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Bacteriological Evaluation of Locally Prepared Smoothies Formulated from Different Fruit Combinations

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The pharmaceutical benefits of fruits to humans is unfolding daily, thus, ensuring its Microbiological quality for the safety of final consumers is of paramount importance. Smoothie combinations made of paw-paw, banana, bene-seed (PPBNBS), orange, water melon, banana (ORWMBN), orange, banana, pineapple (ORBNPN) and paw-paw, watermelon, banana (PPWMBN) were prepared and analyzed as fresh (analyzed immediately after preparation), room temperature (analyzed after 24 hrs of preparation on bench) and refrigerated (kept in the refrigerator for 24 hrs after preparation). The mean colony count for refrigerated smoothie drink was 1.0-9.0 x 10^5 Cfu/mL, 1.0-8.0 x 10^5 for

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smoothie kept at room temperature and 1.0-5.0 x 10^5 Cfu/mL for freshly analyzed drink. The high mean count observed among the refrigerated drink may be interpreted as high concentration of psychrophilic organisms. Nine (9) genera of Microorganisms were isolated and identified. *Rothia, Micrococcus, Paenibacillus, Klebsiella* and *Lactobacillus* had similar 11.11 % rate of occurrence. *Enterococcus* sp were 22.22 % abundant. *Bacillus* and *Staphylococcus* sp both had 33.3 % occurrence, while 44.4 % being the most abundant was recorded for *Streptococcus* sp. Among the identified organisms, Gram positive were the most frequent isolates occurring 8 (88.9%) compared to 1 (11.11%) of Gram negative organisms. The distribution of isolated organisms were not influenced (P = 0.05) by the fruits combinations, neither the analyzed conditions. Although, the orange, banana, pineapple (ORBNPN) combination tend to have more 40% isolates occurrence compared to other three (PPBNBS, PPWMBN and ORWMBN) smoothie combinations with even distribution of isolates at 20% occurrence rate. Though *Streptococcus* sp were the most abundant 44.4 % strain of isolated organisms, *Staphylococcus* sp were presence in all smoothies' combination. The results of the index study report higher than acceptable colony count in fruit drink according to the Microbiological Criteria (GULF standard, 2000). And the isolated organisms are prominent indicators pathogens of foodborne infection. This therefore implies that, smoothie's drink that are not properly handled during preparation can serve as a potential threat and a salience vehicle for foodborne intoxication and possible outbreak.

Keywords: Foodborne indicators pathogens; klebsiella; staphylococcus; bacillus and streptococcus; smoothies drink; different fruits combinations; microbiological quality.

1. INTRODUCTION

Fruits are great sources of phytochemicals essential for human health and are consumed as an important component of a healthy human diet. Their beneficial effects are based on the composition of many vitamins, minerals, dietary fiber, phytochemicals and their protective role against diseases such as diabetes, obesity, hypertension or coronary heart disease [1-3]. Recently, fresh fruits, vegetables as well as smoothies is in high demand [4-6]. This is because consumption of fruits, fresh and healthy food is a preferred healthier lifestyle of most populace.

Smoothies are thick beverage products prepared from a wide range of fruits and vegetables using a blender. It may include other ingredients such as water, crushed ice, fruit juice and sweeteners (such as honey, sugar, syrup), dairy products (such as milk, yoghurt, low fat or cottage cheese), plant milk (such as coconut milk, tiger nut milk, almond nut milk, soy milk), seeds (such as celery seeds), spices (such as ginger, garlic), tea, chocolate, herbal supplements and nutritional supplements [7].

In some developing Countries like Nigeria, smoothies are becoming a preferred choice of drink and are commonly prepared on demand and sold in big shops, hotels and other relaxation spots [8].

Silha et al., 2022 [9] reported that smoothie drinks sold in fast food establishments of Eastern Bohemia are potential source of microbial pathogens while Krahulcova et al., 2021 [10], reported that antibiotic-resistant coliforms bacteria proliferate more in green smoothies and juicy formulations containing more vegetable ingredient compare to others. Many other research articles on smoothies report on its unsafe state. However, information on the Microbiological safety of locally made smoothies that are served in 50-70% homes, 50-80% renounced hotels and seat out-joints is limited in Cross River State. Therefore, the research seeks to investigate the microbial contents of locally made smoothies based on fruit combinations, taking into consideration the influence of temperature on microbial load for public safety.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The fruit samples used in this study were orange, banana, watermelon, bene-seed, pineapple and pawpaw. They were purchased within Ekpo Abasi junction fruit market, Calabar south.

Samples were aseptically wrapped using a sterile polythene bag and conveyed immediately after purchased to Microbiology Laboratory, University of Cross River State (UNICROSS).

The fresh fruits were sorted according to fruit combinations, thereafter, fruits were peeled with sterile knife, seeds removed from fruits that had

seeds, thoroughly washed with running tap water, diced into smaller pieces and weighed on a weighing balance (Digital compact scale; Atom A123). 200 grams weight of each fruit item was homogenized using a Moulex juicer blender (Model JB-70B) with 400mL of water. The blending process was allowed for 5 minutes, and thereafter was turned into sterile bottles as stock contents.

The smoothie stock was divided into three different portions, one was analyzed fresh, the second portion was allowed at room, while the third one was kept under refrigerating temperature and both were analyzed after 24 hours. 1mL of each homogenate was taken and suspended in 9 mL of distil water and diluted down to $10^{-10.}$. 1mL diluent of 10^{-5} and 10^{-6} were pour-plated in already prepared molten semi-solid nutrient (Chaitanya, RDM-NA-01)) and MacConkey (Chaitanya, RDM_MCA-02) agar respectively. After 24 hours culture, the morphological characterization of the emergent colonies were observed and documented, the total heterotrophic bacterial count was also carried out. Cultures were subjected to three consecutive sub-cultures to obtain pure cultures before further analysis was carried out.

2.2 Data Analysis

The student unpaired T-test was used to compare and determined the significant bacterial mean count of different smoothie combinations at different analyzed conditions at P = 0.5. Microsoft Excel 2006 was used for the pictorial representation of: Pie, Bar and Line graphs for better separation and understanding.

3. RESULTS

3.1 Bacterial Cell Count

The fresh portion of smoothie combinations made of pawpaw, banana and bene-seed had bacterial cell count of 1.3-2.0 x10^5 Cfu/mL reported from freshly prepared smoothies.

The colony count from room temperature ranged from 2.0 to 4.4 x 10^5 Cfu/mL while that of refrigerating temperature range 6.0 to 8.0 x 10^5 Cfu/mL. Orange, banana and pineapple smoothie formulation yielded 1.0 to 2.0 x 10^5 Cfu/mL for freshly prepared. The room temperature colony count was 3.0 to 7.0 x 10^5 Cfu/mL while the refrigerated recorded 1.4 to 6.0 x 10^5 Cfu/mL respectively.

Orange, watermelon and Banana fresh smoothie combinations yielded 1.3 to 5.0 x 10^5 Cfu/mL, Room temperature bacteria cell count was 1.8 to 8.0 x 10^5 Cfu/mL and 1.0 to 9.0 x 10^5 Cfu/mL for refrigerated portion.

Pawpaw, watermelon and banana fresh portion had 1.0 to 3.0 x 10^5 Cfu/mL. Room temperature and refrigerated had 2.0 to 6.0 and 1.0 to 4.0 x 10^5 respectively (Table 1).

3.2 Isolated and Identified Microorganisms

Streptococcus sp*, Bacillus* sp*, Staphylococcus* sp*, Enterococcus* sp, *Rothia* sp*, Micrococcus* sp*, Peanibacillus* sp*, lactobacillus* sp and *Klebsiella* sp were the nine prominent genera of microorganisms isolated and identified in this study with respective frequencies of 44.4 % for *Streptococcus*, 33.3 % for *Bacillus and Staphylococcus* while 22.22 % was for *Enterococcus. Rothia, Micrococcus, Paenibacillus, Lactobacillus* sps all had percentage occurrence of 11.11 % respectively (Fig. 1). The occurrence rate of Gram positive isolates was higher 8(88.9 %) compare to 1 (11.11 %) for Gram negative organisms.

3.3 Variations in Isolated Organisms According to Smoothie Combinations

The research records variations in the distributions of isolated organisms among smoothie formulations. The different analyzed conditions such as; fresh, room temperature and refrigerated had no influence on the frequency of isolated organisms. However, the freshly analyzed smoothie made from combination of ORWMBN had 6 ((24.00) (6/25 X 100/1)) followed by 3 (12.0) for PPWMBN and 1 (4.00) for both ORBNPN and PPBNBS. The room temperature of ORWMBN yielded 3 (12.0) followed by 2 (8.00) for ORBNPN and 1 (4.00) for both PPWMBN and PPBNBS. 3 (12.0) and 2 (8.0) was for PPBNBS and ORBNPN and 1(4.00) for both ORWMBN and PPWMBN were obtained from refrigerated smoothie (Fig. 2).

3.4 Sum-total of Isolates Based on Gram Reactions

The study reports higher frequency 8 (88.9 %) of Gram positive organisms compared to 1 (11.1 %) Gram negative bacterial isolates (Fig. 3).

Table 1. Mean bacterial cell counts in colony forming unit (Cfu/mL) from various smoothies analyzed conditions

	Fresh	Room Tempt	Refrigerated
PPBNBS	1.3-2.0 x 10^5 Cfu/mL	2.0-4.4 x 10^5 Cfu/mL	6.0-8.0 x 10^5 Cfu/mL
ORBNPN	1.2-2.0 x 10^5 Cfu/mL	3.0-7.0 x 10^5 Cfu/mL	1.4-6.0 x 10^5 Cfu/mL
ORWMBN	1.3-5.0 x 10^5 Cfu/mL	1.8-8.0 x 10^5 Cfu/mL	1.0-9.0 x 10^5 Cfu/mL
PPWMBN	1.0-3.0 x 10^5 Cfu/mL	2.0-6.0 x 10^5 Cfu/mL	1.0-4.0 x 10^5 Cfu/mL

Keys: ORWMBN = Orange, watermelon, Banana. PPWMBN = Pawpaw, watermelon and Banana. ORBNPN = Orange, Banana and pineapple. PPBNBS = Pawpaw, Banana and pineapple.

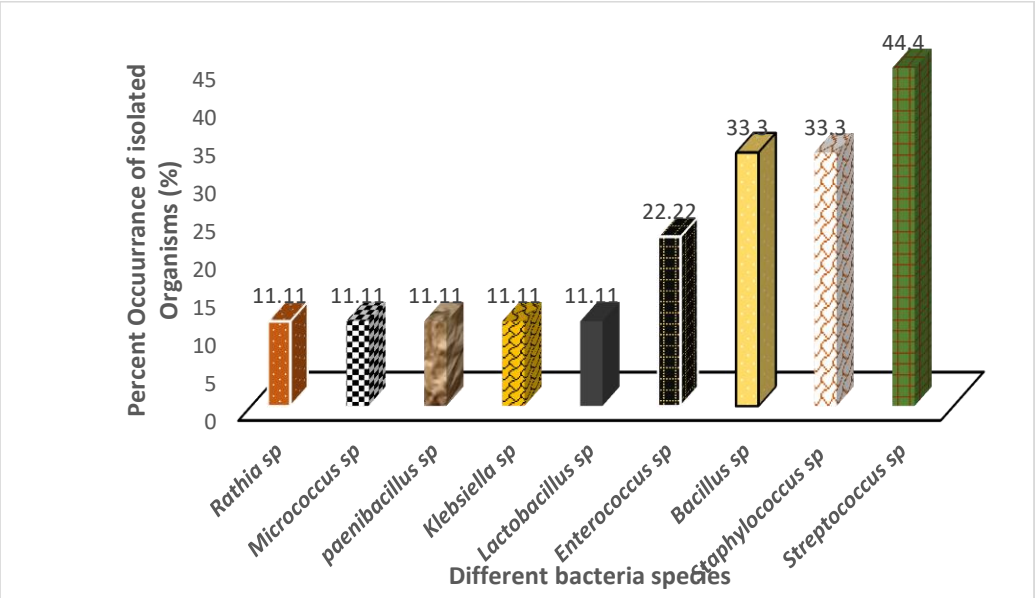

Fig. 1. Total number of bacteria isolates and their percentage occurrence in different smoothies' combinations

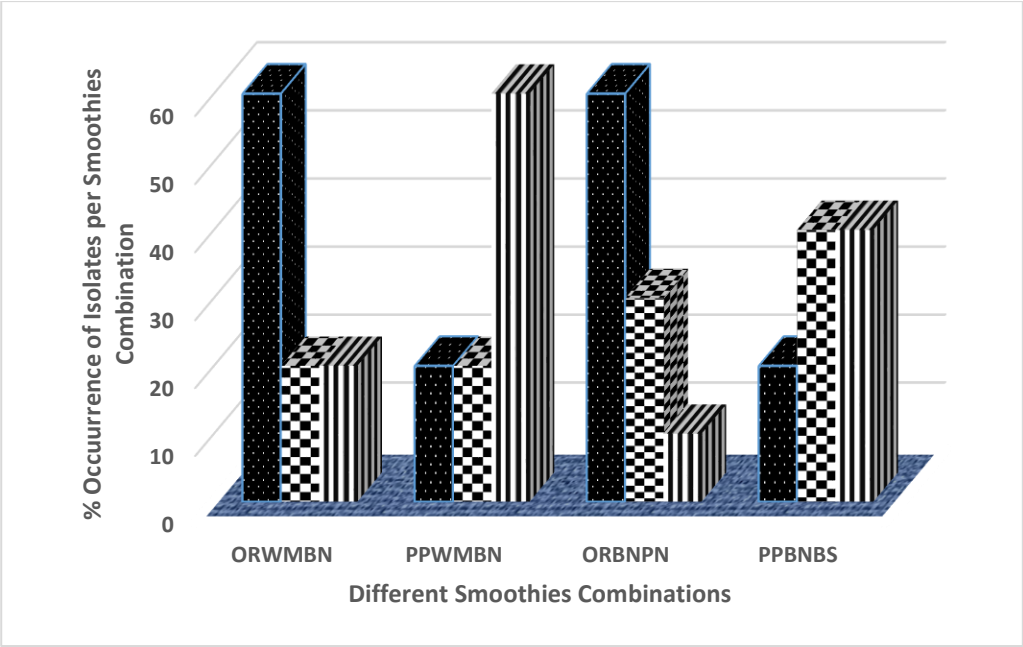

Fig. 2. Different smoothies' combinations and percentage frequencies of isolates

Table 2. Distribution of isolates according to different smoothies combinations analyzed under different (fresh, room temperature and refrigerated) conditions

	ORWMBN			PPWMBN			ORBNPN			PPBNBS			Total no. Of appearance
Isolated Organisms	**F**	**RM**	**R**	**F**	**RM**	**R**	**F**	**RM**	**R**	**F**	**RM**	**R**	
Bacillus sp.	0.00	0.00	11.11	0.00	0.00	0.00	11.11	0.00	0.00	11.11	0.00	0.00	3
Streptococcus sp.	11.11	0.00	0.00	0.00	11.11	0.00	0.00	0.00	11.11	0.00	0.00	22.22	5
Rothia sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.11	0.00	1
Micrococcus sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.11	0.00	1
Staphylococcus sp.	22.22	11.11	0.00	0.00	0.00	33.33	22.22	11.11	0.00	0.00	0.00	0.00	9
Paenibacillus sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.11	0.00	0.00	0.00	0.00	1
Klebsiella sp.	0.00	0.00	0.00	0.00	0.00	0.00	22.22	0.00	0.00	0.00	0.00	0.00	2
Lactobacillus sp.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	11.11	0.00	0.00	0.00	0.00	1
Enterococcus sp.	0.00	0.00	0.00	11.11	0.00	0.00	11.11	0.00	0.00	0.00	0.00	0.00	2
Number of Times	3	1	1	1	1	3	6	3	1	1	2	2	25

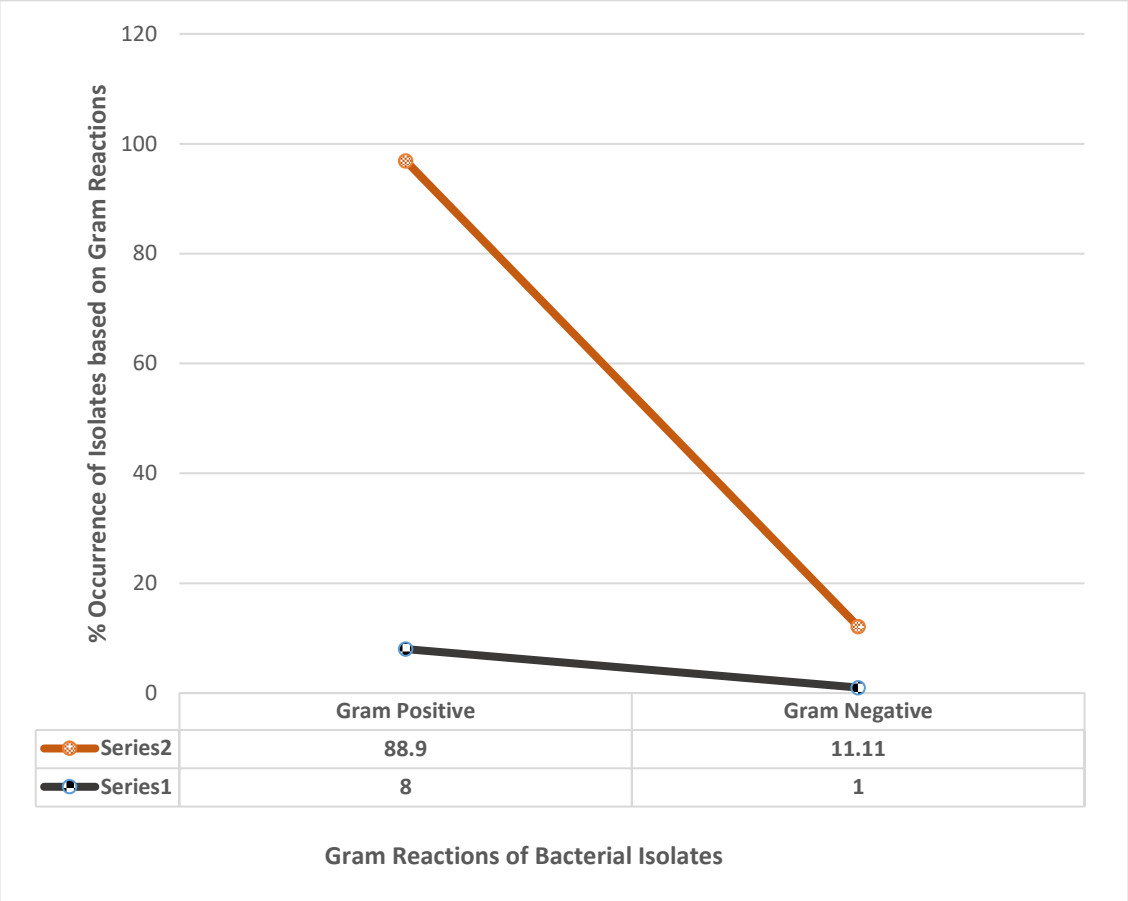

Fig. 3. Sum-total of isolates based of gram reactions

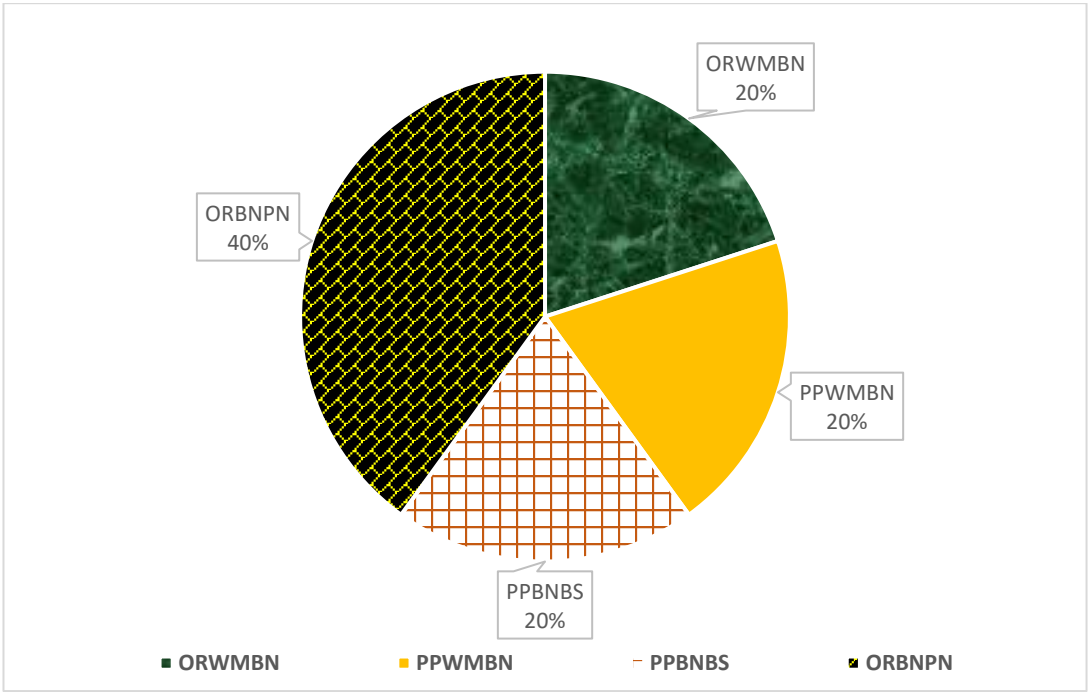

Fig 4: Sum-total of all isolates according to smoothies combinations

3.5 Sum-total of Isolates According to Smoothie Combinations and Conditions

The smoothie combination; ORWMBN, PPWMBN and PPBNBS fresh, room temperature and refrigerated conditions had similar 5 (20.0 %) isolates occurring rate while ORBNPN had the highest 10(40.0 %) percentage isolates (Fig. 4).

4. DISCUSSION

The evaluation of bacterial quality of locally prepared smoothies formulated using four different fruits combinations (ORWMBN, PPWMBN, PPBNBS, ORBNPN) was carried out to ascertain the safety of smoothie drink to final consumers at different conditions.

The cross sum of mean count for refrigerated smoothie was 1.0 to 9.0 X 10^5 Cfu/mL, 1.0 to 8.0 X 10^5 for room temperature and 1.0 to 5.0 X 10^5 Cfu/mL for freshly prepared. The presence of microorganisms in all the three analyzed smoothie conditions are unhealthy according to the microbiological criteria (GULF standard). However, high mean count observed among refrigerated smoothies may be interpreted as high concentration of psychrophiles.

Obasi and odoh [11] conducted an experiment on smoothie combinations made of sweet melon control (SMC:100%), cucumber-smoothie control (CUC:100%), sweet melon-cucumber (SMC:90:10, 80:20, 70:30, 60:40 and 50:50%) and reported total bacterial count ranging from $1.2x10^4$ to $8.0x10^3$ in Makurdi, Benue state, Nigeria.

Bacillus was found in all smoothie combinations at frequency of 1 (11.11 %). The occurrence of *Streptococcus* sp was similar 1 (11.11 %) to that of *Bacillus* except that it's occurrence in PPBNBS was higher 2 (22.22 %). *Rothia* sp and *Micrococcus* occurred just once 1 (11.11 %) in PPBNBS fruits combination. *Paenibacillus* and *Lactobacillus* sp occurred only in ORBNPN 1 (11.11 %). *Klebsiella* appeared only in ORBNPN at the rate of 2 (22.22 %) while *Enterococcus* sp had occurrence rate of 1 (11.11 %) in ORWMBN and ORBNPN respectively. *Staphylococcus* had the highest frequency of 9 (100.0) distributed at the rate of 3 (33.33 %) in ORWMBN and 6 (66.66 %) in ORBNPN. Its occurrence in ORWMBN was in all analyzed (Fresh, room temperature and refrigerated) conditions.

A total of 9 (100.0 %) of prominent strains of microorganisms were isolated and identified in this study. And comprised of; *Rothia, Micrococcus, Paenibacillus, Klebsiella, Lactobacillus, Enterococcus, Bacillus, Staphylococcus,* and *Streptococcus* sp respectively.

Staphylococcus, Bacillus and *Klebsiella* were the most noticeable food borne pathogens isolated in this study. Their presence are serious public threat indicators and their major rout of entry may be poor personal hygiene and handling during preparation. According to most published literature [8], *Staphylococcus aureus* is commonly found in smoothies formulated majorly from Watermelon, pineapple and sweet melon cucumber blends. This is in line with the report of the index study, also reporting the presence of *S. aureus* in ORWMBN and ORBNPN smoothie combination that has water melon and pineapple constituents.

Streptococcus sp was the second most abundant 5 (55.56 %) isolate in all smoothies' combinations. The report of Falkenhorst et al., 2008 [12] noted with interest, Streptococcus among the causative agents of food spoilage based of their outbreak experiment conducted on Group A *Streptococcus* species in Denmark.

Other microorganisms such as *Micrococcus, Rothia, Paenibacillus, Lactobacillus* and *Enterococcus* occurred sparingly, however the potency in any pathogen recovery from diluent of 10^{-5} should not be undermined [13].

The index result reports higher frequency 8 (88.9 %) of Gram positive organisms compared to 1 (11.11 %) Gram negative bacterial isolates. This is scientifically proven, being that Gram positive microorganisms are always the most implicated pathogens in food poisoning [14].

The smoothie combination ORBNPN at all analyzed conditions had 10 (40.0 %, 10/25 X 100/1) time distribution of isolates, other combinations; ORWMBN, PPWMBN and PPBNBS had similar even isolates distribution of 5 (20.0 %). Smoothie combinations have no significance output and or influence on bacterial occurrence, although serving the drink fresh after preparation is reported by this study to have

reduced microbial load. However, observing good hygiene practices during smoothie preparation is essential in reducing microbial load and ensuring safety of the final consumers [4].

5. CONCLUSION

The research report showed unacceptable bacterial colony mean count of 1.0-9.0, 1.0-8.0 and 1.0-5.0 x 10^5 Cfu/mL for smoothie analyzed after 24 hours of preparation and kept under refrigeration, the second kept on bench for 24 hours after preparation before analysis and the third analyzed fresh immediately after preparation. The isolated and identified organisms were majorly Gram positive organisms of medical importance highly implicated in foodborne infections. They were: *Rothia, Micrococcus, Paenibacillus, Lactobacillus, Enterococcus, Bacillus, Staphylococcus,* and *Streptococcus* respectively. Improper handling and preparation of smoothie drink is a silence vehicle for the transmission of foodborne indicator pathogens. This implies that, the public and or the final consumers are the most at risk group. Hence, personal hygiene measures such as proper hand washing during food preparation, thorough washing of purchased fruits as well as using clean utensils during food preparation are important measures of microbial load reduction/infection mitigation.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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