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Microbiological and physicochemical evaluation of starch produced from cassava and maize

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ABSTRACT

The qualitative assessment of cassava and maize starch revealed that the mean counts of viable bacteria were 4.8 x 10^3cfu/g and 3.7 x 10^3cfu/g for cassava starch (CS) and maize starch (MS) respectively. The coliform counts were 2.9 x 10^3cfu/g and 3.0 x 10^3cfu/g for CS and MS respectively while the fungi counts were 3.53 x 10^2cfu/g and 4.2 x 10^2cfu/g for CS and MS respectively. The mean counts for commercial starch was recorded as 1.5 x 10^3cfu/g, 3.33 x 10^2cfu/g and 1.9 x 10^2cfu/g for total viable bacteria, coliform and fungi respectively. The microbial isolates were identified as *Bacillus alvei*, *Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus* and *Saccharomyces cerevisiae* (yeasts). The starch from both products were odourless, tasteless, insoluble in water and in alcohol. There was a slight dissimilarity in color with CS and commercial starch being white and MS appearing slightly yellow. The pH was 7.48 for CS, 6.50 for MS and 5-7 for commercial starch while the ash content was 0.50% for CS, 0.36% for MS and 0.1-0.6 for commercial starch. The moisture content for CS, MS and commercial starch was 6.5%, 8.0% and 10.0-12.0% respectively while the fibre content was 0.023% for CS, 0.048% for MS and 0.2 for commercial starch. The starch also had varying amounts of fat and starch content. The results suggest that starch produced locally from cassava and maize are contaminated with microorganisms and have some physicochemical qualities that do not meet acceptable standard for industrial starch.

KEYWORDS

Starch;
Cassava;
Maize;
Bacteria;
Yeast.

INTRODUCTION

Starch is widely distributed natural product, obtained from plant sources (grain or root crops) which is consumed as food, but is also readily converted chemically, physically and biologically into many useful products. It is extensively used in pharmaceutical formulations. Its widest pharmaceutical application is in the formulation of solid dosage forms where it serves as binders, fillers, disintegrating agents and can also be used as lubricants for tablet production. This is attributable to its peculiar physical and chemical properties, which amongst others confer on its inertness and blandness. When subject to heat treatment in a water suspension, it undergoes some physicochemical changes that make it useful as binder[5]. Pharmacopoeia starch has been obtained from various sources including maize, wheat, sweet potato, sago, plantain, cocoyam, potato, rice and cassava. Some of this starch like corn starch and potato starch are used as disintegrants in the manufacture of compressed tablets[5]. Many authorities use corn starch and potato starch as standards for evaluation of agents which might serve as disintegrants[3]. All additives have effects on tablets and starch is no exception, hence a good understanding of the properties of starches especially new ones developed for use in the pharmaceutical industry is imperative[5]. This study is limited to cassava and maize starch obtained from freshly harvested cassava tubers and maize grains respectively. Cassava demand is estimated to grow at 2.0% annually for food and 1.6% per year for feed in developing countries, while total cassava production is projected to reach 168 million tonnes by 2020 based on the current production. However, this amount can be far surpassed in developing countries with the right policies and incentives. Moreover, with the increasing establishment of starch – utilizing industries in developing countries, the production will have to increase over the projected figures (275.1 million metric tons)[9]. Nigeria produces annually over 6 million tons out of 624 million tons of maize produced worldwide. The world major producers of maize are USA followed by China and Brazil. The major exporters are USA, China, Argentina, Brazil, Hungary and South Korea, Mexico, Egypt and Taiwan. Nigeria does not participate much in this world trade due to its high domestic demand pressure on the maize for industrial uses, animal feeds and consumption. More than 60% of maize produced in Nigeria is consumed by the industrial sector for the production of flour, beer, malt drink, syrup, cornflakes, starch, dextrose and animal feeds. Nigeria accounts for the largest producer of cassava worldwide, regardless of this fact, all pharmaceutical grade starch (PGS) used by most industries in the country is imported which is an unhealthy situation for both the pharmaceutical industry and economy. Over the years efforts have been made to produce local PGS, but the production is still very much underdeveloped and the starch unappreciated in the country compared to the foreign PGS. The present study therefore becomes necessary. The objectives of this study were to assess the microbiological and physicochemical qualities of starch produced from cassava and maize, and identify the microbial contaminants of the product.

MATERIALS AND METHODS

Collection of samples

Freshly harvested cassava (*Manihot esculenta*) tubers and maize (*Zea mays*) were purchased from Central Market, Minna, Niger State, Nigeria. The samples were transported in cellophane bags to the laboratory, where they were washed and processed for extraction of starch. Preparation and handling of samples were done according to standard procedures prescribed[10]. Commercial starch was obtained, which was used as a control for analysis of both cassava and maize starch.

Extraction of starch

Cassava starch

The cassava tubers were debarked and washed with clean water, sliced into small pieces and soaked in distilled water for 24 hours to increase the moisture content and assist the extraction of starch. The tubers were then grated using a grater, a little amount of distilled water was added to the meshed cassava to increase the moisture content and allow the easy extraction of the starch. The mash was filtered, screened and allowed to settle in a clean bowl. The extracted starch was then dewatered with a clean muslin cloth and finally the starch was spread on a sterile tray, covered with a muslin cloth (to protect from files and dust) and air dried for 4 days to a fine powder.

Maize starch

The maize grain was carefully extracted from a properly cleaned maize cob. The maize was washed and dust, chaffs and foreign materials were removed. The fresh cleaned maize was then soaked in distilled water for 48 hours which increased the moisture content and also assisted the extraction of starch. The maize grain was gently blended with a sterile blender with added controlled amount of distilled water. After blending, the maize grain was filtered and allowed to settle, followed by decanting and dewatering. The maize starch was air dried to a fine powder. All forms of utensils and water used in course of extraction were sterilized properly.

Enumeration of microorganisms in starch

Nine milliliters of distilled water was placed in five test tubes and sterilized by autoclaving at 121°C for 15 minutes. One gramme (1g) of cassava starch was introduced into the first test tube and shaken thoroughly to mix and then serially diluted. The same procedures were repeated for the other samples. All the dilutions were then plated on Nutrient Agar (NA), MacConkey Agar (MCA), and Sabouraud Dextrose Agar (SDA), for the enumeration of aerobic heterotrophic bacteria, coliforms and fungi respectively. NA, and MCA plates were incubated at 37°C for 24hours while the SDA plates were incubated at room temperature (28±2°C) for 3-5days. Colonies which appeared after the incubation were counted and subcultured repeatedly on NA and SDA to obtain pure cultures.

Characterization and identification of isolates

Bacteria

The bacterial isolates were characterized based on colonial morphology and biochemical tests which include Gram staining reaction, catalase, methyl red, indole, citrate utilization and carbohydrate utilization test. The isolates were identified by comparing their characteristics with those of known taxa using the schemes of Buchanan and Gibbons[1]. The fungi growth were examined macroscopically and their morphology determined by staining with lactophenol cotton blue. They were further identified using their morphological and biochemical characteristics[8].

Determination of physico chemical properties

The starch was analysed for P^H, moisture content, fibre, crude fat, ash and starch content following standard procedures[10]. All chemicals used were of analytical grade.

RESULTS

Microbial counts and identification

The mean counts of the total viable bacteria for cassava and maize starch were 4.8 x 10^3cfu/g and 3.7 x 10^3cfu/g respectively (TABLE 1). The results revealed that cassava starch had higher bacterial counts than maize starch. The coliforms counts were quite high in both products (2.9 x 10^3 cfu/g and 3.0 x 10^3 cfu/g for CS and MS respectively). The mean fungi population of cassava and maize starch were 3.53 x 10^2cfu/g and 4.2 x 10^2cfu/g respectively (TABLE 1).

TABLE 1: Counts of microorganisms in starch analysed

Time	Cassava starch			Maize Starch			Commercial starch		
	TVB ($x10^3$cfu/g)	CF ($x10^3$cfu/g)	FG ($x10^2$cfu/g)	TVB ($x10^3$cfu/g)	CF ($x10^3$cfu/g)	FG ($x10^2$cfu/g)	TVC ($x10^3$cfu/g)	CF ($x10^2$cfu/g)	FG ($x10^2$cfu/g)
0	7.0	2.0	1.6	6.2	1.2	2.4	0	7.0	1.2
1	5.2	2.5	3.0	4.0	3.8	6.0	0	1.0	1.0
2	4.8	3.0	6.0	3.5	3.2	TNTC	1.0	2.0	2.2
3	4.0	3.0	TNTC	3.0	3.0	TNTC	2.0	0	4.0
4	3.2	3.8	TNTC	1.7	3.8	TNTC	0	0	1.0
Mean	4.8	2.9	3.53	3.7	3.0	4.2	1.5	3.33	1.9

cfu/g: colony forming unit per gramme; TNTC: Too numerous to count; TVB: Total viable bacteria; CF: Coliforms; FG: Fungi

Identification of isolates

The results indicated that various species of bacteria belonging to four genera were detected in the starch samples. The organisms were *Bacillus alvei*, *Escherichia coli*, *Serratia marcescens* and *Staphylococcus aureus*. *Bacillus alvei* and *Staphyloccocus aureus* were more frequently isolated, each having 30% frequency of occurrence. *E. coli* and *S. marcescens* were less frequently encountered. Yeasts (*Saccharomyces cerevisiae*) that occurred in the samples constituted 15% of the total microbial isolates obtained.

Quality factors and characteristics of starch

The starch was odorless, tasteless and insoluble in water and alcohol. The cassava and commercial starch were white in colour while the maize starch possessed a slight yellow colour (TABLE 2).

PHYSICOCHEMICAL QUALITIES OF STARCH

The results indicated that the ash content was higher in cassava (0.50%) than maize (0.36%). Similar ob-

TABLE 2 : Quality factors and characteristics of starch

Parameter	Cassava (*Manihot esculenta*)	Maize (*Zea mays*)	Commercial starch
Colour	White powder	Slightly yellow powder	White powder
Taste	Tasteless	Tasteless	Tasteless
Odour	Odourless	Odourless	Odourless
Solubility	Insoluble in water and alcohol (96%)	Insoluble in water and alcohol (96%)	Insoluble in water and alcohol

servation was made for the PH. It was also observed that maize starch had a higher fat content (0.032%), fibre (0.048%), moisture (8.0%) and starch content (99.9%) than cassava starch. The commercial starch revealed some slight differences in the parameters studied (TABLE 3).

TABLE 3 : Physicochemical qualities of starch samples analyzed

Parameter	Cassava	Maize	Commercial starch
Ash (%)	0.50	0.36	0.1 - 0.6
Fat (%)	0.001	0.032	0.15 - 0.2
Fibre (%)	0.023	0.048	0.2
Moisture (%)	6.5	8.0	10.0 – 12.0
P^H	7.48	6.50	5 – 7
Starch (%)	99.50	99.90	95.0

DISCUSSION

The results obtained from this study have established that viable microorganism (bacteria and fungi) are found in cassava and maize starch. The sources of these organisms may be due to poor handling and injury to the cassava and maize during harvesting, transporting and storage by the farmers. The large population of these microorganisms may be due to the use of contaminated water for the extraction of the starch. It is also possible that the bacteria may have contaminated the starch during the process of air - drying. The presence of coliforms in the starch was probably because the water used for extraction of the starch was contaminated with faecal materials or the tubers might have carried faecal materials with them during harvesting. Lansing[6] reported that coliforms are widespread with some living in soil and water. The subsequent increase in the coliform counts may be as a result of improper storage of the starch. The presence of yeasts in the starch may have come from the environment, where the tubers and cobs were being stored or as a result of the tubers and cob being soaked in water for extraction. Therefore, since most yeasts are chemoorganoheterotrophs and use carbohydrates to synthesize amino acid and protein, they are very likely to contaminate the starch. The microbial contaminants identified were *Bacillus alvei*, *Escherichia coli*, *Serratia marcescens* and *Staphylococcus aureus*. These organisms occur widely in the environment (air, soil, water). *Bacillus alvei* are important food borne pathogens among the *Bacillus* species. The organism forms a unique type of cell called endospores as a strategy for survival in the soil environment where these bacteria predominate. Due to the resistance of their endospores to environmental stress as well as their long term survival under adverse conditions, *Bacillus alvei* can be isolated from a wide variety of sources, from soil, by direct contact or airborne dust[11]. The implication of the occurrence of *Bacillus alvei* in the starch is that they degrade starch and therefore, reduce the quality of the product.

Escherichia coli is an important food borne disease organism, enteropathogenic, enteroinvasive and enterotoxigenic types can cause diarrhea, Transmission of pathogenic *E. coli* usually occurs via faecal – oral contamination[2]. Common routes of contamination of starch include unhygienic handling, farm contamination due to manure fertilization and irrigation of crops with contaminated water or raw sewage.

Serratia marcescens occurring in starch may reduce the quality of the product due to the degradation of the starch by this organism. It is common in the respiratory and urinary tracts of adults and the gastrointestinal system of children[12]. It is also possible that this bacterium may impart colour to the product due to its red pigment.

Staphylococcus aureus is a microbe found on the nasal membrane and skin of humans. They are responsible for many human diseases; it is a most important human pathogen that causes boils, abscesses, wound infection, toxic shock syndrome amongst other diseases. It is also a major cause of food poisoning[6]. The ash content of the cassava starch (0.50%) was higher than that of the maize (0.36%) yet both parameters were with the in accepted range as compared to that of cassava starch (0.6%) stipulated by[10]. The low amount of ash in the starches indicates that the starch has a low amount of inorganic materials. The fibre content of the cassava starch (0.023%) was lower than that of the maize starch (0.048%). This shows a significant difference with the control starch with 0.17% fibre content and also the standard (0.22%) fixed by SON[10]. The pH of the starch ranged from 6.50 - 7.48. These values show a slight significant difference when compared to the standard (pH 4.7 – 6.8) set by SON[10]. Similarly[7], reported that a good starch should have a PH of 4.7 – 5.3 which is contrary to the result obtained from this study. Thus the recorded pH of the present study indicates that the starch components were intact. That is, they were not fermented which would have resulted in the accumulation of some metabolites and the high starch content of the products. The Kisan sahakari Starch Manufacturing Society Limited, KSSMS[4], reported P^H range of 4.5 – 6.5 for corn starch. Onwueme[7] reported the moisture content of cassava starch to be 10-13.5% while[10,4] set the standard for the moisture content of corn starch to be 12.0% and 11% respectively. The results obtained in the present study indicated that the low moisture content (6.5% - 8.0%) may be due to the fact that the starch was over dried in the sunlight for too long. This might have created the chance for pathogens to contaminate and degrade the starch.

CONCLUSION

According to the results obtained, it is apparent that locally produced cassava and maize starch contain microorganisms that may make the starch unfit for pharmaceutical use. In addition some of the physicochemical qualities of the starch analyzed could not meet the standard requirements for industrial starch. These include fiber, moisture, and pH of the starch. Therefore, starches should be properly processed and handled to avoid microbial contamination and spoilage. Besides, it may be necessary for cassava and maize intended for the extraction of starch to be properly monitored from planting stages to harvesting so as to prevent or reduce microbial contamination.

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