes to digest starch [38, 53- 57].The citric acid esterification reaction promotes the cross-linking of starch chains, making starch resistant to enzymatic hydrolysis [56].

CONCLUSION

Chemical modification processes were carried out on four commonly consumed yam varieties in Nigeria for possible attenuation of alpha amylase digestibility. The results obtained showed that acetylation with acetic anhydride could not attenuate the alpha amylase digestibility of the modified yam flour as hypothesized. Phosphorylation with sodium trimetaphosphate and sodium tripolyphosphate impacted differently on each varieties of the yam flour samples. However esterification with citric acid impacted the digestibility of the citrated yam flours for reduced digestibility. Citric acid esterification produced resistant starch type 4 (RS4) and attenuated alpha- amylase digestibility of starch in the four varieties of yam flour investigated. RS4 is a functional starch in the dietary management of type II diabetics. Optimizing the experimental conditions for the production of citric acid esterified yam flour with moderate attenuation in the alpha amylase digestibility will be necessary in the future direction in this research work.

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