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Microbial Populations Associated with The Retting of African Breadfruit (*Treculia africana*) Pulp

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Abstract

The retting of African breadfruit pulp and microorganisms associated with this process were investigated. Samples were collected from the African breadfruit pulp on 2 days interval, plated on different media and isolates were characterized and identified. The pulp underwent natural fermentation which was characterized by the growth of bacteria 10^2-3.5 × 10^{10} CFU/g, yeast 10-4.5 × 10^5 CFU/g, mold 10^2-2.8 × 10^3 CFU/g and lactic acid bacteria 1.0 × 10^2 to 3.8 × 10^5 CFU/g. The pH values ranged from 5.0 to 5.4 and the lactic acid content from 0.30% to 0.52%. The bacteria isolated were *Micrococcus sp., Lactobacillus fermentum, Bacillus subtilis, Streptococcus sp.*, molds like *Aspergillus niger, Rhizopus stolonifer* and the yeast, *Saccharomyces cerevisiae*. The growth of microorganisms was completely inhibited by alcohol sterilized and antibiotic-treated samples.

Keywords:* *Treculia africana; Retting; Pulp; Microorganisms; Titratable acidity

Introduction

Treculia africana (African breadfruit) is a large evergreen tropical food tree species belonging to *Moraceae* family and the genus *treculia*. In West Africa, individual breadfruit trees are found scattered throughout the Southern rain forest zones [1]. The tree can grow up to 20 m high and produce compound fruits of considerable sizes on the tree trunk or on older branches. The fruit head is spherical in shape with a diameter of up to 0.5 m. The fruit is hard and spongy in texture when ripe and contains numerous seeds like orange pips embedded at various depths in the fleshy pulp [2]. African breadfruit seed is of high nutritional value. *Treculia africana* are crops of major importance in Eastern part of Nigeria in particular and some other parts of West Africa which serves as both source of food and as source of income. They are grown not for their pulp which is fibrous,

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strong and takes time to disintegrate [3] but for their seeds which are particularly rich in proteins and oils [4]. When the fruit is matured on its tree, it falls down and it is processed by allowing it to undergo natural fermentation/ret naturally. This is accomplished through biological disintegration in order to separate the seeds from the fleshy pulp (mesocarp). This process is carried out by solid substrate fermentation of the pulp at ambient temperature (28°C to 30°C) for a period of 12-21 days in covered heaps. At the end of the retting period, the retted African breadfruit which contained the seeds was separated from the fleshy core and washed with water up to several times to remove the retted pulp and the mucilage which surrounds each of the seeds. During fermentation problems may be experienced with some fruits in that some batches ferment rather poorly making the recovery of the seeds difficult. This study was undertaken to characterize the microorganisms responsible for the retting of African breadfruit pulp and this will provide the necessary information for the development of starter cultures. These starter cultures will have predictable characteristics that could be used commercially to reduce the fermentation time.

Materials and Methods

Fully matured African breadfruits (ABF) that freshly fell down from its tree was carried from the farm at Emekuku, Owerri to the Microbiology Laboratory of Federal University of Technology, Owerri for analysis. The African breadfruit (ABF) was left in the laboratory to ret naturally for about 12-14 days. The pH and temperature of the retting African breadfruit was monitored during this period.

Determination of the softness of the african breadfruit (ABF) pulp

The ABF pulps were removed from the heaps at regular intervals and the softness was measured using fresh samples as the standard. The degree of softening was classified on a scale from 0(meaning no softening) to 5 (complete softening) after its manual probing using round tipped glass bar of 3 mm diameter [5].

pH and total titratable acidity: Ten grams of the fermenting African breadfruit pulp was homogenized in 100 ml of distilled water and the pH was measured. The sample homogenate was then titrated to pH 8.0 with (0.1 M) sodium hydroxide using phenolphthalein as the indicator. The titratable acidity was expressed as lactic acid equivalents on wet weight basis.

Microbiological analysis: Ten (10 g) of the African breadfruit (ABF) pulp with its seeds were collected at random from the different parts of the naturally retting ABF and homogenized in a sterile waring blender and then 1.0 g of the homogenate was mixed with 10 ml of sterile saline which was serially diluted and plated out.

Isolation of microorganisms from the retting African breadfruit (ABF) pulp

Nutrient agar (Oxoid), De man Rogossa Sharpe agar, potato dextrose agar and malt extract agar were aseptically prepared according to the manufacturer's instructions and used for the isolation of bacteria, lactic acid bacteria, fungi and yeasts. Nutrient agar was incubated at 30°C for 24-48 h and De Man Rogossa Sharpe for 48-72 h, potato dextrose agar (PDA) and malt extract agar (MEA) were incubated at 28 ± 1°C for 3-5 days [6-8].

Identification and characterization of isolates

Bacterial isolates were identified and characterized on the basis of cultural, morphological, biochemical and Grams reaction. The identification scheme was based primarily on those of [9-11]. The pure isolated fungi were identified using cultural and morphological features according to the standard keys in fungal identification [8,12].

Results

TABLE 1. **Changes in chemical properties of fermenting African breadfruit pulp.**

Fermentation time (Days)	**pH**	**Titratable acidity (%lactic acid)**	**Softness A^A A**
0	5.4	0.30	0 0
2	5.4	0.36	0 1
4	5.3	0.85	1 2
6	5.3	0.86	2 3
8	5.2	0.72	3 4
10	5.1	0.65	4 5
12	5.1	0.60	5 5
14	5.0	0.52	5 5
A^A-ABF treated with antibiotics; A-ABF without antibiotics; 0-No softening; 5-Complete softening			

There was high degree of softness in the ABF not treated with antibiotics than that treated with antibiotics as shown in TABLE 1.

During the fermentation of the pulp, a number of changes took place due to a combination of microbial and chemical effects. The surface became putrid after 8 days which was not adequate to allow easy recovery of the seed. The end point of the fermentation (12-14th day) was observed when there was complete liquefaction of the pulp as analyzed by the penetration test. There was increased acidity in the pulp as the number of days progressed from 5.4 to 5.0. The titratable acidity increased from 0.30 to a maximum of 0.86 on day 6 and decreased to 0.52 on day 14 as shown in TABLE 1.

TABLE 2. **Morphological and biochemical characteristics of bacteria isolate in the retting ABF.**

Test	**Isolates**			
	A1	**B**	**C1**	**A2**
	Yellow cocci with raised entire colonies	Rods in chains, slender colonies	Cocci in chains, tiny colonies	Whitish, raised rods.
Grams reaction	-	+	+	+
Spore staining	-	-	-	+
Capsule staining	-	-	-	-
Motility	-	-	-	+
Oxidase test	+	-	-	-
Catalase test	+	-	-	-
Citrate utilization	+	-	-	-
Glucose	+	+	-	-
Gas	-	-	+	-
Urease	-	-	-	-
Coagulase	-	-	-	-
Indole	-	-	-	-
Methyl red	-	-	-	-
Organism	*Micrococcus* sp.	*Lactobacillus* sp.	*Streptococcus* sp.	*Bacillus* sp.

Four bacterial species were isolated. They contributed to the retting of the pulp. These isolates have been associated with fermentation activities (TABLE 2).

TABLE 3. **Characterization and identification of fungal isolates.**

Colonial characteristics	**Microscopic isolate code appearance**	**Organism identified**
Tall White filamentous hypae	Non-septate hyphae X	*Rhizopus stolonifer*
Black spores on short white hyphae	Hyphae septate conidia sterigma Y	*Aspergillus niger*

The two fungi isolated (TABLE 3) contributed to the retting of the pulp. Yeast, *Saccharomyces cerevisiae* was isolated during the retting process. This showed a significant contribution by this yeast (TABLE 4).

TABLE 4. **Morphological, cultural and biochemical characteristics of yeast strain isolated.**

Characteristics	**Isolate z**
Surface	Smooth
Margin	Circular
Colour	Creamy white
Cells	Spherical/oval
Gram stain reaction	+
Glucose	+
Catalase	+
Galactose	+
Maltose	+
Sucrose	+
Lactose	-
Growth at 25°C	+
Growth at 30°C	+
Isolate identified	*Saccharomyces cerevisiae*
+ = Positive result (acid produced)	
− = Negative result (no acid production)	

TABLE 5. **Microbial counts and composition of bacteria during natural retting of African breadfruit pulp.**

Fermentation period (days)	**Total count (cfu/g)**	**Predominant flora**
0	10^2	*Aspergillus sp.*
2	1.1×10^2	*Aspergillus sp., Rhizopus sp.*
4	1.3×10^2	*Aspergillus niger, Rhizopus sp.*
6	1.6×10^2	*Aspergillus niger, Rhizopus sp.*
8	2.3×10^2	*Aspergillus niger, Rhizopus sp.*
10	2.6×10^3	*Aspergillus niger, Rhizopus sp.*
12	2.8×10^3	*Aspergillus niger, Rhizopus sp.*
14	1.5×10^2	*Aspergillus niger, Rhizopus sp.*

The predominant bacteria are presented in TABLE 5. *Micrococcus* sp. and *Streptococcus* sp. were initially present within the first 2 days after which *Lactobacillus* sp. and *Bacillus* sp. dominated until the pulp was completely retted.

TABLE 6. **Microbial counts of LAB on De Man Rogossa Sharpe agar.**

Fermentation period (days)	**Total count (cfu/g)**
0	———
2	1.0×10^2
4	1.6×10^2
6	2.8×10^3
8	3.2×10^4
10	3.8×10^5
12	3.5×10^4
14	2.8×10^3

The total count increased as the fermentation days increased. It was highest on day 10 and declined on day 14 (TABLE 6). There was decreased microbial activity on day 14 which indicated that the nutrients in the pulp had been utilized by the microbes.

TABLE 7. **Microbial counts and composition of molds during African breadfruit pulp retting.**

Fermentation period (days)	**Total count (cfu/g)**	**Predominant flora**
0	10^2	Mixed populations of *Micrococcus* and *Streptococcus species.*
2	2.4×10^2	*Micrococcus sp., Streptococcus sp., Bacillus sp.*
4	2.3×10^4	*Lactobacillus sp., Bacillus subtilis*
6	1.6×10^6	*Lactobacillus sp., Bacillus subtilis*
8	3.0×10^9	*Lactobacillus sp., Bacillus subtilis*
10	3.5×10^{10}	*Lactobacillus sp., Bacillus subtilis*
12	2.8×10^8	*Lactobacillus sp., Bacillus subtilis*
14	1.8×10^3	*Lactobacillus sp., Bacillus subtilis*

Rhizopus sp. was isolated only from day 2 to day 14 while *Aspergillus* sp. was present throughout the retting process (TABLE 7).

TABLE 8. **Number and composition of yeast during natural retting.**

Fermentation period (days)	Total count (cfu/g)	Predominant flora
0	10	*Saccharomyces cerevisiae*
2	1.2×10^2	*S. cerevisiae*
4	1.8×10^2	*S. cerevisiae*
6	3.5×10^3	*S. cerevisiae*
8	4.2×10^4	*S. cerevisiae*
10	4.5×10^5	*S. cerevisiae*
12	3.2×10^3	*S. cerevisiae*
14	1.0×10^2	*S. cerevisiae*

There was a moderate increase in the number of yeast at a maximum of 4.5×10^5 CFU/g on day 10 which drastically reduced to 1.0×10^2 CFU/g on day 14 (TABLE 8). There was a progressive increase in the mold count to a maximum of 2.8×10^3 CFU/g on day 12 after which there was observed reduction on day 14 with a value of 1.5×10^2 CFU/g (TABLE 7). The molds were identified as *Aspergillus* sp. and *Rhizopus* sp. which were dominant to the end of the retting of the pulp.

Discussion

The African breadfruit pulp is hard in the fresh state with the seeds embedded in the pulp. However, through fermentation, the pulp softens and disintegrates thereby making the seed recovery easy after washing with water. The microorganisms that were associated with the retting of African breadfruit (ABF) pulp were isolated and identified as *Micrococcus* sp., *Lactobacillus* sp., *Streptococcus* sp., *Bacillus subtilis, Aspergillus niger, Rhizopus stolonifer* and *Saccharomyces cerevisiae.* These microorganisms were mainly fermentative microorganisms and were identified at different levels and periods during the retting process. This observation agrees with those of [13], who reported that fermentations of oil bean and melon pods involve several microorganisms with *Bacillus sp*. playing the major roles. Similar result was reported by [14] here he stated that *Micrococcus* and *Staphylococcus saprophyticus* failed to bring about retting of the *Colocynthis citrullus* pod. The *Micrococcus* sp. isolated in this work was transient as it was not present on further days of isolation, characterization and identification. There was a slow decrease in pH from 5.4 to 5.0 during the fermentation days (TABLE 1). This was because natural fermentation by adventitious bacteria present in the raw materials may be undesirable microorganisms which resulted in delayed pulp disintegration. There was increase in the lactic acid content as the fermentation time progressed as indicated by the titratable acidity (TABLE 1). Mixed flora of bacteria was found to be responsible for the retting of the African breadfruit pulp. Seven isolates were purified and characterized and most of them were Gram positive rods and cocci that are in clusters and chains. They were subjected to physiological and biochemical tests. Among the isolates, 2 are Gram positive rods, 1 negative cocci and 1 Gram positive cocci. The physiological and morphological characteristics of the isolates were given in TABLE 2. The characteristics of the fungi isolated from the retting ABF were stated in TABLE 3. However, yeast (*Saccharomyces cerevisiae*) were isolated and the morphological and biochemical characteristics of the yeast are contained in TABLE 4. During the retting process, there was a remarkable increase in the yeast counts with the value of 4.5×10^5 CFU/g in 10 days (TABLE 8). There was also increase in the counts at day 14. This could be as a result of the depletion of the substrate in the pulp. The mold number gradually increased to a maximum of 2.8×10^3 CFU/g which was recorded on the 12^{th} day of fermentation (TABLE 7). This could be as a result of its insignificant contribution to the retting of the pulp. *Micrococcus sp.* and *Streptococcus sp.* were present in the first 2 days but could not be isolated on later days of fermentation (TABLE 5). *Bacillus subtilis* was dominant during the retting process. The fact that *Bacillus* sp. has the ability to initiate fermentation singly or in combination was equally

reported by [11]. This supports the observation in this work that *Bacillus subtilis* appears to be predominant throughout the duration of the fermentation, unlike other identified species. This is because *B. species* (*B. subtilis*) has been reported to be capable of secreting several types of hydrolytic enzymes such as proteases, amylases and pectinases [11,15,16]. Pectinases can lead to liquefaction of plant tissues and occurs in several *Bacillus spp* including *B. subtilis*. This pectinase strongly disintegrates the retting pulp of African breadfruit (ABF) and it has a strong demucilaginating effect on the ABF pulp. This enhances the retting process. The yeast isolated in the retting African breadfruit could produce pectinolytic enzymes as they (yeast and bacteria) attained their growth peaks at same days (TABLES 5 and 8). This agrees with the report of [17] who stated in his work that the degradation of pulp during cocoa fermentation might not only be by pectinolytic enzymes produced by *Bacillus* sp. but that of yeast, too. *Bacillus subtilis* constituted one of the most important organisms involved in this retting process as it has been reported to be important in the fermentation of carbohydrate materials [18,19].

Conclusion

Different groups of microbes and microbial fermentations were observed to be associated with the softening of the African breadfruit pulp. This will be of immense benefit as to choose the appropriate starter culture to initiate fermentation and significantly accelerate the retting process.

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