Tropical Journal of Pharmaceutical Research May 2018; 17 (5): 883-890
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v17i5.19

Original Research Article

Physicochemical and microbiological evaluation of acid-modified native starch derived from *Borassus aethiopum* (Arecaceae) shoot

Chukwuemeka P Azubuike[1]*, Adebowale O Adeluola[1], Mmesoma S Mgboko[1], Shadrack J Madu[2]

[1]Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, College of Medicine Campus, PMB 12003, Surulere, Lagos, [2]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmacy, University of Maiduguri, PMB 1069, Maiduguri, Borno State, Nigeria

For correspondence:* ***Email:* *cazubuike@unilag.edu.ng;* ***Tel:*** *+234-8033618556*

Sent for review: 7 February 2018 *Revised accepted: 17 April 2018*

Abstract

Purpose: *To evaluate the physicochemical properties and microbiological quality of Borassus aethiopum shoot acid-modified starch (AMS) for potential pharmaceutical applications.*
Methods: *Modification of Borassus aethiopum native starch (NS) was carried out using 6 % w/v HCl at 37 ± 2 ^{o}C for 192 h. The AMS was characterised for their morphological, micromeritics, rheological, thermal properties as well as their microbiological quality using standard protocols.*
Results: *AMS demonstrated increased aqueous solubility, crystallinity and slight increase in flow properties. There was a reduction in swelling and hydration capacities, amylose content as well as viscosity of the modified starch. Scanning electron microscopy analysis showed that the integrity of the modified starch granules were maintained and there was no disruption of the granular structure. Fourier transform infrared spectrophometer data confirmed the hydrolysis of NS with the increase in the intensity of the O-H stretch. AMS met United States Pharmacopoeia requirements in terms of microbiological quality, however, there was presence of Aspergillus niger.*
Conclusion: *Modification of Borassus aethiopum shoot starch by acid treatment led to desirable improvement in some of its physicochemical properties which could improve its functional properties in pharmaceutical industries.*

Keywords: *Native starch, Acid-modified starch, Borassus aethiopum, Microbiological quality, Physicochemical properties*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Starch is a major carbohydrate reserve in plants and one of the most versatile biopolymers. It has an inexhaustible supply [1]. Major starch sources employed for pharmaceutical purposes include roots, tubers, cereals, grains, fruits and legumes. Starch usually occurs as granules, they exist naturally in plant cells [2]. The accumulation pattern of starch granules in plant tissues as well as its composition and other physicochemical parameters vary depending on the botanical

source [3]. Starch is made of two polymers: amylose (15 – 30 %) and amylopectin (70 – 85 %) [4].

Starch is used in biopolymer industries due to its ease of modification, biocompatibility, ease of use, biodegradability, its intrinsic physicochemical properties as well as its non-toxicity [5]. In its native form, starch has limited industrial applications, and is usually modified to create novel properties as well as improved functionality [6,7]. The undesirable characteristics of native starch for pharmaceutical use include poor flowability and solubility, instability at high temperature and poor resistance among others [8]. Hence their use is limited and unsuitable for specific industrial applications [9].

Modification produces starches that are better suited for specific industrial applications and wide range of applications. Acid modification of starch is a chemical modification involving hydrolyzing starch with hydrochloric or sulphuric acid at certain temperature below the gelatinization temperature of the starch [10]. Various studies on effects of acid hydrolysis on starch from conventional sources have shown that acid hydrolysis yields starch with increased solubility and gel strength, increase in relative crystallinity, decreased viscosity, decrease in swelling capacity and even increase in compressibility [10,11].

To the best of our knowledge, there has been no investigation on acid modification of *Borassus aethiopum* (BA) starch (non-conventional source of starch). *Borassus* a dioecious palm tree of African origin grows well in West Africa and has been utilized for both medicinal and non-medicinal purposes [12]. In this study, various physicochemical properties as well as microbiological purity of acid modified *Borassus aethiopum* (AMS) starch were studied.

EXPERIMENTAL

Materials

Borassus aethiopum shoots were collected from Hong Local Government Area of Adamawa State, Nigeria in May 2017, identified and authenticated by by Mr. O.O. Oyebanji at the herbarium of the Department of Botany, University of Lagos, Nigeria and a voucher specimen (no. LUH 7409) assigned and deposited at the herbarium.

Commercial brand pregelatinized starch (PGS) was a gift from Phamatex Industry Limited, Lagos, Nigeria. All other reagents used in the study were of analytical grade.

Extraction of starch from the fresh *Borassus aethiopum* shoots

The procedure used by Muazu *et al* [13] was employed in the extraction of *Borassus aethiopum* native starch (NS). Briefly, 2 kg of fresh BA shoots were washed, peeled and reduced to small sizes. They were then soaked in water preserved with sodium metabisulphate (0.075 %) for 24 h prior to pulverization in a mill. The pulverized mixture was subsequently stirred and filtered using double fold clean cheesecloth and then allowed to settle while the starch sediments. This was followed by decanting the water and centrifuging the suspension at 4000 rpm for 5 min.

Preparation of *Borassus aethiopum* acid-modified starch (AMS)

Acid hydrolysis of NS was carried out by suspending the 500 g of the native starch in 1000 mL of 6 % w/v aqueous HCl at 37 ± 2 ^{o}C for 192 h [14]. The acid modified starch was stored in an airtight container.

Characterization of starch samples

Pharmacopoeial properties

Some pharmacopoeial properties (solubility, pH, ash content and moisture content) were carried out using methods detailed in a previous study [15]. The parameters were assessed in triplicates for NS, AMS and PGS.

Amylose content

The amylose content of NS and AMS was determined using the method described by Okunlola and Akingbala [16]. Briefly, 1 mL 95 % ethanol and 9 mL 1 N NaOH was added to a 100 mL volumetric flask containing 100 mg of sample. The sample was heated for 10 min and made up to volume with distilled water. Thereafter, 1 mL of 1 N acetic acid and 2 mL iodine solution was added to a 5 mL portion of the starch solution and made up to 100 mL with distilled water. The absorbance at 620 nm wavelength was determined using the UV/VIS spectrometer (JP Selecta, Barcelona, Spain).

Micromeritics

The micromeritics properties (bulk and tapped densities, Carr's compressibility index and Hausner ratio) of the starches were determined

using the procedures employed by Azubuike *et al* [15].

Rheological and related properties

Hydration capacity

Each sample (1 g) of starch powder was weighed and poured into centrifuge tubes. Distilled water (10 mL) was then added and mixed for 2 min. The mixture was centrifuged at 100 rpm for 10 min. The supernatant obtained was decanted and the sediment weighed.

Re-dispersion time

Distilled water (10 mL) was added to the sediment obtained from the determination of the hydration capacity. The tube was gently agitated with the same intensity until the sediment was re-dispersed. The time taken for the sediment to re-disperse was recorded as the re-dispersion time.

Swelling power

An aqueous suspension of starch (2 % w/v) in a water bath (Surgifriend Medicals, England) was maintained at 77 °C for 1 h. The suspension was cooled at 25 ± 2 ^{o}C and centrifuged at 5000 rpm for 9 min. The liquid supernatant was collected in an aluminium dish and evaporated at 60 °C for 28 h. The swollen starch sediments were weighed to determine the swelling power.

Viscosity

Viscosity was measured with a viscometer (DV-E, China) using a method described by Reddy and Bhotmange [17]. The viscosity of a 2 %w/v starch suspension was read at 100 rpm.

Morphological studies

Granule morphology of the starch samples was examined using scanning electron microscope (Pro X, Netherlands). The starch powder samples were mixed with ethanol to obtain a 1 % suspension. The suspension was smeared on an aluminium stub with a double sided adhesive tape and was coated with gold powder.

Fourier transform infrared spectroscopy (FTIR)

The starch samples (5 mg) were individually blended with solid KBr (50 mg) and compressed into discs. The FTIR spectra were obtained by recording the transmittance in the range of 4000 to 400 cm^{-1} under dry air in a FTIR spectrometer (Bruker, South-Africa).

Differential scanning calorimeter

Differential scanning calorimeter (Mettler Toledo, UK) was used to analyse the thermal properties of the starch samples [18]. Briefly, the starch sample was weighed into an aluminium pan, hermetically sealed and equilibrated at room temperature for 1 h. It was heated at the rate of 10 ^{o}C/min from 30 to 120 ^{o}C with an empty sealed pan as a reference.

Microbiological evaluation

Microbiological assessment of the starch samples were carried out using the method employed by Ozolua *et al* [19]. Eosine methylene blue agar (EMBA), Cetrimide agar (CET), Salmonella Shigella agar (SSA) and Tryptone soy agar (TSA) were used for the detection of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* and *Shigella spp*, and total aerobic microbial counts (TAMC) respectively. These plates were incubated at 35 ± 2 ^{o}C and observed daily for 72 h. Sabouraud dextrose agar (SDA) was used for detection of total yeast and mould (TYMC) counts. The plates for determination of TYMC were incubated at 27 ± 2 °C [20].

Statistical analysis

The measurements were in triplicate, mean comparison of the test samples with the standard was carried out using one-way analysis of variance (ANOVA). Significant differences (p < 0.05) were determined by Tukey test. Statistical analysis was carried out using OriginPro 2016 software (OriginLab Corporation Northampton, MA 01060 USA).

RESULTS

Pharmacopoeial and physicochemical properties

The results of pharmacopoeial and physicochemical properties of the three starch samples are presented in Table 1. The AMS and PGS formed a translucent suspension in aqueous medium and were insoluble in ethanol while NS was turbid in aqueous medium but also insoluble in ethanol. The starches tested positive for iodine test. The pH values of both NS and AMS varied significantly with the standard starch (p < 0.05). There was no significant difference in the ash and moisture content values of the NS and AMS while the ash value of PGS was much lower.

Table 1: Pharmacopoeial and physicochemical properties of the starches

Sample code	NS	AMS	PGS
pH	3.49±0.02	5.89±0.01	6.82±0.01
Moisture content (%L) ± SD	13.71±0.05	13.50± 0.03	11.65±0.07
Ash value (%) ± SD	1.55±0.49	1.40±0.07	0.79±0.03
Amylose content(%)	23.99	12.64	14.70
Bulk density (g/mL) ± SD	0.76 ± 0.07	0.69 ± 0.05	0.61 ± 0.17
Tapped density (g/mL) ± SD	0.95 ± 0.09	0.94 ± 0.03	0.72 ± 0.15
Carr's index (%) ± SD	21.0 ± 11.5	20.0 ± 2.2	16.0 ± 8.9
Hausner ratio ± SD	1.29 ± 0.2	1.26 ± 0.03	1.20 ± 0.12
Hydration capacity ± SD	1.80 ± 0.20	1.68 ± 0.24	5.45 ± 0.70
Swelling capacity ± SD	7.75 ± 3.75	1.57 ± 0.49	12.44 ± 0.13
Viscosity (mPa s) ± SD	30.5 ± 6.42	22.5 ± 3.48	51.3 ± 10.62
Re-dispersion time (s) ± SD	41.6 ± 6.1	14.0 ± 1.0	_

NS- *Borasus aethiopum* native starch, AMS-acid modified starch, PGS- Standard pre-gelatinized starch.

Amylose content

The amylose content (Table 1) of *Borassus aethiopum* native starch decreased from 23.99 % to 12.64 % upon acid modification. The amylose content of the PGS was 14.7 %.

Micromeritics

The bulk density of NS varied significantly with those of AMS and PGS ($p<0.05$). However, there was no significant difference between the tapped density of NS and AMS. There was statistically significant difference ($p < 0.05$) between the two *Borassus aethiopum* starches and the PGS in the results obtained for Carr's index and Hausner's ratio (Table 1).

Hydration, swelling and viscosity

The results for hydration and swelling capacities presented in Table 1 showed that acid hydrolysis of *Borassus aethiopum* native starch resulted in a decrease in both capacities. The results of the viscosity (Table 1) of the starches showed that upon modification, the viscosity of the *Borassus aethiopum* native starch was reduced.

Morphological features

The results for the SEM are presented in Figure 1. The results showed that AMS still retained its granular structure after acid treatment.

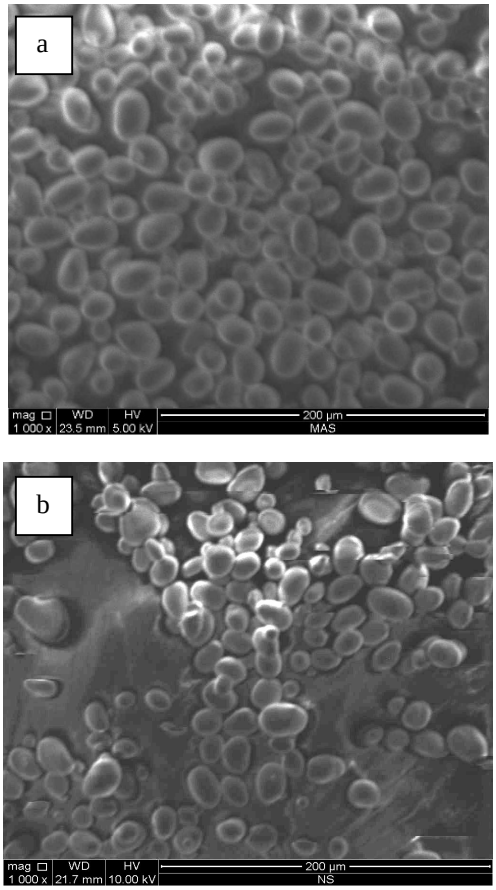

Figure 1: SEM micrographs of (a) NS and (b) AMS. 1000x magnification. NS- *Borasus aethiopum* native starch, AMS-acid modified starch

Fourier transform infrared spectra

The results of the FTIR study of the starch samples powders are presented in Figure 2. The NS, AMS and PGS had basic peaks and bands that are characteristics of starch granules although there were some minor differences in some of the peaks and band intensities.

Thermal characteristics

The DSC curves of the starch samples are shown in Figure 3 while the onset, peak and endset temperatures of the first endothermic peak are presented in Table 2.

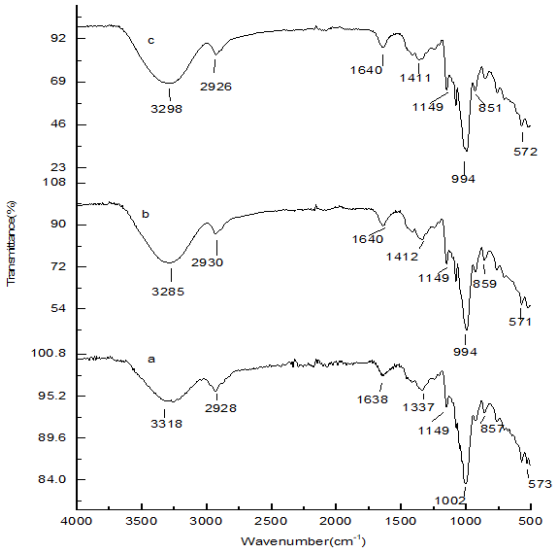

Figure 2: Stacked FTIR spectra of (a) NS, (b) AMS and (c) PGS. NS- *Borasus aethiopum* native starch, AMS-acid modified starch, PGS- Standard pre-gelatinized starch

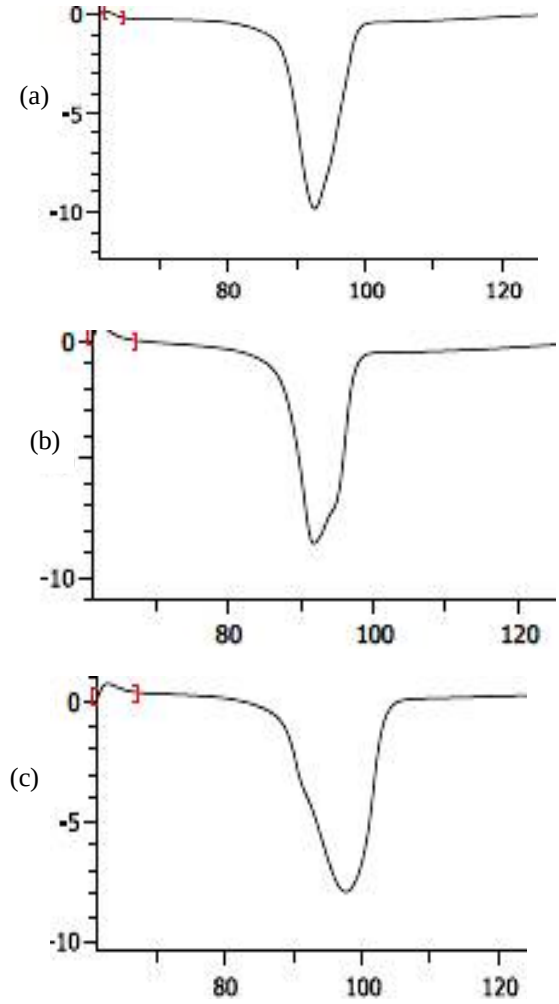

Figure 3: DSC thermogram of (a) NS, (b) AMS and (c) PGS**;** x-axis is temperature (^{o}C) while y-axis is heat flow (mW)

Table 2: Thermal properties of standard pre-gelatinized starch, native starch and acid-modified starch

Sample code	To onset temperature (°C)	Tp peak temperature (°C)	Tc endset temperature (°C)	Range (Tc-To)
NS	87.94	92.16	98.42	10.48
AMS	87.63	91.37	97.19	9.56
PGS	88.07	97.22	103.05	14.98

NS = *Borassus aethiopum native starch,* AMS = acid-modified starch, PGS = standard pregelatinized starch

The results of the microbial evaluation of the starches are shown in Table 3. There was no growth on EMBA, CET and SSA media for the two samples, however, the presence of *Aspergillus niger* on the Sabouraud dextrose agar was observed for NS and AMS samples.

Table 3: Microbiological quality of native and acid modified starch

Medium	Native starch (cfu/g)	Acid-modified starch (cfu/g)
SSA	0	0
TSA	0	$3x10^{2}$
SDA	$1x10^{2}$	$1x10^{2}$
EMBA	0	0
CET	0	0

SDA = Sabouraud dextrose agar. EMBA = Eosine methylene blue agar. TSA = Tryptone soy agar. CET = centrimide agar. SSA = Salmonella Shigella agar.

DISCUSSION

The solubility of NS in water was lower compared to AMS. Acid modification improves solubility of starch in an aqueous medium [10]. This might be due to the cleavage of the glyosidic bonds converting the polymer into monosaccharides. When AMS is employed in tablet formulation, the increase in solubility will result in an increased hydrophilic network in the tablet matrix. Thus the increased solubility of the AMS showed that AMS might be a better disintegrant if this mechanism of disintegration is considered.

The moisture content of starch is very important as it can affect the flow property as well as the mechanical properties of starch, it can also support microbial growth and spoilage thereby rendering the product unsafe for human consumption. The moisture content of the three starch samples were below the upper limit 15 % recommended by the BP [21].

The pH of PGS was within BP specification [21] which states that the pH of starch should be ranged from 4.50 to 7.50. Although the pH of the

NS was not in this range, that of modified starch was within the BP specification [21].

Ash value is very important in the examination of purity in powdered formulation ingredients. There is no documented specification on ash value by both BP [21] and USP [20]. However, the ash value of the AMS was slightly lower than that of the NS which implies that acid modification reduces the amount of inorganic matter in starch hence improving its purity.

The lower amylose content of the modified starch compared to the native starch might be due to the destruction of the starch helical structure during hydrolysis, which then reduced the amount of the amylose [16]. This is similar to the findings reported by Odeku and Picker-Freyer [11]. Due to this reduced amylose content, the crystalline regions would be forced into a closer packing during compression and will result in increase in tablet strength. Hence AMS can be employed as a binding agent in pharmaceutical formulations.

In terms of flow character, powders with Carr's index values of 16-20 and 21-25 are considered to have fair and passable flow character respectively while those with Hausner ratio values of 1.19-1.25 and 1.26-1.34 are considered to have fair and passable flow character [20]. From the study, PGS had fair flow character while the *Borasus aethiopum* starches had passable flow character, hence a glidant is required to improve their flow. Modification only slightly improved the flow of NS powder hence acid hydrolysis of the native starch had slight influence in its flow property.

The swelling power of AMS was almost five and eight times respectively lower than those NS and PGS. This decreased swelling power of acid modified starch might be due to the increase in high proportion of soluble dextrin of both small and medium chain length in starch granules and also increase in crystallinity which restricted the percolation of water within the starch matrices [10]. In terms of disintegrant potentials, PGS and NS with higher swelling power could be better disintegrants when employed in tablet formulations if swelling mechanism is predominately the mechanism of disintegration. However, no single mechanism is responsible for the mode of action of many disintegrants. Acid hydrolysis of starch reduced the hydration capacity because of the increase in the crystalline regions.

The reduced viscosity observed with AMS is likely due to the extensive disruption of the amorphous regions in the starch granules and the conversion of the amylose to low molecular weight chains. AMS had the lowest re-dispersion time and it might be attributed by the reduced viscosity exhibited by the starch.

The micrographs of the native and the acid modified starches were oval in shape and showed intact granules, however, granules of AMS were more closely packed, and the granule size was slightly increased.

The FTIR spectra of the starches are characteristic of granular starches although acid treatment caused minor shift in peak/band positions/intensities. The peak at 900 - 950 cm^{-1} in the infrared spectra of all the starch samples is an evidence of the glycosidic linkage. The sharp band 2800 - 3000 cm^{-1} of all the starch granules is characteristic of C-H stretch associated with the ring hydrogen atoms. A more detailed description of FTIR spectrum of starch has been reported in literature [22]. For acid modified starch, there was an increase in the intensity of the O-H stretch (3000 - 3600 cm^{-1}) which is indicative of hydrolysis [22]. The intensity of the C-H stretch (2800 - 3000 cm^{-1}) of the acid modified starch was increased compared to the native starch. This change in the intensity may be attributed to the change in the amylopectin and amylose

DSC thermograms of the NS, AMS and PGS showed an initial endothermic peak in the region of 85 to 105 ^{o}C for all the samples. The endothermic peak at this region is due to gelatinization [23]. However, there was no significant difference in the onset, peak and endset temperatures for AMS and NS.

Absence of growth on the EMBA and CET media is indicative of absence of *Escherichia coli* and *Pseudomonas aeruginosa* respectively while absence of growth on SSA medium is indicative of absence *Salmonella spp* and *Shigella spp.* According to USP [20], for microbial enumeration tests for modified starch, the total aerobic microbial count should not exceed 10^3 cfu/g while total combined moulds and yeasts count should not exceed 10^2 cfu/g and for the tests for specified microorganisms, there should be absence of *Salmonella species* and *Escherichia coli*. The samples met these specifications. However, in tropical weather conditions, storage conditions for the drug product such as starch samples will determine the sustainability of microbial integrity of the product [24]. The presence of *Aspergillus niger* in both starches could have been as a result of some degree of fungal contamination of the plant shoots during the process of storage and transportation. This is

capable of causing spoilage on the starch and/or the final drug product in which they are used as excipient on storage [25].

CONCLUSION

Acid modification of *Borassus aethiopum* native starch improved its physicochemical and functional properties as evidenced in increased crystallinity and aqueous solubility, and slightly improved flow property. Moreover, the changes in other physicochemical properties such as viscosity, swelling and hydration capacities upon acid modification of the native *Borassus aethiopum* starch may enhance its application as am excipient in the pharmaceutical industry.

DECLARATIONS

Acknowledgement

The authors are grateful to Phamatex Industries Limited for providing some of the facilities/materials used for the study.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. CPA and AOA conceived and designed the study, MSM and SJM collected and analysed the data, CPA and MSM wrote the manuscript. All authors read and approved the manuscript for publication.

REFERENCES

1. *Zhu F. Impact of ultrasound on structure, physicochemical properties, modifications, and applications of starch. Trends Food Sci Technol 2015; 43(1): 1-17.*
2. *Clasen SH, Müller CM, Parize AL, Pires AT. Synthesis and characterization of cassava starch with maleic acid derivatives by etherification reaction. Carbohydr Polym 2018; 180: 348-353.*
3. *Alcázar-Alay SC, Meireles MAA. Physicochemical properties, modifications and applications of starches from different botanical sources. Food Sci Technol (Campinas) 2015; 35(2): 215-236.*
4. *Lawal OS, Lechner MD, Kulicke WM. Single and multi-step carboxymethylation of water yam (Dioscorea alata) starch: Synthesis and characterization. Int J Biol Macromol 2008; 42(5): 429-435.*
5. *Te Wierik GHP, Bergsma J, Arends-Scholte AW, Boersma T, Eissens AC, Lerk CF. A new generation of starch products as excipient in pharmaceutical tablets. I. Preparation and binding properties of high surface area potato starch products. Int J Pharm 1996; 134(1-2): 27-36.*
6. *Masina N, Choonara YE, Kuma, P, du Toit LC, Govender M, Indermun S, Pillay V. A review of the chemical modification techniques of starch. Carbohydr Polym 2017; 157: 1226-1236.*
7. *Dey A, Sit N. Modification of foxtail millet starch by combining physical, chemical and enzymatic methods. Int J Biol Macromol 2017; 95: 314-320.*
8. *Bos CE, Bolhuis GK, Van Doorne H, Lerk CF. Native starch in tablet formulations: properties on compaction. Pharm World Sci 1987; 9(5), 274-282.*
9. *Santana ÁL, Meireles, MAA. New starches are the trend for industry applications: a review. Food Public Health 2014; 4(5): 229-241.*
10. *Odeku OA, Akinwande BL. Effect of the mode of incorporation on the disintegrant properties of acid modified water and white yam starches. Saudi Pharm J 2012; 20(2): 171-175.*
11. *Odeku OA, Picker-Freyer KM. Characterization of acid modified Dioscorea starches as direct compression excipient. Pharm Dev Technol 2009; 14(3): 259-270.*
12. *Jatau DF. Profitability assessment of Borassus aethiopum (Mart) marketing in Adamawa State, Nigeria. J Agri Soc Sci 2008; 4(4): 159-164.*
13. *Muazu J, Zakama SG, Dzarmah UJ. Extraction and characterization of the starch from the shoot of Borassus aethiopum as a prospective pharmaceutical raw material. World J Pharm Sci 2014; 2(8): 704-707.*
14. *Atichokudomchai N, Varavinit S. Characterization and utilization of acid-modified cross-linked Tapioca starch in pharmaceutical tablets. Carbohydr Polym 2003; 53(3): 263-270.*
15. *Azubuike CP, Aloko S. Preliminary studies on binding potentials of defatted cake derived from Blighia sapida seeds in ascorbic acid tablets. J Rep Pharm Sci 2017; 6(2): 167-179.*
16. *Okunlola A, Akingbala O. Characterization and evaluation of acid-modified starch of Dioscorea oppositifolia (Chinese yam) as a binder in chloroquine phosphate tablets. Braz J Pharm Sci 2013; 49(4): 699-708.*
17. *Reddy DK, Bhotmange MG. Viscosity of Starch: A Comparative Study of Indian Rice (Oryza Sativa L.) Varieties. International Review of Applied Engineering Research 2014; 4(5): 397-402.*
18. *Sindhu R, Khatkar BS. Morphological, pasting and thermal properties of common buckwheat (Fagopyrum esculentum Moench) flour and starch. Int J Innov Res Adv Stud 2016; 3: 160-164.*
19. *Ozolua RI, Omogbai EK, Akerele JO, Okhamafe AO. Microbiological and toxicological studies on cellulose*

generated from agricultural wastes. Afr J Biotechnol 2005; 4: 1147-1151.

20. *United States Pharmacopoeia. United States Pharmacopoeia National Formulary (USP 41 NF 36). Rockville, Md: United States Pharmacopoeial Convention, Inc; 2018*
21. *British Pharmacopoeia Commission. British Pharmacopoeia 2017. London: UK. TSO Publishers: 2017*
22. *Fang JM, Fowler PA, Tomkinson J, Hill CAS. The preparation and characterization of a series of chemically modified potato starches. Carbohydr Polym 2002; 47: 245-252*
23. *Zhu F, Mojel R, Li G. Physicochemical properties of black pepper (Piper nigrum) starch. Carbohydr Polym 2018; 181: 986-993.*
24. *Aghili SR, Jabbari Amiri MR, Abastabar M. Risk of Fungal Contamination of Ointments and Tablets after Opening of the Package for Use in Hospitals. Iranian J Health Sci 2016; 4(4): 1-13.*
25. *Dao H, Lakhani P, Police A, Kallakunta V, Ajjarapu SS, Wu KW, Ponkshe P, Repka MA, Murthy SN. Microbial Stability of Pharmaceutical and Cosmetic Products. AAPS PharmSciTech 2018; 19(1):60-78.*