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Evaluation of Microbial Availability and Linkages to the Physicochemical Quality of Natural and Deteriorated Rubber Latexes

Augusta O. Mmuoegbulam[1*], Celestina C. Ugwu[2], Ignatius F. Obi[1] and Emmanuel E. Igwe[1]

[1]Department of Microbiology, Faculty of Biological Sciences, University of Calabar, Calabar, Nigeria.
[2]Department of Applied Microbiology and Brewing, Faculty of Natural Sciences, Enugu State University of Science and Technology, Enugu, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author AOM designed the study, wrote the protocol and the first draft of the manuscript. Authors AOM and IFO carried out the practical work. Authors IFO and EEI performed the statistical analysis while authors AOM, CCU, IFO and EEI managed the analyses of the study. Authors AOM and IFO managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Natural and deteriorated rubber latex samples were analyzed for bacteriological and physicochemical quality. Bacterial isolates obtained after 24 hours of incubation ranged from $1.5x10^6$ to $1.58x10^8$ CFU/mL, corresponding to three weeks-deteriorated and natural rubber latexes respectively. However, after 48 hours of incubation, the bacterial counts in the natural rubber latex, one week deteriorated rubber, two weeks deteriorated and three weeks deteriorated rubbers were $1.7x10^8$ CFU/mL, $1.094x10^8$ CFU/mL, and $7.1x10^7$ CFU/mL respectively. Heterotrophic fungal counts ranged from $3.2x10^7$ to $7.8x10^7$ CFU/mL corresponding to three weeks-deteriorated and natural rubber latexes respectively. Statistical analysis for bacterial and fungal isolates showed that there was no significant difference in the mean bacterial and fungal counts

**Corresponding author: E-mail: oluchidinma@yahoo.com;*

within the samples at 95% confidence interval. Microorganisms isolated from natural and deteriorated rubber samples include *Acetobacter* spp., *Enterococcus* spp., *Aspergillus* spp., *Actinomyces* spp., *Penicillium* spp., *Saccharomyces* spp. Physicochemical analysis showed a weakly acidic condition of natural rubber latex and an extreme degree of hardness in all the deteriorated rubber samples.

Keywords: Natural rubber latex; deteriorated rubber latex; bacteriological quality; physicochemical analysis; bacterial and fungal isolates.

1. INTRODUCTION

The natural rubber which is derived from an Indian word "caoutchouc" can be defined as a coagulated or precipitated product from the latex of rubber tree (*Hevea brasiliensis*). The rubber plant which is a native of Brazil was introduced to Nigeria around 1895. It is a variety of plant belonging to the genus *Hevea* and the family Euphoribiaceae [1]. The natural rubber is made from runny, milky liquid called latex that oozes from rubber plants when they are cut. Natural rubber latex refers to the white sap coming out from the *Hevea brasiliensis* tree and contains minority but relevant components, especially proteins, carbohydrate, phospholipids and inorganic compounds in variable amounts. However, based on the seasonal effects, clone and the state of the soil, the average composition of latex has been given as 25 to 35% (wt/wt) polyisoprene; 1 to 1.8% (wt/wt) protein; 1 to 2% (wt/wt) carbohydrates; 0.4 to 1.1% (wt/wt) neutral lipids; 0.5 to 0.6% (wt/wt) polar lipids; 0.4 to 0.6% (wt/wt) inorganic components; 0.4% (wt/wt) amino acids, amides, etc.; and 50 to 70% (wt/wt) water [2].

According to Koyoma and Steinbuchel [3], rubber particles are formed specifically in the cytoplasm of specialized cells called latifiers which are found in the rubber plant. Thus, latex is an endogenous milky fluid synthesized and accumulated under pressure in a network of laticifer cells [4]. Rubber latex contains a large number of chemical compounds from P, C, N, O, S, Ca, K, Mg, Co, and Fe, either due to their role in latex biosynthesis or just because they are absorbed from the soil. Natural rubber is used in a large variety of products due to its flexibility, resistance, impermeability and insulating properties [5].

The latex from rubber is a vital material in the automobile industry as it is used in the manufacture of tyre, car bumpers, seats etc. [6]. It takes several distinct steps to make a product out of natural rubber. First, the latex is collected from the rubber trees using a traditional process called rubber tapping. This involves making a wide U-shaped cut in the tree bark. As the latex drips out, it is then collected in a cup. The collected latex from many trees is then filtered, washed and reacted with acid to bring about the coagulation of the rubber particles [7].

Natural rubber consists of C_5H_8 units (Isoprene), each of which contains one double bond in the cis configuration with poly-isoprene of *Hevea brasiliensis* containing two additional trans-isoprene units in the terminal region. Although dehydrated natural rubber of *H. brasiliensis* has been reported to contain approximately 6% nonpolyisoprene constituents, Rose et al. [7] stated that out of approximately 2,000 plants that synthesize poly (cis – 1, 4 – isoprene), only natural rubber of *Hevea brasiliensis* (99% of the world market) and guayule rubber of *Parthenium argentatum* (1% of the world market) are produced commercially.

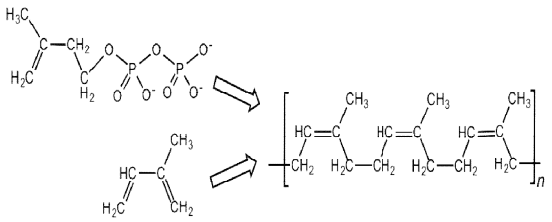

Fig. 1. Chemical structure of cis-polyisoprene - the main constituent of natural rubber

Rubber latex contains a large number of microorganisms. Microorganisms such as fungi, bacteria and actinomycetes are capable of degrading natural rubber by producing extra cellular enzymes. Actinomycetes such as *Streptomyces* spp. are capable of degrading natural rubber as they produce variety of enzymes. Microorganisms gain access to the latex mostly as a result of poor technical skill by personnel during tapping and processing in the factory [8]. The commonest microorganisms are bacteria such as *Streptococcus, Escherichia coli* and other related coliforms [9]. The fungus

(*Schizosaccharomyces*) also affects the latex by degrading it. According to Rose and Steinbuchel [1], fungi degrading natural rubber have been isolated from soil and deteriorated tyres.

It has been documented that the crumb and matrix of virgin rubber material form interfacial sulphur crosslinks. This therefore causes a problem in the recycling of old tyres by blending ground spent rubber and the virgin rubber followed by vulcanization. Thus, microorganisms capable of breaking sulphur-sulphur and sulphur-carbon bonds are been used to devulcanize waste rubber so as to make the surface polymer chains more flexible and increase their binding upon vulcanization. Holst et al. [10] have studied many sulphur oxidizing species for this purpose and Borel et al. [11] reported attempts by Faber to grow *Fusarium solani* upon vulcanized rubber tyres.

For microorganisms to thrive in latex, certain factors are taken into consideration such as temperature, nutrient availability, pH, moisture content and aeration. The survival of these organisms through the subsequent stages of processing leads to mechanical instability of the latex due to breakdown of the constituent materials of the latex and depletion of oxygen level. This mechanical instability could lead to the destruction of the refined product of rubber due to the loss of flexibility.

Tree tapping creates room for microbial infection as the cambium of the tree will be affected during the process and so is exposed to infections by microbes [8]. Poor hygienic conditions also lead to the introduction of microbes into the latex. For example when buckets used for collection of latex from the field are not clean, it results to enzyme accumulation which contaminates the newly collected latex by precoagulating it and thereby leading to inferior quality of coagulum. Enzyme accumulation is as a result of the presence of organisms in the bucket, utilizing a substrate of its choice to produce the enzyme. Production of amylase could be as a result of utilization of carbohydrate (starch), or protease production as a result of utilization of protein in the latex present in the bucket.

However, preservatives such as phenolic compounds and simple inorganic compounds can be used to preserve rubber latex from putrefaction and coagulation. Some of these could also serve as anti-coagulants.

2. MATERIALS AND METHODS

2.1 Study Site

The rubber samples, both deteriorated and natural were collected from Pamol Rubber Estate which is located in 8th miles on the outskirts of Calabar which is the capital of Cross River State, Nigeria. The city of Calabar lies between longitudes 8° 20^{1} 00^{11} E, and latitudes 4° 50^{1} 00^{11} N. Samples were collected in sterile containers and transported immediately to the laboratory for analysis.

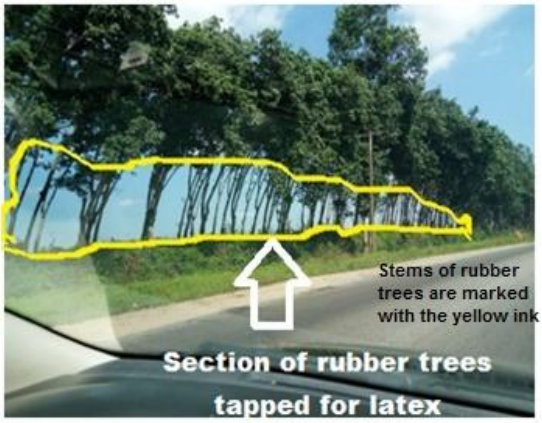

Fig. 2. Rubber trees in Pamol Rubber Estate

2.2 Sample Collection

The natural rubber samples were collected aseptically in four different parts as natural rubber latex (freshly tapped), first stage of deteriorated rubber latex (a day old), second stage of deteriorated rubber latex (a week old), and third stage of deteriorated rubber latex (2 weeks old). The rubber samples collected in sterile rubber containers were transported immediately to the laboratory and stored in the refrigerator till experiment was carried out. All samples were analyzed within four to six hours of collection.

2.3 Digestion Technique

1 g of deteriorated rubber samples were cut into a conical flask and 30 mL of nitric acid (HNO_3) and 10 mL of hydrochloric acid (HCL) were added to the conical flask. The mixture was placed in a hot air oven at 100°C until digestion was completed. The mixture was then made up to 100 mL by adding 60 mL of deionized water.

2.4 Isolation and Maintenance of Microbial Isolates

Culture media used include: Nutrient agar for bacterial growth and Potato Dextrose agar for fungal growth. Triple Sugar Iron agar was also used for sugar fermentation.

1 ml of natural rubber latex and a gram of deteriorated rubber latex were weighed using a micropipette and a sterile foil paper respectively. 1 ml of natural rubber latex added into 9 ml of sterile distilled water was serially diluted while 1 g of deteriorated rubber latex was added to 100 ml of sterile distilled water in a conical flask which served as the aliquot. From the aliquot, 1 mL was taken into the first tube containing 9 mL sterile distilled water to obtain a 10^{-1}. Same procedure was repeated until 10^{-10} dilution was obtained. Known dilutions were then used for inoculation into the agar plates. Each sample was plated in duplicate using the pour plate method. The nutrient agar was used for the isolation of bacteria while potato dextrose agar was used for fungal isolation. After plating, nutrient agar plates were incubated at 37°C for 24 hours and potato dextrose agar (PDA) plates were incubated at 27°C for 7 days.

At the end of the incubation period, emerging colonies were enumerated using the colony counter and discrete colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates.

Pure colonies of bacteria and fungi were maintained on nutrient agar and potato dextrose agar slants respectively and then stored in a refrigerator at 4°C for further identification.

2.5 Identification of Isolates

Fungal isolates were examined macroscopically for cultural characteristics such as shape, colour, size and consistency. Bacterial isolates were characterized based on microscopic appearance, colony morphology and gram staining reactions as well as appropriate biochemical tests as described by Cheesbrough [12]. The isolates were identified by comparing their characteristics with those of known taxa, as described by Cruickshank et al. [13] and Holt [14].

2.6 Physicochemical Analyses

The parameters analyzed were pH, Temperature (°C), Conductivity (µS/cm), BOD (mg/L), Total Hardness, Iron (mg/L), Ammonium (mg/L), Nitrite (mg/L), Nitrate (mg/L), and Sulfide (mg/L). The physicochemical results were determined using a spectrophotometer (HACH- DR 5000 model) with specific reagent for each parameter.

3. RESULTS

3.1 Heterotrophic Plate Count

The heterotrophic plate count of the natural rubber latex and deteriorated rubber samples gave counts for bacteria and fungi. Tables 2 and 4 show the occurrence of bacterial and fungal isolates in the natural, 1st stage, 2nd stage, and 3rd stage deteriorated rubber latexes while Table 3 shows the different most probable fungal isolates in the samples.

Table 1. Results of physicochemical analysis of natural and deteriorated rubber latex

S/N	Parameters/Units	NRL	DRL 1	DRL 2	DRL 3
1	pH	5.02	ND	ND	ND
2	Temperature (°C)	30.7	30.2	29.5	28.7
3	Conductivity (µS/cm)	8.74	190.3	185.9	147.5
4	BOD (mg/L)	0.39	7.064	6.89	7.18
5	Total Hardness	75.5	ND	ND	ND
6	Iron (mg/L)	4.49	1.86	1.44	2.27
7	Ammonium (mg/L)	0.76	0.04	0.47	0.56
8	Nitrite (mg/L)	0.578	0.216	0.232	0.67
9	Nitrate (mg/L)	12.9	7.7	11.2	10.3
10	Sulfide (mg/L)	617	41	125	70

Key: ND: Not detected
NRL: Natural rubber latex
DRL 1: One week deteriorated rubber latex
DRL 2: Two weeks deteriorated rubber latex
DRL 3: Three weeks deteriorated rubber latex

Table 2. Occurrence of bacterial isolates in samples

Isolate	Samples of occurrence	Total	Percentage occurrence (%)
Acetobacterium sp.	NRL	1	10
Enterococcus sp.	NRL & DRL1	2	20
Acetobacter sp.	NRL	1	10
Flavobacterium sp.	NRL	1	10
Moraxella sp.	DRL2	1	10
Actinomyces sp.	DRL1, DRL2 & DRL3	3	30
Pseudomonas sp.	DRL2	1	10

Key: NRL: Natural rubber latex
DRL1: One week deteriorated rubber latex
DRL2: Two weeks deteriorated rubber latex
DRL3: Three weeks deteriorated rubber latex

3.2 Heterotrophic Bacterial Count

It was observed from the plate count as shown in Fig. 3 that the heterotrophic bacterial count after 24 hours of incubation ranged from 1.5 x 10^6 CFU/mL to 1.58 x 10^8 CFU/mL which represents the lowest and the highest bacterial counts recorded in the three weeks deteriorated rubber and natural rubber latexes respectively. The counts in the one week and two weeks deteriorated rubber were found to be 7.0 x 10^6 CFU/mL and 2.5 x 10^6 CFU/mL respectively. However, after 48 hours incubation, the bacterial counts in the natural rubber latex, one week deteriorated rubber, two and three weeks deteriorated rubbers were 1.7x10^8 CFU/mL, 1.09 x 10^8 CFU/mL, 8.4x10^7 CFU/mL and 7.1x10^7 CFU/mL respectively as shown in Fig. 4.

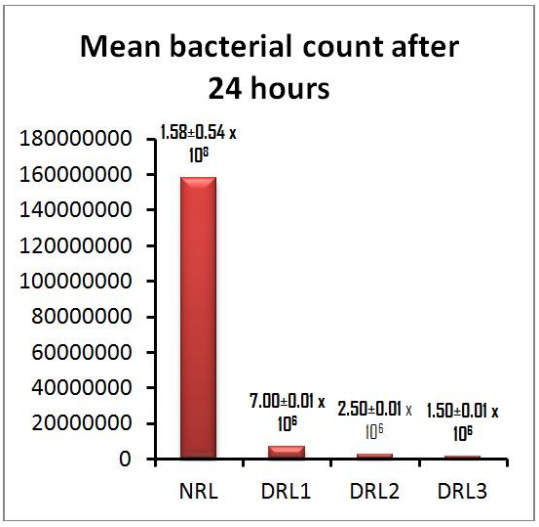

Fig. 3. Total heterotrophic bacterial count after 24 hours of incubation
Key: NRL: Natural rubber latex
DRL1: One week deteriorated rubber latex
DRL2: Two weeks deteriorated rubber latex
DRL3: Three weeks deteriorated rubber latex

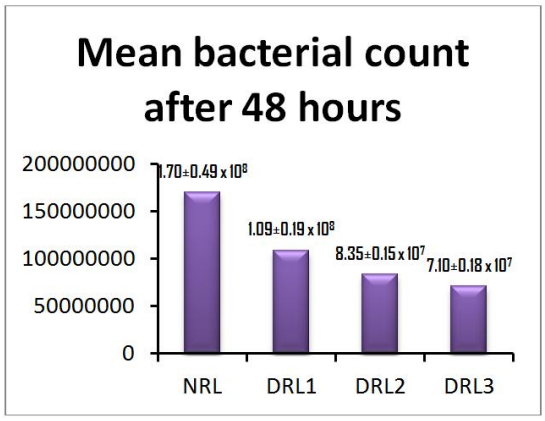

Fig. 4. Total heterotrophic bacterial count after 48 hours of incubation
Key: NRL: Natural rubber latex
DRL1: One week deteriorated rubber latex
DRL2: Two weeks deteriorated rubber latex
DRL3: Three weeks deteriorated rubber latex

3.3 Heterotrophic Fungal Counts

Heterotrophic fungal count ranged from 3.2 to 7.8 x 10^7 CFU/mL representing the lowest and the highest fungal counts which were obtained from the three weeks deteriorated rubber and the natural rubber latex respectively. However, the fungal count in the one week deteriorated rubber was observed to be 6.7 x 10^7 CFU/mL. The two weeks deteriorated rubber was observed to have a fungal count of 5.9 x 10^7 CFU/mL (Fig. 5).

3.4 Frequency of Occurrence for the Bacterial and Mould Isolates

The percentage occurrence of bacterial and mould isolates in this study as obtained from the results presented on Tables 2 and 4 are given in Figs. 6 and 7 respectively.

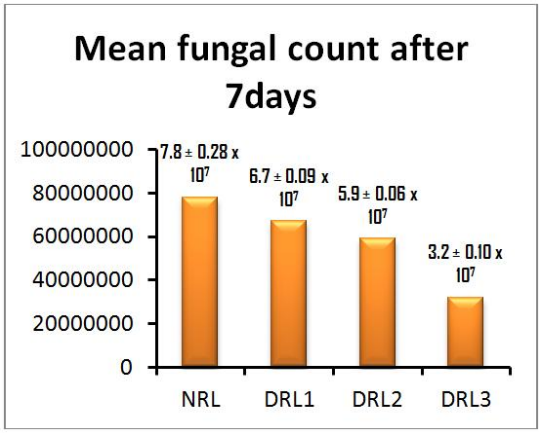

Fig. 5. Total heterotrophic fungal count after 7 days of incubation

Key: NRL: Natural rubber latex
DRL 1: One week deteriorated rubber latex
DRL 2: Two weeks deteriorated rubber latex
DRL 3: Three weeks deteriorated rubber latex

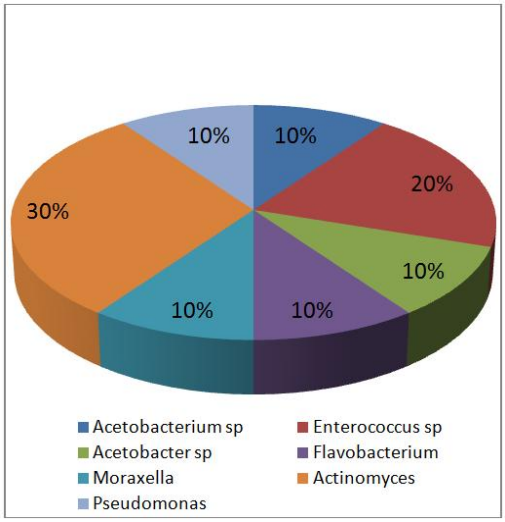

Fig. 6. Percentage occurrence of bacterial isolates in the rubber samples

Table 3. Identification and characterization of mould isolates from natural and deteriorated rubber latexes

S/N	Macroscopic or colonial morphology	Probable organism
1	Colonies were black in colour projecting out dust on top of the medium. The black colour darkens more as the colonies become older.	*Aspergillus niger*
2	The colonies were fluffy white with a yellowish orange pigmentation on the reverse side	*Fusarium* sp.
3	The colonies were small, round, moist and greenish-blue in pigmentation	*Aspergillus* sp.
4	The colonies were flat, dry, filamentous and brownish in pigmentation	*Aspergillus* sp.
5	The colonies were round and greenish	*Penicillium* sp.

Table 4. Occurrence of mould isolates in rubber samples

Isolate	Samples of occurrence	Total occurrence	Percentage occurrence (%)
Aspergillus sp	NRL, DRL1, DRL2 and DRL3	4	57
Fusarium sp	DRL2 and DRL3	2	29
Penicillium sp	NRL	1	14
Total		7	100

Key: NRL: Natural rubber latex
DRL1: One week deteriorated rubber latex
DRL2: Two weeks deteriorated rubber latex
DRL3: Three weeks deteriorated rubber latex

Table 5. Occurrence of yeast isolates in rubber samples

Isolate	Samples of occurrence	Total occurrence	Percentage (%)
Candida sp	NRL, DRL1, DRL2, DRL3	2	50%
Saccharomyces sp	NRL, DRL1, DLR2, DLR3	2	50%

Key: NRL: Natural rubber latex
DRL1: One week deteriorated rubber latex
DRL2: Two weeks deteriorated rubber latex
DRL3: Three weeks deteriorated rubber latex

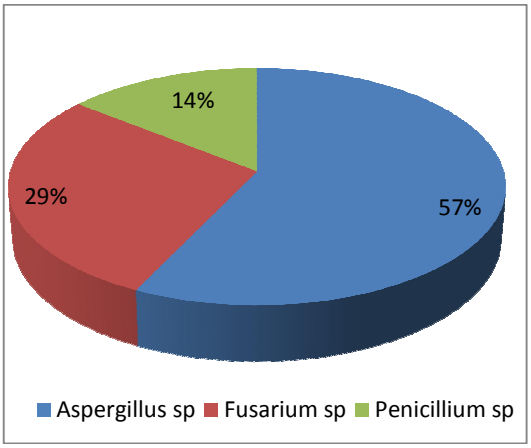

Fig. 7. Percentage occurrence of mould isolates in rubber samples

3.5 Statistical Analyses

Table 6 shows the statistical analysis of bacterial and fungal counts for natural and deteriorated rubber latexes (expressed in mean count ± standard deviation).

Table 6. Statistical analysis of fungal and bacterial count

Sample category	Bacteria	Fungi
NRL	170.0±49.5	77.5±27.6
DRL1	108.5±19.1	66.5±9.2
DRL2	83.5±14.8	59.0±5.7
DRL3	71.0±18.4	32.0±9.9

Key: NRL: Natural rubber latex
DRL1: One week deteriorated rubber latex
DRL2: Two weeks deteriorated rubber latex
DRL3: Three weeks deteriorated rubber latex

3.5.1 ANOVA analysis for bacterial isolates

There was no significant difference in the mean bacterial counts within the samples; hence F_{cal} (3.64) was less than F_{crit} (9.28) "at 95% confidence interval".

3.5.2 ANOVA analysis for fungal isolates

There was no significant difference in the mean fungal counts within the samples; hence F_{cal} (3.28) was less than F_{crit} (9.28) "at 95% confidence interval".

4. DISCUSSION

4.1 Bacteriological Quality of Samples

As shown in Figs. 2 to 4, natural rubber latex had a higher number of microbial loads than the deteriorated rubber. This therefore implies that the natural rubber latex is a nutritious medium that allows the growth of microorganisms. Natural rubber latex is composed of proteins, carbohydrates phospholipids and inorganic compounds which support microbial growth. The reduction in microbial load of the deteriorated samples is due to the depletion of nutrient in the samples as a result of the initial growth of the organisms inherent in the natural rubber latex. This reduction may also be as a result of antimicrobial metabolites produced by the initial group of microorganisms that deteriorate natural rubber latex.

In terms of occurrences, *Actinomyces* spp had the highest percentage of occurrence (30) appearing in all the stages of deteriorated rubber latex samples (Table 2). This shows that *Actinomyces* spp were the most dominant species in the deteriorated rubber latex. This may also have been because of the metabolites produced by the degraders of natural rubber latex and these metabolites are favorable to the growth of this bacterial species; the metabolites served as precursor metabolites for the organisms.

4.2 Fungal Quality of Rubber Samples

Based on the fungal count, *Aspergillus* sp was the most prevalent mould by occurring in all the samples with percentage occurrence of 57 (Table 4). *Aspergillus* species are known to produce antimicrobial agents during their growth and these account for their presence in both natural and deteriorated rubber latexes. For yeast, *Candida* and *Saccharomyces* spp had the same percentage of occurrence (Table 5). This accounts for the degradation of simple sugar to organic acid and alcohol by both *Saccharomyces* and *Candida* spp. *Candida albican* ferments glucose and maltose to acid and gas, sucrose to acid and does not ferment lactose. *Sacharomyces* spp ferment glucose to alcohol. This acidic environment as well as the presence of simple sugar for consumption makes these two organisms proliferate together at equal distributions (i.e. 50% of occurrences). According to Chengalroyen and Dadds [15], the growth of microorganisms that utilize natural rubber as a sole carbon source is a slow process. Thus, incubation periods extending over days or even weeks are required to obtain enough cell mass. This therefore accounts for few growth of microorganisms after 24 hours of incubation as observed in Table 1.

4.3 Physicochemical Quality of Rubber

Being the first of its kind, this research shows the physicochemical quality of natural and deteriorated rubber latexes which is not very common in other literatures. From the results obtained in Table 1, the pH of natural rubber latex is weakly acidic (5.02), implying that microorganisms capable of degrading natural rubber latex thrive in weakly acidic condition while deteriorating the natural rubber latex. However, the pH of the deteriorated rubber latex could not be determined as a result of the concentration of the acids (HCL and HNO_3) used for the digestion of the deteriorated rubber samples.

Natural rubber latex was observed to be hard by having a total hardness of 75.5. This is due to the chemical structure of the elastomers. However, in the process of degradation, the total hardness of the rubber moisture was increased, and this is reflected in the inability of obtaining the results for total hardness in all the deteriorated rubber samples. This means that higher quantity of magnesium and calcium ions were produced in the deteriorated rubber latexes.

Generally, Nitrogen containing compounds follow a trend of conversion. For instance, nitrate could be present as a result of oxidation of other forms of nitrogen, including nitrite, ammonia, and other “organic nitrogen compounds such as amino acids”.

5. CONCLUSION

Natural rubber latex serves as a nutritious medium for the growth and proliferation of rubber degrading microorganisms. This is as a result of the components found in natural rubber latex. Microorganisms specifically gain access to latex mostly as a result of poor technical skill by personnel during tapping and processing in the factory. Microbial degradation of natural rubber is mainly carried out by microorganisms such as bacteria and fungi.

Among the microorganisms isolated from natural and deteriorated rubber samples, bacteria such as *Actinomyces* spp. were predominant in the deteriorated rubber samples while *Enterococcus* spp. were predominant in the natural rubber latex. For fungal isolates, *Aspergillus* spp., *Saccharomyces* spp. and *Candida* spp. were predominant in both the natural and deteriorated rubber latexes.

Degradation of natural rubber latex by bacteria and fungi occurs under acidic condition and only organisms that can thrive under such conditions will be involved in the degradation and deterioration of the natural rubber latex. The natural rubber has a high total hardness which explains the chemical structure of its elastomers.

6. RECOMMENDATIONS

Having successfully carried out this research work, we therefore recommend the following:

- There should be generally accepted standards for comparing the physicochemical parameters found in natural and deteriorated rubber latexes. This will help in enhancing the quality of rubber products as rubber samples will be assessed at different levels of production. It will also help in the advancement of the quality of rubber products.
- Tappers should be trained and equipped with the necessary tools used for tapping as this will enable proper latex collection and prevent its contamination.

This study could serve as a beginning stage for the above proposed recommendations.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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