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Microbiological Suitability of African Bitter Yam Starch

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Starch was extracted from African Bitter Yam *(Dioscorea dumetorum)* and its physical properties were determined. Its ability to support growth of microorganisms was assessed and compared with maize starch BP. Both starches were used to prepare compressed tablets by wet granulation method. Some physical characteristics of the tablets were determined. The tablets were further tested for the ability to support microbial growth soon after production and after storage at ambient conditions for four weeks. Both starches as well as lactose tablets containing them did not support the growth of coliform organisms and *Salmonella* species. Maize starch BP supported growth of microorganisms more than African Bitter Yam starch. All the tablets complied with the British Pharmacopoea standards for uncoated tablets. Tablets containing maize starch BP and those containing African Bitter Yam starch did not support the growth of coliform organisms and *Salmonella* species. Tablets containing Yam starch supported much less microbial growth than those of maize starch BP. It is inferred that African Bitter Yam starch can be considered as pharmaceutical grade starch.

Key words: African Bitter Yam, *Dioscorea dumetorum,* Microbiology.

Introduction

Pharmaceutical grade starch consists of polysaccharide granules extracted from the caryopsis of mature maize grain *Zea mays,* of rice *Oryza sativa* or of wheat *Triticum aestivum* or from the tubers of potato *Solanum tuberosum.* Starch is essentially inert but serves numerous purposes in the pharmaceutical industry. Starch and its modifications are the most important pharmaceutical excipients. In tablet technology, starch serves as filler, binder and disintegrant (Gossinger and Staum 1980; Juslin *et al* 1981; Kottke *et al* 1993). The British Pharmacopoea (BP) suggests that in tropical and sub-tropical countries where maize starch, rice, wheat or potato starches may not be available, Tapioca starch, obtained from the rhizomes of *Manihot utilissima,* may be used provided it complies with the specifications of pharmaceutical grade starches. It is known that the pharmaceutical properties of a starch may depend on its botanical source. Consequently, starches from various other sources have been extracted and extensively studied (Mittal and Ocran 1968; Nasipuri 1975; Esezobo and Ambujam 1982; Esezobo 1986). Starch from African Bitter Yam (ABY) *Dioscorea dumetorum,* family *Dioscoreaceae,* has been studied as disintegrant and binder in tabletting (Iwuagwu *et al* 1986). with encouraging results. ABY grows wildly and is cultivated with high yield in the southeastern region of Nigeria on a small scale. Since many people do not eat it, its starch will be economical to the pharmaceutical industry.

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Microbial contamination of a pharmaceutical product may have its origin in the raw materials, equipment and the packaging. It may also result from lack of good manufacturing practice as well as its method of use. Starch as a raw material of natural origin is usually supplied to the manufacturer of pharmaceuticals as a non-sterile product. It is, therefore, important that it does not support the growth of microorganisms unduly which may have pathologic consequences or undermine the aesthetic value of the preparations. The United States Pharmacopoea (USP) prescribes that pharmaceutical grade starch must be devoid of *Salmonella* species and *E. coli.*

The BP specifies that *E. coli* must be absent from pharmaceutical grade starch. Although both compendia are silent on the gorwth of fungi on starch, effort should be made by the manufacturers to control growth of mould on starch. This is predicated on the ability of some fungi to produce a plethora of mycotoxins, which can precipitate pathogenic states in humans e.g. Aflatoxins.

Starch has been implicated in the contamination of tablets of barbiturates, tranquilizers, digitalis and alkaloids (Kallings *et al* 1966) and potato starch has been reported to be much more heavily contaminated (coliforms up to 120 cells g^{-1} in 32 of 38 samples and total bacterial counts up to 20,000 cells g^{-1}) than wheat starch, while no coliform organisms were found in maize starch.

The present study investigates the microbial suitability of ABY starch as a pharmaceutical tablet excipient in comparison to the starch BP as standard. The use of the latter as a

standard was advised by its popular use in the pharmaceutical industry as filler, disintegrant and binder.

Materials and Methods

ABY starch was extracted from mature tubers of the yam according to the method earlier described by Iwuagwu *et al* (1986). Maize starch BP (courtesy of May and Baker, Lagos, Nigeria), lactose (A.B. Knight & Co., UK), Saboraud agar, McConkey agar, nutrient agar, nutrient broth and salt agar (all from Oxoid, London, UK) were purchased from the vendors. Water was sterile and double distilled and all other chemicals were analytical reagent grade.

The ABY starch was defatted by slurring in warm acetone and stirring for 6 h. The acetone was squeezed out and the starch dried in a hot air oven (Kottermann, Germany) at 50°C for 12 h to a moisture content of about 2.0% and characterised. The Saboraud agar, nutrient broth, nutrient agar, McConkey agar and 7% salt agar were prepared according to the manufacturer's instructions and sterilized at 121°C for 30 min in an autoclave. The angle of repose was determined by a modification of the method of Jones and Pilpel (1966). The swelling capacity was estimated by the method of Bowen and Vadino (1984). The hydration capacity (water reaction capacity) was determined by a method similar to that of Ring (1985). The pH of the starch sample was determined by the BP method using an electronic meter (Phywe, Germany). The true density was determined by the specific gravity bottle method in which the light liquid paraffin of known specific gravity was used. The size of the ABY starch grains was determined using a camera lucida. The sizes of 40 randomly selected grains were determined and the mean and stanard deviation computed.

Both starches were subjected to microbial tests. 1 g of starch was evenly suspended in 10 ml of nutrient broth from which 10^{-1}, 10^{-2}, and 10^{-3} dilutions in water were made. 0.1 ml of the 10^{-3} dilution of the starch broth was inoculated onto plates containing Saboraud agar. The latter were incubated at ambient temperature of 32°C for 72 h, while the former was incubated at 37°C for 18 h.

Four batches of lactose granules were prepared by the wet granulation method based on the formula listed in Table 1. Lactose was mixed intimately with the appropriate disintegrant starch in planetary mixer (Moulinex, France) and wetted with starch paste to an acceptable consistency. Wet massing was carried out for 10 min. The wet mass was passed through a 1.40 mm aperture sieve. Wet granules were dried in the hot air oven (Kottermann, Germany) at 50°C for 16 h and the dried granules were passed through a 1.18 mm aperture sieve. Dry granules were further dried for 4 h to a moisture content of 1.5 to 1.8%.

Tablets were made by mixing (Rotomixer, Foster Equipment, UK) the granules with 1% w/w magnesium stearate and compressing in a single punch tabletting machine (Koln Hiehl, Germany) at a fixed compression pressure to compacts weighing 560-580 mg. The tablets were stored in sterile glass bottles fitted with lined plastic screw caps for at least 48 h at ambient temperature and relative humidity (32°C/75%) before characterisation.

The tablets were assessed for friability (Erweka TA, Germany), hardness (Schleuniger 2E-205, Switzerland) and disintegration time (Mark IV, Manesty Machines, UK).

Microbial testing of the tablets was carried out on batches I and II as follows: six plates of each of Saboraud agar, McConkey agar, nutrient agar and 7% salt agar were prepared and paired such that each pair would correspond to a particular dilution. One tablet was dispersed in 10 ml water and serially diluted aseptically in three other test tubes to 10^{-1}, 10^{-2} and 10^{-3} respectively. 0.1 ml of each dilution was aseptically inoculated into the plates and incubated. The Saboraud agar plates were incubated at room temperature of 32°C for 48 h, while those containing McConkey agar, nutrient agar and 7% salt agar were incubated at 37°C for 48 h. Subsequently, the colonies were counted by direct counting method. The procedure was repeated after 4 weeks of storage for both the batches.

Table 1
Formula for the wet granulation of lactose

Ingredients	Amount per tablet (mg)			
	I	II	III	IV
Lactose	500	500	500	500
Maize starch BP	50	-	50	-
African bitter yam starch	-	50	-	50
10% Maize starch paste	qs	-	-	qs
10% ABY starch	-	qs	-	qs

Table 2
Some physical characteristics of ABY starch

Characteristics	Results
Angle of repose	48.0°
pH	6.25
True density	1.2 g ml^{-1}
Swelling capacity	1.45
*Grain size	4.37(1.7)
Water retention capacity	2.98

*Standard deviation in parenthesis

Results and Discussion

Some physical characteristics of ABY starch are listed in Table 2. The angle of repose of a powder heap is an index of its flowability (Neumann 1967). Free flowing powders have angles of repose between 25° and 50°. The ABY starch is free flowing and will not impede the flow of tablet granulation. The pH of the yam starch, which is in the neutral range, is ideal since it will not alter the pH of liquid environment to the extent of compromising drug stability or its bioavailability. With a specific gravity of 1.52 g cc^{-1} the yam starch is heavy and this may be contributory to its good flow characteristics (Neumann 1967). The swelling capacity as well as the water retention capacity of the yam starch are suggestive of excellent disintegrant characteristics.

The microbial counts of the starches are listed in Table 3. The organisms isolated from the starches were *Bacillus subtilis* and moulds. There was no growth on McConkey agar for both the starches, indicating the absence of enterobacters such as *E. coli, Pseudomonas aeruginosa* and *Salmonella typhi*. There was no growth in 7% salt agar for both the starches. This was indicative of the absence of *Staphylococcus aureus*. Moulds were present in both the starches. However, the count for the maize starch BP was more than twice the number for ABY starch as shown by the count in the Saboraud agar plates. The number of the colonies of the fungi was found to decrease as the concentration of inoculum decreased. No yeast was identified in either of the starches. There was microbial growth in the nutrient agar containing both the starches. More growth (about 1.5 times) of bacteria was observed on the maize starch BP plates than on the ABY starch plates.

Table 4 lists some physical characteristics of the tablets prepared as earlier detailed. All the tablets had short disintegration times well within the BP specified 15 min for uncoated tablets. Although there was no significant difference ($p > 0.05$) between the disintegration times of various batches of tablets, batches II and IV which contained ABY starch as disintegrant, exhibited longer disintegration time than batches I and III. This could imply that ABY starch may be slightly weaker disintegrant than maize starch BP. Batches II and IV were stronger than batches I and III tablets ($p < 0.05$). The strength of the tablets correlated with the disintegration time. This finding is in agreement with that of Iwuagwu *et al* (1986). The friability values of all the tablets were within an acceptable range of 0.77-1.67%. These values of friability suggest that the tablets would be able to resist abrasive shock occasioned by transportation and handling.

The results listed in Table 5 indicate that microbial growth occurred in Saboraud and nutrient agar media containing tablets made with both the starches. It is significant to note that in Saboraud agar, tablets made with maize starch supported more growth at all dilutions than tablets made with ABY starch. Indeed, dilution of the latter to 10^{-2} and 10^{-3} resulted in no growth on the plates. No growth was observed in the McConkey agar and 7% salt agar. It was observed that both the starches supported more growth before than after the tabletting. This observation may suggest that tablet-manufacturing process, which involves drying and compression in which considerable heating is involved, reduces the degree of contamination of the tablets. The microbial counts of the tablets after 4-week storage period were similar to the initial

Table3
Microbial counts of the starches (before tabletting)

Medium	No. of colonies* Maize starch BP	ABY starch
Nutrient agar (bacterial count)	9×10^{-2} cfu	6×10^{-2} cuf
McConkey agar (bacterial count)	no growth	no growth
Saboraud agar:		
(fungi count)	11cfu	5 cfu
(yeast count)	no growth	no growth
7% Salt agar (bacterial count)	no growth	no growth

*All counts were means of at least duplicate experiments and are expressed in colony forming units (cfu).

Table 4
Some physical characteristics of the tablet

Batch No.	Parameters *Disintegration time (min.)	Friability (%)	**Hardness (kp)
I	1.35	0.84	10.2 (1.2)
II	3.24	1.67	15.4 (1.0)
III	1.46	1.18	10.5 (1.2)
IV	2.48	0.77	14.2 (1.0)

*, Mean of 6 determinations; **, mean of 10 determinations; standard deviations are listed in parentheses.

Table 5
Microbial counts (cfu) of lactose tablets containing starch disintegrants

Medium	Saboraud agar		Nutrient agar		McConkey agar		7% Salt agar	
*Disintegrant	I	II	I	II	I	II	I	II
Dilutions:								
10^{-1}	75	4	28	5	-	-	-	-
10^{-2}	15	-	13	2	-	-	-	
10^{-3}	2	-	7	1	-	-	-	-

*Disintegrant I, maize starch; Disintegrant II, African bitter yam starch

counts. The crushed as well as the intact tablets which were exposed to the atmosphere did not support any growth of microorganisms. The granules and tablets remained dry and the absence of significant amounts of moisture may have been responsible for the absence of growth on the samples. When stored under dry conditions, spoilage due to growth of the microorganisms is unlikely to occur (Blair *et al* 1988). Usually, storage of tablets under tropical conditions of high temperature and relative humidity precipitates a dramatic loss of microbial integrity within a relatively short period of time (Bos *et al* 1989). In a recent study, it was shown that the addition of a suitable preservative prevented spoilage of tablets due to the growth of natural contaminants during storage under tropical conditions (Bos *et al* 1991). The results of this investigation may indicate that ABY starch is microbiologically superior to maize starch BP and may therefore be suitable as a pharmaceutical excipient. It may therefore be used instead of the official starches as such.

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