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Studies on Microbiological Quality and Stability of Folic Acid Tablets Formulated with Tapioca Starch

Kubili Mshelbwala[1], Kenneth Chibuzor Ofokansi[2], Franklin Chimaobi Kenechukwu[2]

[1]Department of Pharmaceutics and Pharmaceutical Microbiology, Faculty of Pharmaceutical Sciences, Almadu Bello University, Zaria, Kaduna State, Nigeria

[2]Drug Delivery Research Unit, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka 410001, Enugu State, Nigeria.

ABSTRACT

The purpose of this study was to assess the microbiological quality and stability of folic acid tablets formulated with tapioca starch as a binder in a comparative study with corn starch BP as the standard binder. This investigation was carried out by evaluating the microbiological quality of the starches, the granules and the folic acid tablets prepared by wet granulation, at specified periods of time under various temperature and humidity conditions. Results indicate that the imported maize starch B.P. had a level of microbial contamination which is comparable with the locally extracted cassava starch. The level of microbial contamination, which was higher when non-sterile distilled water was used as the diluting medium than when sterile distilled water was employed, was shown to be dependent on the both the temperature and relative humidity, with high humidity supporting microbial growth in the products. Folic acid tablets formulated with the two different starches exhibited lower level of microbial contaminants than the raw starches, indicating possible denaturation of the mainly vegetative organisms during tableting. Further bioevaluation is ongoing in our laboratory on the effect of compression during tableting on the level of microbial contamination.

KEY WORDS: Tapioca starch, tablets, stability, folic acid, microbiological quality.

INTRODUCTION

In recent times, focus on plant research has increased all over the world and a lot of evidence has been collected to show immense potential of medicinal plants used as phyto-medicines for the treatment of disease [1] as well as traditional excipients for the manufacture of various dosage forms [2]. Cassava starch is obtained from the rasped tubers of *Manihot utillisima,* a tropical root tuber commonly used as food in Nigeria and other tropical countries. The plant is cultivated and grows to a height of about 2.4 m. The roots or tubers that contain the starch radiate from the stem just below the surface of the ground. The starch has been widely studied with respect to its suitability as an excipient in pharmaceutical dosage forms particularly in tableting technology [3 – 7]. The International Starch Institute has demonstrated for the first time on an industrial scale, that tapioca (cassava) starch can be produced with the same purity and yield as that of the potato starch industry [8].

Folic acid (also known as vitamin B9 or folacin) are forms of the water-soluble vitamins. Folic acid is itself not biologically active, but its biological importance is due to tetrahydrofolate and other derivatives after its conversion to dihydrofolic acid in the liver. Absorption of folic acid by the body is facilitated by enzymes associated with the mucosal cell membrane. More specifically, absorption primarily occurs in the mucosa of the upper intestine, known as the jejunum and duodenum. Insufficient folic acid in the diet and the inability to absorb folic acid can cause anemia or birth defects, namely, anecephaly and spina bifida, the latter

*Author for correspondence; chimafrankduff@yahoo.com;frankline.kenechukwu@unn.edu.ng;**Tel**: +234-8038362638

resulting in brain development abnormalities [9]. Tablets are solid dosage forms containing medicinal substances with or without suitable diluents. Tablets are simple and convenient to use. They provide an accurately measured dosage of the active ingredient in a convenient portable package, and can be designed to achieve sustained release, protect unstable medications or disguise unpalatable ingredients [10, 11]. They may be classified according to the method of manufacture as either compressed tablets or molded tablets. The compressed tablets are the most widely used dosage form in the world and the vast majority of all tablets manufactured are made by compression. The compressed tablet is the most popular dosage form in use nowadays. About two-thirds of all prescriptions are dispensed as solid dosage forms, and half of these are compressed tablets [12]. Tablet dosage forms are the most popular and preferred drug delivery systems in terms of precision of unit dose, low cost, patient compliance, and good physical and chemical stability and account for 70 - 80 % of all pharmaceutical dosage forms [13]. Tablets have remained the most common dosage form by which medicaments are usually administered to patients because of their advantages over the other dosage forms [14].

It is always very important to assess the stability (short-term or long-term) of pharmaceutical formulations. Stability could be viewed from the degradation of the active ingredients or a change in the physicochemical properties of the formulations [15, 16]. Expectedly, stability test which involves examining for quality and potency at suitable time intervals, is ought to be conducted for a period corresponding to the normal time that the prodcut would remain in stock or in use. Since this test is time-consuming and expensive, accelerated stability tests are employed during the product development stage to enable rapid prediction of long-term stability of the product. It is then possible to identify the most stable formulation without resorting to the lenghty conventional stablity tests. No published work is available in the literature concerning the stability of folic acid tablets formulated with a native starch.

Consequently, the objective of this study was to investigate the microbiological quality and stability of folic acid tablets formulated with tapioca starch as a binder in a comparative study with corn starch BP as the standard binder. This investigation was carried out by evaluating the microbiological quality of the starches, the granules and folic acid tablets prepared by wet granulation, at specified periods of time under various tempearture and humidity conditions.

MATERIALS AND METHODS

Materials

The materials used include folic acid (Hoffman La Roche Ltd, Basel, Switzerland), Maize starch BP (BDH Chemicals Ltd, Pool, UK), Lactose BP (AB Knight and Co, London, UK). Tapioca starch was processed in the formulation laboratory of the Department of Pharmaceutics and Pharmaceutical Microbiology of Ahmadu Bello University, Zaria.

Preparation and physicochemical properties of Cassava starch

The tubers of cassava (*Mannihot utilissima*) were procured from the Institute for Agricultural Research, Ahmadu Bello University, Zaria.The Tapioca starch was prepared according to an established procedure [4] and as reported [7]. Preliminary physicochemical tests were then performed on the cassava and maize starches following standard procedures [3-6].

Preparation of starch mucilage

Starch mucilage was prepared following a method already reported [5] by weighing amounts of starch powder that would produce 5 %w/w of the starch binders in the formulation. Each weighed quantity was then suspended in the required amount of distilled water in a beaker and heated with continuous stirring until the mucilage was formed. The mucilage was used while still hot for a more effective binding.

Preparation of granules

The formulation compositions of different batches of the granules prepared by wet granulation method [4] and tablets are presented in Tables 1 and 2. Briefly, accurately weighed quantities of folic acid and the starches were mixed together in an automatic mixer followed by addition of 5 % binder mucilage and further mixing.The resulting wet mass was transferred to a granulator fitted with an appropriate sieve. The granules formed were dried and sieved followed by addition of magnesium stearate and Talc powder, and mixing in a hand mixer.

Granules size analysis

The sieve method of analysis was employed [4-6]. Briefly, four sieves (500 µm, 355 µm, 250 µm, and 180 µm pore sizes) were arranged in order of decreasing size in a stack such that the sieve with the largest pore size was at the top. A 100 g of the

granules was poured into the topmost sieve and the Endecott sieve shaker was then switched on for 15 min. The amount of the granules left on each of sieve was weighed and the percentage of the granules on that sieve was calculated.

Table 1: Formulation compositions of the tablets

Batch code	Ingredient (mg)					
	Folic acid	Starch	Lactose	Binder	Talc	Magnesium stearate
I	2.50	54.10	-	0.156	3.24	0.36
II	2.50	42.00	12.00	1.093	3.24	0.36
III	2.50	30.00	24.00	1.015	3.24	0.36
IV	2.50	18.00	36.00	0.78	3.24	0.36
V	2.50	-	54.00	0.468	3.24	0.36

Table 2: Composition of starch and lactose in the tablets (total weight = 60 mg)

Batch code	Starch (%)	Lactose (%)	Folic acid (%)
I	90	0	4.1
II	70	20	4.1
III	50	40	4.1
1V	30	60	4.1
V	0	90	4.1

Tablet compression

The granules were compressed into tablets on a single punch Manesty tabletting machine (Manesty, England) fitted with a circular 5.5 mm set of punches and die, according to an established method [3]. The compression pressure was maintained at 4.5 kg/sq.cm and the speed of compression was 70 tab/min.

Bioevaluation

Microbiological tests were carried out on the raw starches and the formulations (granules and tablets) using standard procedures [15, 16]. The pour plate method was employed and the suspending media were sterile distilled water and non-sterile distilled water. A 5 % starch suspension was thoroughly mixed on a Gallenkamp whirl mixer and 2 ml volume was withdrawn and serially diluted in sterile nutrient agar (N/A) for the bacteria and in Sabouraud dextrose agar (SDA) for the fungi. The plates were incubated at 37 ˚C for 48 h for bacteria and at 24-26 ˚C for 96 h (for fungi). The plates were observed daily for presence or absence of growth. Viable counts were taken at the end of the incubation period using the Gallenkamp colony counter. Each experiment was replicated and the mean colony/spores taken and the colony forming units per ml (CFU/ml) or spore forming units per ml (SFU/ml) as the case may be were calculated. The entire procedure was repeated using the granules and the tablets.

Identification and confirmatory tests for micro-organisms

Identification and confirmatory tests were carried out, using standard procedures [17], on the starches to detect the presence or absence of the following micro-organisms in the samples: *Escherichia Coli*, *Salmonella spp. Staphylococcus aureus,*and *Pseudomonas aeruginosa* for bacteria and *Candida albicans*, *Aspergillus niger*, *Rhizopus mucor* and *Penicillium spp* for fungi.

Stability studies

Time and relative humidity-dependent microbiological studies were also carried out on the raw starches and the formulations, following the ICH guidelines [18] and in accordance with previous study [12].

RESULTS AND DISCUSSION

The tapioca starch passed all the preliminary tests (iodine and acidity tests) carried out to confirm the presence of starch [3]. There was no cyanide detected in the cassava starch sample. Cassava contains cyanide in different quantities [8]. The species of cassava cultivated in the northern parts of Nigeria could be eaten raw or cooked [19]. Most pharmaceutical industries have objected to the use of cassava starch due to the presence of cyanide [8]. There was no evidence of the presence of cyanide in the starch employed in the present study. The cyanide present in the species employed in this study could have been destroyed during the process of starch extraction, consistent with previous report [19]. The Tapioca starches were spherical with a particle density and size range of 1.446 – 1.461 g/cm^3 and 3.50 – 10.50 µm respectively. Their moisture content varied between 8.09 and 20.0 % and with poor flow properties. The steeping period is known to influence the physical characteristics of the starches [7].

Table 3 shows the moisture absorption and desorption properties of the local cassava starch and imported maize starch B.P. It is evident from the Table that the two starches absorbed moisture gradually and lost all the moisture almost at the same rate under the experimental conditions employed. Cassava starch absorbed larger amounts of moisture than maize starch B.P. at all levels of relative humidities employed. At the highest RH investigated i.e. 85 %, cassava starch

absorbed 12.0 % while the maize starch absorbed 11.1 % of moisture. The moisture absorbed is reversible as shown by the amounts of moisture desorbed from the starches (Table 3).

While Figs. 1a-b depict the bacterial count which was carried out first in the months of August and September and second in the months of November and December in both sterile and non-sterile distilled water, respectively; Figs. 2a-d show the level of fungi in the starches. Generally, there were higher bacterial and fungal counts in the months of August and September than in the months of November and December. For bacterial count, in the first case in the two starches, there were more bacteria in the starches formulated in non-sterile water than in sterile distilled water. Comparing the bacterial count in the two starches prepared with sterile distilled water, the imported maize starch B.P. had higher bacterial count than the local cassava starach. In the second case using sterile distilled water, the bacterial count in the cassava starch was higher than that in the imported maize starch in both sterile and non-sterile distilled water. More so, the level of fungal contaminants was lower in the imported maize starch B.P. than in the local cassava starch.

Table 3: **Moisture absorption and desorption properties of the starches**

Parameter	Relative humidity (%)	Starch	
		Maize	Cassava
Moisture absorption	0	0	0
	33	6.2	7.3
	76	9.1	10.1
	85	11.1	12.0
Moisture desorption	76	4.2	6.1
	33	6.3	7.1
	0	12.3	13.5

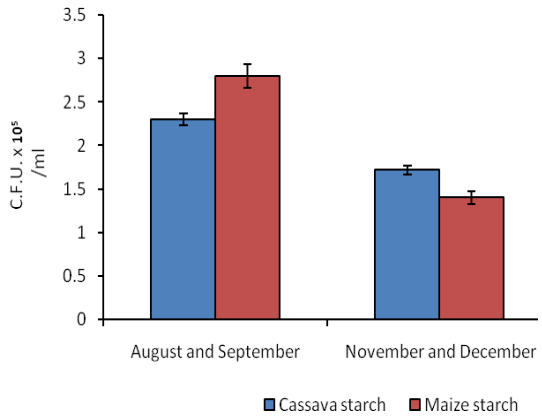


Figure 1a: Level of bacteria count in the starches prepared using sterile distilled water.

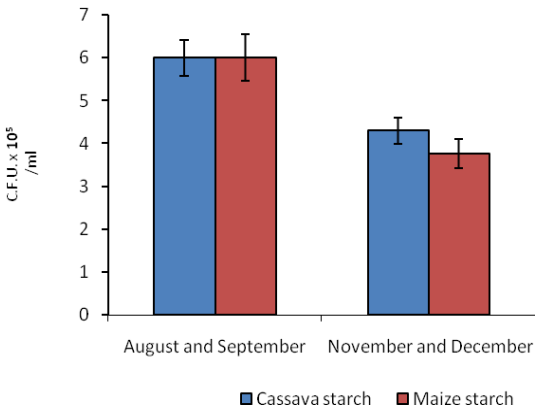
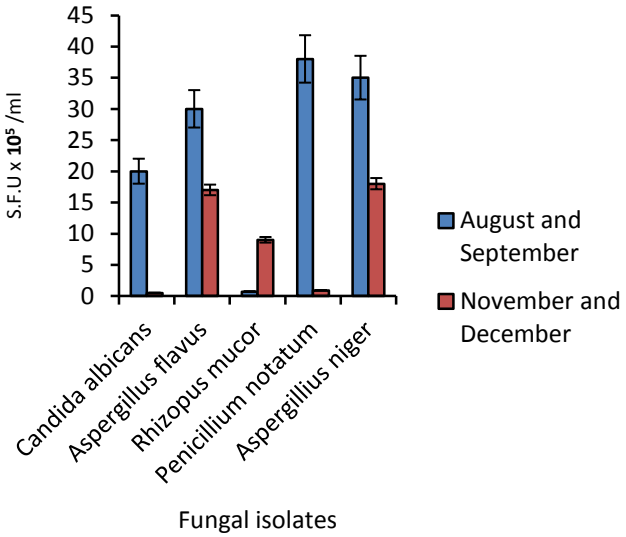


Figure 1b: Level of bacteria count in the starches prepared using non-sterile distilled water.



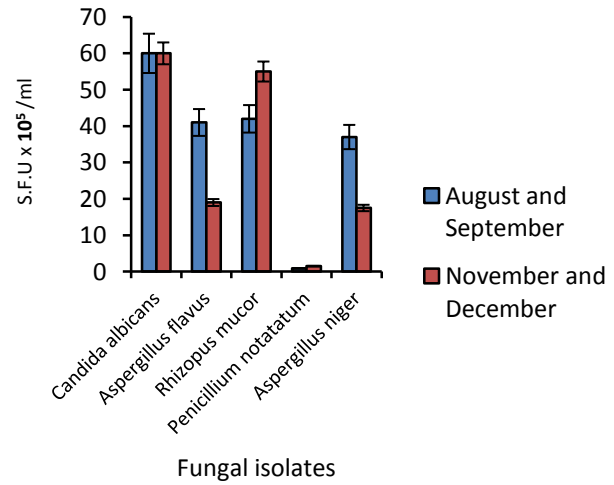


Figure 2a: Level of fungal SFU recorded in cassava starch prepared using sterile distilled water
Figure 2b: Level of fungal SFU recorded in cassava starch prepared using non-sterile distilled water

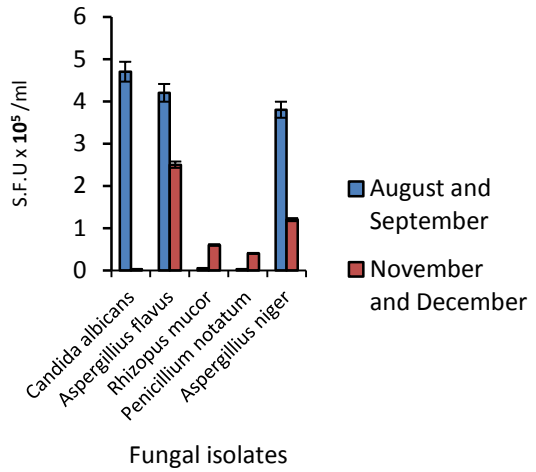


Figure 2c: Level of fungal SFU recorded in maize starch prepared using sterile distilled water

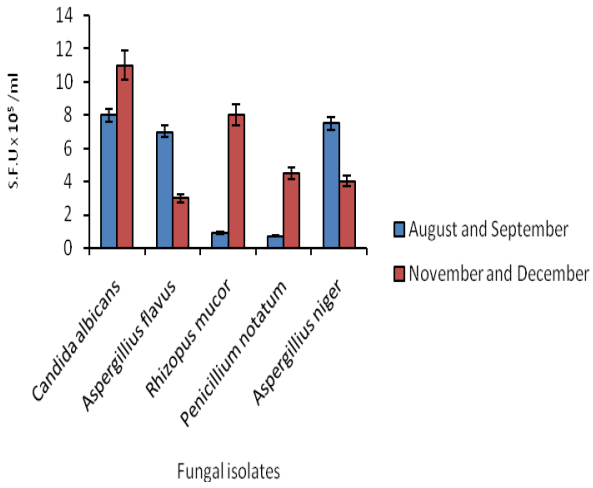


Figure 2d: Level of fungal SFU recorded in maize starch prepared using non-sterile distilled water

Fig. 3 shows the level of bacterial contaminants in the folic acid granules and tablets prepared with the starches. The test was carried out in sterile distilled water only. The granules prepared with the imported maize starch B. P. recorded higher number of bacterial count than the granules prepared with the local cassava starch. The effect of relative humidity on the level of bacterial contaminants is shown in Fig. 4. The level of bacterial contaminants in the tablets prepared with cassava starch was higher than in the tablets prepared with maize starch B. P. Comparing the bacteria count in the granules and tablets prepared with the starches, there was marked reduction in the bacterial count in the tablets prepared with maize starch B.P. The reverse was the case with granules and tablets prepared with cassava starch where the tablets recorded higher bacteria count than the granules. As illustrated in Fig. 4, at 0 % R.H., the bacteria count in the two starches was between 1 x 10^4 to 2 x 10^4 CFU/ml. However, at 85 % R.H., the two bacteria count increased considerably to between 3 x 10^4 to 5 x 10^4 CFU/ml with tablets formulated with maize starch B.P. recording a higher number than tablets prepared with cassava starch. Fig. 5a-b show the level of fungal contaminants in the folic acid tablets stored under 0 % and 85 % relative humidities for five weeks. The figure indicates that, at 0 % R.H., the level of fungi was less in all the tablets formulated with the two different starches as compared to that at 85 % R.H. Tablets formulated with cassava starch, however, recorded a lower level of fungal contaminat than tablets prepared with maize starch B.P. at both 0 % and 85 % R.H. Identification/confirmatory tests revealed the presence of bacteria (*Staph aureus* and *E. coli*) and fungi (*Candida albicans*, *penicillium notatum*, *Asp. flavus*, *Asp. niger* and *Rhizopus mucor*) in the cassava and maize starches. However, the maize starch B.P. had less level of contaminants than cassava starch in sterile media. In fact, in the months of August and September in sterile media, there were only two types of contaminants identified in the maize starch B.P., namely *Asp. flavus* and *Candida albicans*.

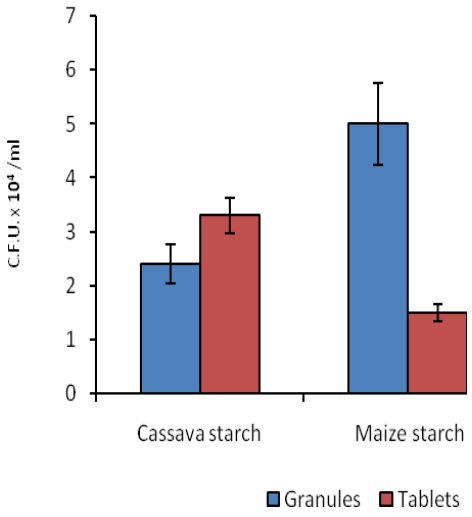


Fig. 3: Level of bacterial count recorded in cassava starch based folic acid granules and tablets.

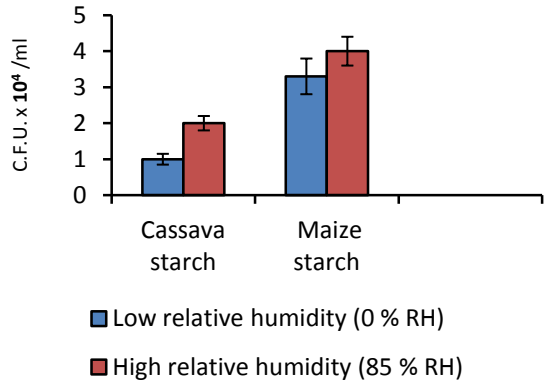


Fig. 4: Viable count of bacteria recorded in cassava starch based folic acid tablets upon storage.

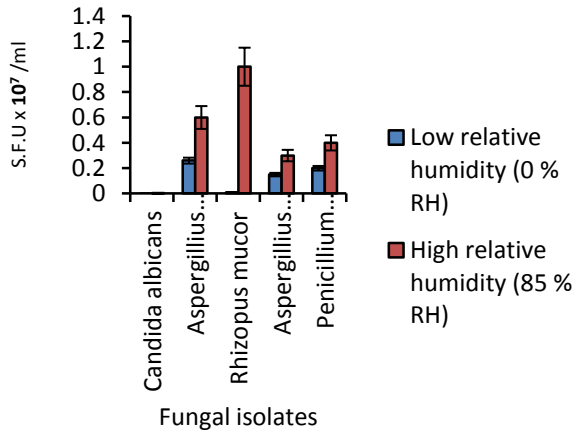


Fig. 5a: Level of fungal contaminants recorded in cassava starch based folic acid tablets upon storage

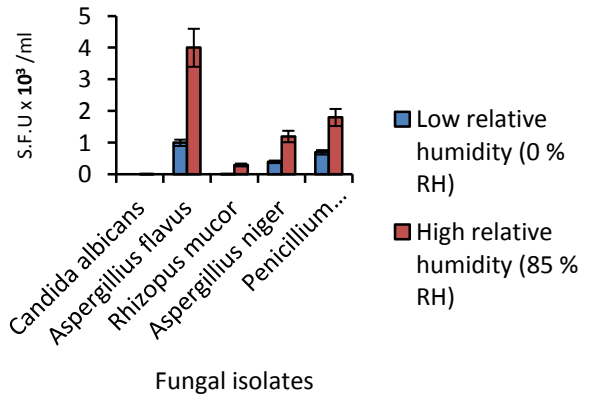


Fig. 5b: Level of fungal contaminants recorded in maize starch based folic acid tablets upon storage.

It is discernible from the results of the bioevaluation that the levels of both bacteria and fungi isolated were higher when non-sterile distilled water was used than when sterile distilled water was used. This was due to the killing of a lot of the bacteria during the sterilization process [17]. It was also observed that the level of contaminant was higher during the months of August and September than during the months of November and December. This was due to the high humidity plus the high temperature which provided a good breeding ground for the bacteria during the months of August and September as against the dry weather condition, low humidity and low temperature during the months of November and December; environmental conditions that are not conducive for bacterial growth [17]. Results equally indicate that, during the months of August and September, the imported maize starch B.P. recorded higher level of bacterial contaminant than the cassava starch. The reason for this is not certain but may be related to chanced contamination during tablet production [17]. Ideally, the imported maize starch ought to have recorded a lower amount of bacterial contaminants than the cassava starch. However, during the months of November and December, there was higher bacterial contamination in the cassava starch than in the imported maize starch B.P. In the case of fungi, maize starch B.P. recorded less number of fungi than the cassava starch. In the months of November and December, the two different starches recorded less number of SFU/ml of fungi possibly as a result of lower level of relative humidity and lower temperature which were not conducive for fungal growth. Therefore, the weather affected the growth of the fungi since they are all vegetative in nature.

When the level of contaminants from the raw material, the granules and the tablets were compared, the starting raw material contained higher level of contaminats than the granules and the tablets contained the least level of contaminants in the case of bacteria. The only exception was the cassava starch-based tablet which contained a higher level of contaminants in the tablets than the granules, which may be attributed to chanced contamination during tableting. There might have been gradual destruction of the bacterial cells during the process of granulation and tableting [17]. The process of drying and tableting involved heat [3, 5, 9-12, 19] and since most of these micro-organisms were vegetative they might have been destroyed in the process. The higher bacterial growth recorded under 85 % R.H. than the 0 % RH is further proof that high humidity supports bacterial growth whereas low RH retards bacterial growth.

The contaminants isolated from the starch samples, granules and tablets call for the observation of current Good Manufacturing Practices (cGMP). If cGMP is not followed, it could lead to microbiological spoilage of pharmaceuticals [17, 20]. This condition is caused by the presence of specific types of pathogens in the pharmaceutical products. Bacterial contaminants of raw materials and the finished products have been known to lead to microbial spoilage, which is manifested when the presence of active pathogenic organism is high or when a higher level of opportunists is observed (when the level of opportunists are high, they can become pathogenic); when toxic microbial metabolites are present; when there is detectable physical and chemical changes which have occurred in the product [20]. cGMP requires that correct raw materials should be used and appropriate procedures adopted by adequately trained personnel working with suitable and necessary facilities. Moreover, pharmaceutical raw materials should be free from potential pathogens namely *Salmonella*, *Escherichia coli* and *Pseudomonas aeruginosa* [20]. Therefore, the detection and isolation of these pathogenic microorganisms from samples calls for extra care in the manufacturing process. The level of opportunistic organisms present under given suitable conditions could become pathogenic.

On the whole, the results of this study indicate that cassava and maize starches contained both high level of opportunists and some active pathogens. Furthermore, the final products (tablets) had lower level of contaminants than the starting raw materials (starches), an implication that a lot of the microorganisms were destroyed during the processes of granulation and tableting.

CONCLUSIONS

This study has shown that the imported maize starch B.P. had a level of microbial contaminants which is comparable with the locally extracted cassava starch. The level of microbial contamination, which was higher when non-sterile distilled water was used as the diluting medium than when sterile distilled water was employed, was shown to be dependent on both the temperature and relative humidity, with high humidity supporting microbial growth in the products. Folic acid tablets formulated with the two different starches exhibited lower level of microbial contaminants than the raw starches, indicating possible denaturation of the mainly vegetative organisms during tableting. Further bioevaluation is ongoing in our laboratory on the effect of compression during tableting on the level of microbial contamination.

REFERENCES

1. Dahanuka SA, Kulkarni RA, Rege NN. Pharmacology of medicinal plants and natural products, Indian J. Pharmacol., 2002; 32: 508-512.
2. Kottke MK, Chueh HR, Rhodes CT. Comparison of disintegrant and binder activity of three corn starch products, Drug Dev. Ind. Pharm., 1992; 18: 2207–2223.
3. Okor RS, Obarisiagbon J. Evaluation of tapioca powder as a tablet base, Nig. J. Pharm., 1981; 12: 17-24.
4. Saringat B, Eseldin IK, Ching HS, The study of some mechanical properties of Tapioca starch, Acta Scientia, 1997; 7: 35-54.
5. Saringat B, Eseldin IK, Ching HS. Compaction behaviour of modified tapioca starch. Acta Scientia, 1997; 7: 55-72.
6. Eichie FE, Okor RS. Effect of acid treatment on the consolidation and plastoelasticity of tapioca powder, Trop. J. Pharm. Res., 2002; 1: 45-48.
7. Alebiowu G. Steeping period influence on physical, compressional and mechanical properties of tapioca starch, J. Pharm. Res., 2007; 6: 139 -144.
8. International starch institute, (2009). “Tapioca (cassava) starch”. Accessed on the 21/02/2009 through http://www.starch.dk/isi/applic1.htm
9. Gowtham M, Vasanti S, Rohan RD, Ashwath N, Paridav M. Formulation and evaluation of immediate release folic acid tablets, Der Pharmacia Lettre, 2011; 3(6): 157 – 162.
10. Gohel MC, Bansal G, Bhatt N. Formulation and evaluation of orodispersible taste masked tablets of famotidine, Pharm. Bio. World, 2005; 3:75-80.
11. Patel R, Baria A. Formulation development and process optimization of theophylline sustained release matrix tablet, Int. J. Pharm., 2009; 1: 30-41.
12. Vikramjit S, Nawazish A, Monika S, Alam MS, Ali MS, Md. Alam MI. Development, evaluation and stability studies of zidovudine and lamivudine tablet dosage form, J. App. Pharm Sci., 2012; 2(9): 149-154.
13. Yüksel N, Türkmen B, Kurdoğlu AH, Başaran B, Erkin J, Baykara T. Lubricant efficiency of magnesium stearate in direct compressible powder mixtures comprising cellactose® 80 and pyridoxine hydrochloride, FABAD J. Pharm. Sci., 2007; 32: 173-183.

14. Okoye EI, Onyekweli1 AO, Olobayo OK, Arhewoh MI. Brittle fracture index (BFI) as a tool in the classification, grouping and ranking of some binders used in tablet formulation: Lactose tablets., Sci. Res. Essays, 2010; 5 (5): 500-506.
15. Attama AA, Okafor CE, Builders PF, Ogbonna O. Formulation and *in vitro* evaluation of a PEGylated microscopic lipospheres delivery system of ceftriazole, Drug Deliv., 2009; 16:448-457.
16. Umeyor CE, Kenechukwu FC, Ogbonna JDN, Builders PF, Attama AA. Preliminary studies on the functional properties of gentamicin in SRMS-based solid lipid microparticles, Int. J. Novel Drug Deliv. Tech., 2011; 1: 130-142.
17. Okore VC. Evaluation of chemical antimicrobial agents. In: Pharmaceutical Microbiology: Principles of the pharmaceutical applications of antimicrobial agents. Vincent C. Okore (ed.), 1st edn., Enugu: EL 'DEMAK Publishers, 2005.
18. Singh J, Tripathi KT, Sakia TR. Effect of penetration enhancers on the *in vitro* transport of ephedrine through rat skin and human epidermis from matrix based transdermal formulations, Drug Dev. Ind. Pharm., 2003; 19:1623-1628.
19. Isah AB, Abdulsamad A, Gwarzo MS and Abbah HM. Evaluation of the disintegrant properties of microcrystalline starch obtained from cassava in metronidazole tablet formulations, Nig. J. Pharm. Sci., 2009; 8(2):26-35.
20. Sharp J. Contamination and contamination control. In: Good pharmaceutical manufacturing practice: rationale and compliance. Sharp J (ed.), CRS Press: Boca Raton, Florida, USA; 2005, pp. 85-98.