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Studies on Different Concentrations of Alcohol-Alkaline and Acid-Alcohol Methods of Modification on some Functional Properties of Starch from Selected Underutilized Legumes

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ABSTRACT

Starch has been employed in so many industries such as food, medical and pharmaceuticals due to its bioavailability and renewable property. It undergoes modifications to attain its maximum potential regarding its solubility, viscosity and gelatinization. Starch from *Cajanus cajan*, *Mucuna pruriens, Vigna subterranea* and *Sphenostylis stenocarpa* were modified by varying the concentration of acid (between 20 - 36%), alcohol (between 50 - 70%) and alkaline (between 20 - 50%) following the alcohol-alkaline and acid-alcohol chemical modification methods. The physicochemical properties of both native and modified starches were compared. The cold water solubility (CWS) of starches modified by the acid-alcohol method increased with increasing concentration of the reactants. The solubility ranged from 53 - 81 % while the CWS of starches treated with the alcohol-alkaline methods increased from 54 - 78% with an increase in the concentration of the reactants. pH, water absorption capacity, gelatinization temperature, moisture content and swelling power were analyzed. The yields of the native starch legumes ranged from 41- 49% and that of the modified starches was in the range of 84 - 99%. The pH of modified starches was in the range of 4.3-8.7, the water absorption capacity increased after modification especially for acid-alcohol treated starches with a decrease in the gelatinization temperature. Also, the swelling power increased. The alcohol-alkaline modified starches had higher swelling power than the acid-alcohol treated starches which showed a swelling power of 3.89 – 6.35. Similarly, the moisture content was higher in the modified starches than the native starches.

Keywords*:* Starch, Alcohol- alkaline, Acid- alcohol, Cold water solubility, underutilized legumes.

Introduction

Starch is an abundant polysaccharide biomolecule; it comprises mainly of amylose and amylopectin[1] with tubers, roots, cereals and legumes as sources. It is a renewable and accessible biomolecule which has numerous applications, some of which include: pharmaceutical, food, industrial, and medical applications. In pharmaceutical industries, it has been used as a binder, adsorbents, diluents, and disintegrants.[2] Native starch are modified to improve their solubility in cold water, enhance their viscosity, improve their gelatinization temperature, and ensure stability of starch.[2] Thus, starch modification combats the downsides of native starch which limits its application. Starch can be modified by applying physical, chemical, enzymatic or genetic modification techniques.

Cold Water Solubility (CWS) is important in the evaluation of properties of starch[3] and is related to the molecular structure and composition of starch.[4]

Jane and Seib[5] patented a method for preparing granular cold water soluble starches by alcohol-alkali treatments. The technique of acid hydrolysis has been used industrially to produce ‘soluble starch’.[5] However, the use of acid-alcohol treatment is gradually gaining popularity owing to higher recovery of modified starch. Subsequently, studies have been carried out on cold water-soluble starch by alcohol-alkaline and acid-alcohol modifications, alongside various physical and chemical modifications.[6-13] This work seeks to improve cold water solubility of starch isolated from underutilized and neglected legumes by varying the concentration of the acid-alcohol and alcohol-alkaline modification methods. It also evaluated the impact of these modifications on the functional properties of the modified starch.

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Materials and Methods

Materials

The plant materials were obtained from the Orie-Igboeze market in Igboeze South Local Government Area of Enugu state. They were identified by the curator at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka with identification number Interceede/0092.

Starch isolation:

starch was isolated from the legumes by the modified method of Sun, Chu, Xiong, and Si (2015).[14] The seeds of *Vigna subterranea*, *Cajanus cajan*, *Sphenostylis stenocarpa* and *Mucuna pruriens var pruriens* were separately de-stoned and milled with a milling machine (Thomas Willey laboratory Mill Model 4) to obtain fine legume flour, after which it was soaked in distilled water (1:3 weight/volume). This was

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filtered using muslin cloth. The solution was left to stand for about 3hrs and was subsequently washed with distilled water and allowed to stand for another 3 hrs. This process was repeated 5 times until the supernatant with neutral pH was obtained. The starch was collected and dried at 40°C for 48 h.

Chemical modification of starch

Acid-alcohol modification

This method was adopted as described by the method of Martin, 2015.[12] Starch (25 g) was suspended in 100 ml methanol in a 250 mL volumetric flask, the suspension was stirred and maintained at 25°C; the reaction was started by adding 1ml of concentrated HCl (36% by weight) and allowed to proceed for 216 h. The reaction was stopped by adding 14 mL 1 M sodium bicarbonate and then cooled in an ice bath; the starch was allowed to sediment and washed with 50% ethanol repeatedly for four times. The starch was oven dried at 40°C and the starch yield was calculated in percentage.

50% Acid- alcohol treatment

The initial method[12] was modified by varying the concentration of acid to 20% and that of alcohol maintained at 50%:
25 g starch was suspended in 100 mL 50% methanol in a volumetric flask, the suspension was stirred and maintained at 25°C; the reaction was started by adding 1 mL of 20% HCl and allowed to proceed for 216 h. The reaction was stopped by adding 14 mL 1 M sodium bicarbonate and then cooled in an ice bath; the starch was allowed to sediment and washed repeatedly with 50% ethanol four times. The starch was oven dried at 40°C and the starch yield was calculated in percentage.

70 % Acid-alcohol treatment:

The initial method[12] was modified by varying the concentration of alcohol to 70% and that of acid maintained at 36% with an increased volume:
25 g starch was suspended in 100 mL 70% methanol in a volumetric flask, the suspension was stirred and maintained at 25°C; the reaction was started by adding 2 mL of 36% HCl and allowed to proceed for 168 h. The reaction was stopped by adding 14 mL 1 M sodium bicarbonate and then cooled in an ice bath; the starch was allowed to sediment and washed repeatedly with 50% ethanol four times. The starch was oven dried at 40°C and the starch yield was calculated in percentage.

Alcohol-alkaline modification:

100 mL of 40% ethanol solution was added to a beaker containing 25 g of starch; it was stirred into slurry. 55 mL 3 M NaOH solution was added at 4 mL/min and the mixture was allowed to stand for 15 min with gentle stirring and an additional 40 % ethanol was added slowly to the suspension and stirred for 10 min. The supernatant was carefully decanted and the starch was washed with a fresh solution of ethanol and neutralized with 3 M HCl. The starch was washed again with 95% ethanol solution, dehydrated and oven-dried at 50°C.[6]

20% Alcohol-alkaline modification

The above experiment was repeated using 1.5 M NaOH and 1.5 M HCl in place of 3M NaOH and 3M HCl respectively.

50% Alcohol-alkaline modification

The initial experiment[7] was further repeated, this time with 50 % ethanol, 4 M NaOH, and 4 M HCl in place of 40 % ethanol, 3 M HCl and 3 M NaOH respectively.

Cold water solubility (CWS)

The CWS was determined as reported by Eastman and Moore.[15]

Determination of functional properties

The swelling power of starch was determined as reported by Schmiele *et al*.[16] The starch yield was determined according to Ji *et al*.[17] The pH and gelatinization temperature were determined according to Akpa and Dagde.[18] Water absorption capacity was determined according to the method of Olu-Owolabi *et al*.[19] The moisture content was determined as reported by Musa *et al*.[9]

Statistical analysis

Data were collected in triplicates and expressed as mean ± standard deviation. One-way analysis of variance was used in separating the mean and comparing the differences. Statistical Product and Service Solution (SPSS) version 16.0 was used as the statistical package. The level of significance was observed at $p < 0.05$.

Results and Discussion

The cold-water solubility (CWS) was dependent on the concentration of the acid-alcohol used. The CWS varied with the concentration of ethanol and NaOH in the reaction mixture for the alcohol-alkaline and acid-alcohol modifications. For the Acid-alcohol modification protocol, the cold water solubility of the modified legume starches had Acid-alcohol 70% treatment on the starches giving the best solubility, followed by the Acid-alcohol 50% treatment pattern on starches and then the Acid-alcohol modification protocol adopted from Lin *et al*.[4]
For the Alcohol-alkaline treatment protocol, the cold water solubility of the modified legume starches had 20% Alcohol-alkaline modification as the treatment with the best solubility; followed by the 50 % alcohol-alkaline modification and then the alcohol-alkaline modification adopted from Chen and Jane[6] (Table 1). *Vigna subterranea*, 50% acid-alcohol treatment had the highest CWS of 80.9% while *Mucuna pruriens* Acid-alcohol treatment had the least CWS of 53.1% (Table 1). The low CWS of the native starches can be attributed to it having a more rigid structure.[20] The role of ethanol is in inhibiting the starch particle swelling and maintaining the integrity of the starch particle and the unduly inhibition of starch granule swelling would delay the dissociation of starch double helix structure.[3] The increased solubility for most of the modification protocols; unlike the native starch granules could be due to an increase of the lower molecular weight fraction containing hydroxyl groups after modification.[21] Jivan *et al*.[29] also reported an increase in CWS upon alcohol-alkaline modification of potato starch.
The swelling power (SP) mostly increased with increase in solubility. 20% Alcohol-alkaline modification protocol on the legume starches showed a swelling power of 4.44 – 6.17, the 50% modification protocol ranged from 3.89 – 6.35 while the initial alcohol-alkaline modification adopted from Chen and Jane[6] ranged from 3.8 - 6.9 (Table 2). *Sphenostylis stenocarpa* 50% Alcohol-alkaline had the highest swelling power of 6.35 greater than the control starch which had an SP of 4.64. The acid-alcohol modified starches gave lower swelling power (2.69 – 4.28) than their native starches (3.97- 4.7). The reduction in the swelling power after some modification protocols (especially the acid – alcohol, 50% and 70% modified starches which ranged from 2.69 – 4.28) may be due to the reduction in the number of binding sites on the granules resulting from the destruction of amorphous region of the granules from the acid attack;[22] or may be attributed to structural disintegration within the granules of the starch during the modification process [23]or may be due to an increase in high proportion of soluble dextrins of both small and medium chain lengths in starch granules.[30]
The Water Absorption Capacity (WAC) increased with increasing Cold Water Solubility (CWS). The WAC of the native starches (2.01 – 2.63) were less than the WAC of the alcohol-alkaline modified starches which ranged from 2.16 – 5.49. The acid-alcohol modified starches gave low absorption values which ranged from 2.03 – 2.63 (Table 3). While highly absorbing starches (such as potato) have broad applications for instance in laundry starches, starches with low WAC (such as some legumes) can be useful also in food industries for instance, in bread making where a low absorbing starch (such as legumes) is required to prevent a dry dough formation.[24]
Gelatinization temperature is the temperature at which the starch forms a completely transparent gel. The gelatinization temperature decreased with increasing cold water solubility (Table 4); this could be as a result of dispersion by swelling and gelatinization in water by the starch polymeric material resulting in a significant hydrolytic cleavage of 1, 4-glycosidic bonds in the starch.[25]

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Table 1: Cold water solubility (mean ± SD) of native and modified underutilized legume starches

Starch Form	***Cajanus Cajan***	***Mucuna pruriens***	***Vigna subterranea***	***Sphenostylis Stenocarpa***
Alcohol Alkaline (A/A)	64.30 ± 0.71	54.40 ± 0.48	54.10 ± 3.53	63.60 ± 1.48
20 % A/A	72.40 ± 0.59	65.50 ± 0.08	78.70 ± 0.5	70.70 ± 0.66
50% A/A	72.80 ± 1.16	63.10 ± 1.29	75.70 ± 0.29	65.60 ± 1.18
Acid – Alcohol (Ac/Al)	54.90 ± 0.45	53.10 ± 0.61	54.00 ± 1.41	65.50 ± 1.41
50% Ac/Al	61.50 ± 3.57	63.50 ± 1.76	80.90 ± 0.49	60.30 ± 2.3
70% Ac/Al	62.80 ± 0.62	69.20 ± 2.42	66.50 ± 3.02	70.00 ± 0.89
Control	80.40 ± 1.23	80.40 ± 1.23	80.40 ± 1.23	80.40 ± 1.23
Native	21.10 ± 3.10	12.100 ± 1.04	19.20 ± 0.86	14.30 ± 1.68

Table 2: Swelling power of native and modified underutilized legume starches

Starch Form	***Cajanus cajan***	***Mucuna pruriens***	***Vigna subterranea***	***Sphenostylis stenocarpa***
Alcohol Alkaline (A/A)	4.03 ± 0.64	3.96 ± 0.33	5.07 ± 1.21	4.54 ± 0.17
20% A/A	4.75 ± 0.64	4.44 ± 0.1	6.17 ± 0.82	5.92 ± 1.10
50% A/A	4.11 ± 0.51	3.89 ± 0.18	4.79 ± 0.38	6.35 ± 2.21
Acid – Alcohol (Ac/ Al)	2.69 ± 1.03	2.94 ± 1.81	3.90 ± 0.29	4.21 ± 0.23
50% Ac/Al	3.78 ± 0.22	4.28 ± 0.26	3.30 ± 0.22	4.12 ± 0.3
70% Ac/Al	2.98 ± 0.2	4.01 ± 0.22	3.97 ± 0.13	3.36 ± 0.13
Control	4.64 ± 0.4	4.64 ± 0.4	4.64 ± 0.4	4.64 ± 0.4
Native	4.66 ± 0.31	4.23 ± 0.54	3.97 ± 0.5	4.70 ± 0.28

Table 3: Water absorption capacity of native and modified underutilized legume starches

Starch Form	***Cajanus cajan***	***Mucuna pruriens***	***Vigna subterranea***	***Sphenostylis stenocarpa***
Alcohol Alkaline (A/A)	2.16 ± 0.09	2.23 ± 0.49	1.86 ± 0.28	2.15 ± 0.09
20% A/A	5.49 ± 3.5	2.66 ± 0.11	2.96 ± 0.21	2.62 ± 0.40
50% A/A	3.52 ± 0.31	3.66 ± 0.3	3.27 ± 0.24	3.08 ± 0.25
Acid – Alcohol (Ac/Al)	2.1 ± 0.16	2.21 ± 0.15	2.2 ± 0.38	2.12 ± 0.05
50% Ac/Al	2.03 ± 0.38	2.41 ± 0.04	2.39 ± 0.25	2.33 ± 0.08
70% Ac/Al	2.6 ± 0.23	2.51 ± 0.25	2.63 ± 0.18	2.21 ± 0.56
Control	3.29 ± 0.65	3.29 ± 0.65	3.29 ± 0.65	3.29 ± 0.65
Native	2.05 ± 0.11	2.01 ± 0.43	2.28 ± 0.15	1.74 ± 0.4

Table 4: Gelatinization temperature of native and modified underutilized legume starches

Starch Form	***Cajanus cajan***	***Mucuna pruriens***	***Vigna subterranea***	***Sphenostylis stenocarpa***
Alcohol Alkaline (A/A)	39.00 ± 0.20	44.00 ± 0.31	38.00 ± 0.38	39.00 ± 0.02
20% A/A	49.00 ± 0.05	44.00 ± 0.2	48.00 ± 0.14	49.00 ± 0.15
50% A/A	40.00 ± 1.28	38.00 ± 0.13	48.00 ± 0.08	39.00 ± 0.37
Acid – Alcohol (Ac/Al)	40.00 ± 0.57	38.00 ± 0.09	39.00 ± 0.26	42.00 ± 0.24
50% Ac/Al	41.00 ± 0.43	45.00 ± 1.62	39.00 ± 0.17	42.00 ± 0.08
70% Ac/Al	41.00 ± 0.26	54.00 ± 1.3	39.00 ± 0.54	42.00 ± 0.10
Control	35.00 ± 0.06	35.00 ± 0.06	35.00 ± 0.06	35.00 ± 0.06
Native	73.00 ± 0.51	71.00 ± 0.17	69.00 ± 0.08	70.00 ± 0.13

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Cajanus cajan and *Sphenostylis stenocarpa* Alcohol-alkaline modification had the least gelatinization temperature of 39°C while *Mucuna pruriens* 70% Acid-alcohol modification had the highest gelatinization temperature of 54°C.

The pH of the modified starches ranged from 4.3- 8.7. The different acid-alcohol modified starches had a highly acidic pH (Table 5). The neutrality of the starches increases its applications both for food and industrial purposes. The amount of moisture present in the starch was evaluated. Acid-alcohol modified starches gave the highest moisture content compared to the native and alcohol-alkaline modified starches (Table 6). Starches with low moisture content are observed to have higher shelf-life than starches with high moisture content. Moisture content is an important component in relation to quality, shelf life and industrial application.[26; 27] The low moisture contents in some of the modified starches maybe a result of the destruction of the amorphous region of the starch granules by some of the reagents.[28] The Starches with low moisture content have applications in paper making, binding, and food industries where low moisture is required to improve the quality and prolong the shelf life.[27]

Vigna subterranea, Cajanus cajan, Sphenostylis stenocarpa and *Mucuna pruriens* starches gave a yield of 46.05%, 41.2%, 48.6% and 40.6% respectively.

The modified starches had starch yield in the range of 84- 99%. The alcohol-alkaline (20%) modification resulted in the highest yield. The native starch obtained was white in colour with no smell and a yield of 40.6%, 41.2%, 46.05% and 48.6% for *Mucuna pruriens var pruriens, Cajanus cajan, Vigna subterranean* and *Sphenostylis stenocarpa* respectively. The yield is considered to be appreciable (≥ 40%) especially when compared with starches from some other legume sources [31]. Wani *et al*.[31] reported a yield of 16 – 47% in a review on a variety of legumes comprising kidney bean, chick pea, lentil, green gram, moth bean, smooth pea, black bean, pinto bean, navy bean, cow pea, faba bean, black gram and broad bean.

The starch yield of *Cajanus cajan* had a higher yield value of 41.2% than that reported by Lawal[32] with a yield of 22.5%. The starch yield of *Vigna Subterranea* was 46.5% and that was slightly higher than 45.57 % reported by Piyarat[33] this could be due to variations in cultivar and granular composition. Lawal and Adebowale,[34] reported a starch yield for *Mucuna* bean in the range of 36.8 - 46.0% and that obtained in the study falls within that range. The slight variation in the values obtained may be as a result of the location of the raw material sources, the varying specie of legumes, period of harvest and the loss experienced during isolation.

Table 5: pH of native and modified underutilized legume starches

Starch Form	***Cajanus cajan***	***Mucuna pruriens***	***Vigna subterranea***	***Sphenostylis stenocarpa***
Acid Hydrolysis	3.6 ± 0.57	3.7 ± 0.78	4 ± 0.85	3.5 ± 0.49
Alcohol Alkaline (A/A)	8.1 ± 0.42	7.4 ± 0.21	7.2 ± 0.21	7.1 ± 0.42
20% A/A	7.1 ± 0.14	6.8 ± 0.28	6.8 ± 0.85	7 ± 0.85
50% A/A	7 ± 0.85	7.1 ± 1.27	7.3 ± 0.42	8.7 ± 0.42
Acid – Alcohol (Ac/Al)	5.9 ± 0.78	4.9 ± 1.06	4.35 ± 1.2	5 ± 1.13
50% Ac/Al	8.2 ± 0.28	7.3 ± 0.14	5.8 ± 0.85	5.9 ± 0.14
70% Ac/Al	6.2 ± 0.28	6.7 ± 0.99	5.4 ± 0.28	6.6 ± 0.28
Control	7.5 ± 0.21	7.5 ± 0.21	7.5 ± 0.21	7.5 ± 0.21
Native	5.3 ± 0.35	6.5 ± 0.42	6.4 ± 0.78	5.8 ± 0.92

Table 6: Moisture content of native and modified underutilized legume starches

Starch Form	***Cajanus cajan***	***Mucuna pruriens***	***Vigna subterranea***	***Sphenostylis stenocarpa***
Alcohol Alkaline (A/A)	10.4 ± 0.11	9.1 ± 0.42	8.2 ± 0.13	10.9 ± 1
20 % A/A	5 ± 0.98	7.5 ± 1.25	13.9 ± 0.69	7.8 ± 1.7
50 % A/A	7.2 ± 0.3	10.1 ± 0.03	10.3 ± 0.6	15.6 ± 0.4
Acid – Alcohol (Ac/Al)	15.7 ± 1.34	13.5 ± 0.39	12.6 ± 0.82	14.7 ± 0.56
50 % Ac/Al	10.4 ± 2.55	13.9 ± 0.05	14.4 ± 0.26	13.2 ± 1.21
70 % Ac/Al	11.8 ± 1.13	11.6 ± 0.03	16.6 ± 0.55	20.4 ± 1.05
Control	6.8 ± 0.13	6.8 ± 0.13	6.8 ± 0.13	6.8 ± 0.13
Native	8.8 ± 0.17	13.6 ± 0.18	13 ± 0.09	11.9 ± 0.1

Table 7: Yield of native and modified underutilized legume starches

Starch Form	***Cajanus cajan***	***Mucuna pruriens***	***Vigna subterranea***	***Sphenostylis stenocarpa***
Alcohol Alkaline (A/A)	95.95 ± 0.28	95.75 ± 2	99.13 ± 2.44	94.13 ± 0.26
20% A/A	99.84 ± 0.3	90.25 ± 1.41	92.29 ± 0.63	95.52 ± 0.17
50% A/A	76.77 ± 1.38	89.38 ± 0.73	91.46 ± 0.24	90.86 ± 0.28
Acid – Alcohol (Ac/Al)	96.02 ± 0.37	90.65 ± 0.21	91.28 ± 0.19	95.68 ± 0.81
50 % Ac/Al	94.31 ± 0.42	95.95 ± 0.19	91.36 ± 0.86	88.64 ± 0.61
70% Ac/Al	97.24 ± 0.94	94.46 ± 0.22	99.58 ± 0.52	98.56 ± 2.51
Native	41.2 ± 2.13	40.6 ± 1.17	46.05 ± 0.69	48.6 ± 1.08

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Conclusion

Applications of native starches are limited by their insolubility in cold water, tendency to easily retrograde, and loss of ordered structure after gelatinization. These could be overcome by physically and chemically modifying them. The study showed the variations in the acid-alcohol and alcohol-alkaline modifications being better than the acid-alcohol modification and alcohol-alkaline modifications reported by Martin[12] and Chen and Jane[5] respectively. This suggests that legumes can be good sources of starch (native starch yield: 41.2 – 48.6%). These various modification had enhanced qualities such as cold water solubility (53.1 – 80.9%), water absorption capacity (1.86 – 5.49), moisture content (5 – 20.4), swelling power (2.69 – 6.35) and gelatinization temperature (38 – 54^{o}C) after modification. This implies that these legumes have applications ranging from pharmaceutical, to industrial to food purposes. The properties of the alcohol-alkaline and acid-alcohol modified starches suggest that these underutilized legume starches could be used in the production of cold water soluble starch with applicable functional properties thereby increasing its value addition amongst other applications.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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