ENUMERATION OF MICROBIAL QUALITY OF YOGHURT INCORPORATED WITH *Moringa oleifera* SEED FLOUR DURING STORAGE

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B. C. Obasi[1]*, B. A. Sunday[1] and T. C. Brown[2]
[1]Department of Food Science and Technology, Federal University Wukari, PMB 1020, Taraba State, Nigeria
[2]Department of Microbiology, Federal University Wukari, PMB 1020, Taraba State, Nigeria
*Corresponding author: blessed200067@yahoo.com

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Abstract: Yoghurt is one of the most popular fermented dairy products widely consumed all over the world. It is obtained by lactic acid fermentation of milk by the action of a starter culture containing *Streptococcus thermophilus* and *Lactobacillus delibrueckii*. This study investigated the effect of *Moringa oleifera* seed flour on the microbial quality of freshly produced and shelf life stability of yoghurt after the storage period of 28 days under refrigeration and ambient temperature (5±1 and 27±1^{0}C), respectively. *Moringa oleifera* seed flour was incorporated into yoghurt at concentrations of 0.5, 1, 1.5, 2 and 2.5%. The microbial enumeration was observed using Nutrient, MacConkey and Potato dextrose agar media. The result indicated that addition of *Moringa oleifera* seed flour at various concentration has remarkable effect on bacteria, yeast and mold count. Microbial count (total aerobic count) decreased with advance storage period for samples A_1 –E_1 at the various concentrations for bacteria cells rang from 3.70 -2.30 X 10^6, 4.06 – 2.21 X 10^6, 4.35 – 2.10 X 10^6, 5.30 – 1.40 X 10^6 and 6.30 – 1.15 X 10 cfu/ml for samples stored under refrigeration temperature, respectively. The result for samples stored under ambient temperature for bacteria cells count ranged from 1.30 X 10^6 – 9.97 for control, 3.70 X 10^6 - 2.80 X 10^6, 4.06 X 10^6 – 2.68 X 10^6, 4.35 X 10^6 – 2.45 X 10^6, 5.30 X 10^6 – 2.33 X 10^6 and 6.30 X 10^6 – 2.25 X cfu/ml for samples A_2 – E_2, respectively. However, samples with concentration of 2.5% stored in the refrigerator and ambient temperature showed high inhibitory effect of (1.15 X 10^6 and 2.25 X 10^6) after the storage period. Yeast and mold count were detected only from samples with concentration of 2% (1.24 X 10^6) and 2.5% (1.84 X 10^6) from ambient temperature after the period of storage, but absent from all samples stored under refrigeration temperature, except control samples (fresh) which showed growth in the third (3rd) and fourth (4th) week of storage (1.22 X 10^6 and 1.53 X 10^6) period. Notably, Coliform counts were not detected in all samples for both control (fresh) and treated samples throughout the storage period. Therefore, it can be concluded that the use of *Moringa oleifera* seed flour can serve as a preservative since it led to retarded microbial growth in the yoghurt produced, thereby, improving its quality and shelf-life stability.

Keywords: Yoghurt, *Moringa oleifera*, microbial quality, storage, shelf-life, preservative

Introduction

Yoghurt is one of the most popular fermented dairy products widely consumed all over the world. It's obtained by lactic acid fermentation of milk by the action of a starter culture containing *Streptococus thermophilus* and *Lactobacillus dellbruekii sub-spp. bulgaricus*. The role of these two genera in yoghurt manufacture can be summarized as milk acidification and synthesis of aromatic compounds (Srerraltha and Padma, 2009). It is generally accepted that the yoghurt should contain 10^7 cfu/ml of viable bacteria (Fadela *et al.*, 2009).

Several medicinal plants have been reported to have antimicrobial properties. Amongst them, *Moringa oleifera*, is one of the most widely distributed species of a monogeneric family *Moringaceae* (Singh *et al.*, 2013). The tree is characterized as a fast-growing, drought-tolerant type, native to north-western India, and is widely cultivated in tropical and subtropical areas where its young seed pods and leaves are regarded as a nutritional powerhouse (Radovich, 2011).

Several compounds have been isolated from the seeds of *Moringa oleifera* including niazirin, niazirinin, 4-[4'-O-acetyl-α-L-rhamnosyloxy) benzyl] isothiocyanate, niaziminin A and B (Faizi *et al.*, 1995). Moringa has long been recognized in traditional medicine worldwide as having value both as a preservative and treatment agent of several health conditions, including the treatment of inflammation, infectious diseases, cardiovascular, gastrointestinal, and haematological and hepatorenal disorders (Belewu *et al*., 2014). Several scientific articles has been published describing the antimicrobial properties of *moringa* seeds, which can translate to its use as an anti-ageing, antifungal and antimicrobial herb (Arora et al., 2013).

Yoghurt are capable of supporting the growth of diverse species of microorganisms due to their high moisture content and neutral pH. Microorganisms found on yoghurt include bacteria or fungi that have grown on and colonized the milk by utilizing nutrients exuded from animal tissues. These could be pathogenic or spoilage-causing microorganisms. Fungi commonly causing spoilage of yoghurt include *Candida albicans* and various species of the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Serretia marcescens*. These affect the product causing devastating losses (Bukar *et al.*, 2010). Besides causing huge economic losses, some fungal species could produce toxic metabolites in the product, constituting a potential health hazard for humans. In order to slow down yoghurt spoilage and minimize the associated adverse health effects, great caution should be taken to follow strict hygiene, and good manufacturing practices during production, storage, transport, and marketing (Kaylegian *et al*., 1995). Milk being a rich medium for growth of spoilage and pathogenic microorganisms, it is capable of being a source of illness/sickness for the large population of consumers. There is therefore a need to study the shelf life of yoghurt in order to determine its quality and safeguard the health and wellbeing of the numerous people consuming the product.

Materials and Method

Sample collection

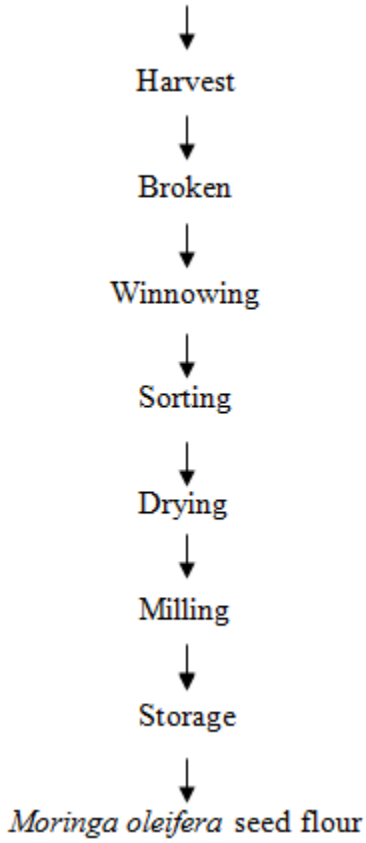
High quality dry pods of M*oringa oleifera* seed and 1500 g of full cream powdered milk were purchased from Wukari market, Taraba State. The starter culture consisting of mixed *Lactobacillus bulgaricus* and *Streptococcus thermophillus* was obtained from Seboreh Farm in Mayo-belwa, Adamawa State.

Sample preparation

Preparation of Moringa oleifera seed flour

The pods of *Moringa oleifera* seeds were broken using hands to expose the winged and coated seeds. This was left to stand for 24 h after which period the seed coat was removed using local mortar and pestle. The broken seed coat and wings were blown off by winnowing to obtain the seeds. The moringa seeds sample was sun dried for period of three weeks; the seeds were ground using a stainless blender. The grinding was repeated continuously until a fine powder was obtained to ensure homogeneity. The powder was sieved through 250 µm mesh sieves to remove any remaining seed coat. The ground and sieved flour was stored in air-tight containers, until when needed for analyses.

Moringa oleifera seed pod;



Source: Fahey (2005)

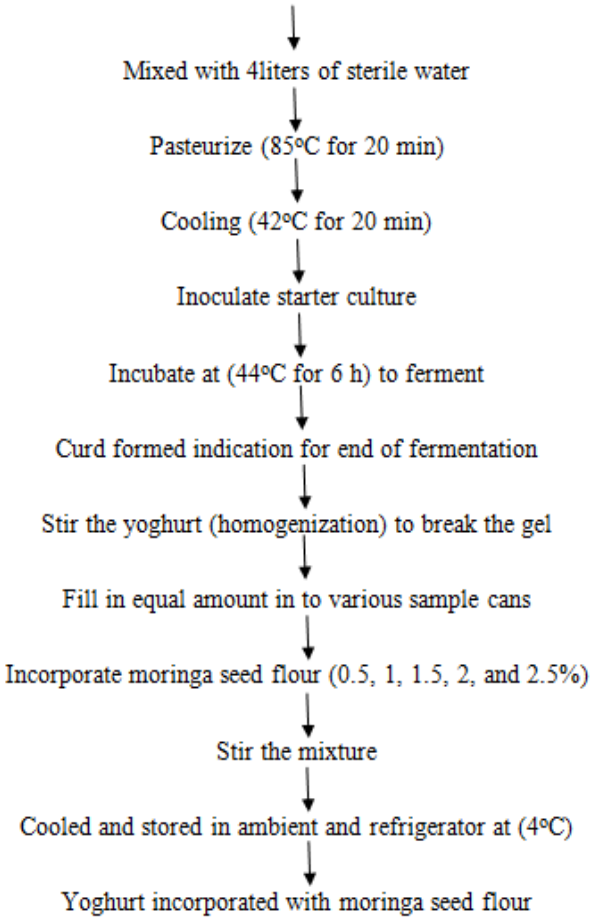
Fig 1: Flow chart for preparation of *Moringa oleifera* seed flour

Production of yoghurt

The sample of yoghurt was produced according to the international standard of yoghurt as described by (Belewu *et al.*, 2012). Loya full cream powdered milk (1500 g) was reconstituted with 4 liters of sterile water and heated to a pasteurization temperature of 85°C for 20 min and then allowed to cool at 42 – 45°C for 20 min before inoculation with 2% starter culture of (*Lactobacillus bulgaricus* and *Streptococcus thermophillus*). The milk was incubated at 44°C for four hours until lactic acid is produce, the pH reaches 4.6 and the milk is set with a characteristics flavor and aroma of yoghurt.

The finished (homogenized) yoghurt was divided into 6 portions; each portion was incorporated with moringa seed flour at different concentrations of 0.5, 1, 1.5, 2 and 2.5% and filled into plastic containers at 200 mls each. The samples were labelled accordingly, control$_1$, A1to E_1 (Refrigeration temperature) and control$_2$, A_2 to E_2. (ambient temperature). Where A_1 and A_2 − 0.5%, B_1 and B_2 −1%, C_1 and C_2 1.5%, D_1 and D_2 − 2%, E_1 and E_2 – 2.5%. All the sample were stored at ambient temperature (27±1°C) and refrigeration temperature (5 ±1°C) for 28 days. The fore going protocol was adopted from (Belewu *et al*., 2014) and the flow chart is shown below.

Full cream powdered milk;



Source: Belewu *et al*. (2012)

Fig. 2: Flow chart diagram for the production of yoghurt

Enumeration of microorganisms

Procedures of Obasi *et al.* (2014) were used to enumerate microorganisms from yoghurt samples incorporated with moringa seed flour. This involved the e of MacConkey agar, Nutrient agar, and Potato dextrose agar for the enumeration of coliform count, total viable count, and total fungi count, respectively. Each of the various samples of yoghurt both treated and control were analyzed. Stock culture was prepared by taking 10 ml of the yoghurt samples mixed with 9 ml of sterile distilled water in a glass test tube. Each test tube was serially diluted in sterile distilled water and 0.1 ml amounts of appropriate dilutions was poured into sterile agar media: Nutrient agar and MacConkey agar (for total aerobic and coliform count) plated in duplicates and incubated at 37°C for 24− 48 h. For yeast and mold count, 0.1 ml amount of appropriate dilutions was spread on potato dextrose agar (PDA). Enumeration of yeast and mold were done after three. All enumerations were expressed as colony forming units per millilitre (cfu/ml) of plated samples.

Results and Discussion

Microbial load of yoghurt incorporated with Moringa oleifera seed flour (MOSF)

The results in Tables 1 and 2 show effect of *moringa* seed flour on microbial quality of yoghurt for fresh and during

storage at ambient (27±1ºC) and Refrigeration (5±1ºC) temperatures. The total aerobic count for all samples incorporated with moringa seed flour from refrigeration temperature ranged from (3.70×10^6– 6.30×10^6) cfu/ml for fresh, (3.15×10^6–2.65×10^6) for week one, (2.95×10^6–2.40×10^6) week two, (2.95×10^6–1.65×10^6) week three, and (2.30×10^6–1.15×10^6) and week four storage, while control$_1$ ranged from (1.30×10^6 –8.02×10^6) (Table 1). Total count for samples incorporated with moringa seed flour from the ambient temperature ranged from (3.70×10^6–6.30×10^6) for fresh, (4.95×10^6–4.30×10^6) for one week, (3.70×10^6–3.15×10^6) for week two, (3.12×10^6–2.40×10^6) for week three and (2.80×10^6 –2.25×10^6) after four weeks of storage, while control$_1$ ranged from (1.30×10^6 –9.97×10^6) (Table 2).

Table 1: Total Aerobic count of bacterial cells, yeast and mold (cfu/ml) in yoghurt incorporated with MOSF stored in refrigeration (5±1ºC) temperature

Properties and storage period (weeks)	Control$_1$	A$_1$	B$_1$	C$_1$	D$_1$	E$_1$
Total aerobic count						
Zero	1.30×10^6	3.70×10^6	4.06×10^6	4.35×10^6	5.30×10^6	6.30×10^6
1	4.15×10^6	3.15×10^6	3.05×10^6	2.93×10^6	2.78×10^6	2.65×10^6
2	6.75×10^6	2.95×10^6	2.85×10^6	$2.70x\ 10^6$	2.50×10^6	2.40×10^6
3	7.98×10^6	2.95×10^6	2.55×10^6	2.35×10^6	1.95×10^6	1.65×10^6
4	8.02×10^6	2.30×10^6	2.21×10^6	2.10×10^6	1.40×10^6	1.15×10^6
Yeast and mold count						
Zero	ND	ND	ND	ND	ND	ND
1	ND	ND	ND	ND	ND	ND
2	1.20×10^6	ND	ND	ND	ND	ND
3	1.31×10^6	ND	ND	ND	ND	ND
4	2.59×10^6	ND	ND	ND	1.24×10^6	1.84×10^6
Coliform count						
Zero	ND	ND	ND	ND	ND	ND
1	ND	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND

ND = Not detected; A$_1$, B$_1$, C$_1$, D$_1$, E$_1$ = Yoghurt with 0.5, 1, 1.5, 2 and 2.5% *Moringa oleifera* seed flour; Control$_1$ = yoghurt without moringa seed flour at refrigeration temperature; MOSF = *Moringa oleifera* seed flour

Table 2: Total Aerobic count of bacterial cells, yeast and mold (cfu/ml) in yoghurt incorporated with MOSF stored in ambient (27±1ºC) temperature

Properties and storage period (week)	Control$_2$	A$_2$	B$_2$	C$_2$	D$_2$	E$_2$
Total aerobic count						
Zero	1.30×10^6	3.70×10^6	4.06×10^6	4.35×10^6	5.30×10^6	6.30×10^6
1	6.05×10^6	4.95×10^6	4.80×10^6	4.73×10^6	4.50×10^6	4.30×10^6
2	8.40×10^6	3.70×10^6	3.55×10^6	3.44×10^6	3.30×10^6	3.15×10^6
3	8.70×10^6	3.12×10^6	2.90×16^6	2.77×10^6	2.52×10^6	2.40×10^6
4	9.97×10^6	2.80×10^6	2.68×10^6	2.45×10^6	2.33×10^6	2.25×10^6
Yeast and mold count						
Zero	ND	ND	ND	ND	ND	ND
1	ND	ND	ND	ND	ND	ND
2	1.20×10^6	ND	ND	ND	ND	ND
3	1.31×10^6	ND	ND	ND	ND	ND
4	2.59×10^6	ND	ND	ND	1.24×10^6	1.84×10^6
Coliform count						
Zero	ND	ND	ND	ND	ND	ND
1	ND	ND	ND	ND	ND	ND
2	ND	ND	ND	ND	ND	ND
3	ND	ND	ND	ND	ND	ND
4	ND	ND	ND	ND	ND	ND

ND = Not detected; A$_2$, B$_2$, C$_2$, D$_2$, E$_2$ = Yoghurt with 0.5, 1, 1.5, 2, 2.5% *Moringa oleifera* seed flour; Control$_2$ = yoghurt without moringa seed flour at ambient temperature; MOSF = *Moringa oleifera* seed flour

Control sample showed low aerobic count (1.30×10^7) for fresh, but count increased with advanced storage to (9.97×10^6) from ambient and (8.02×10^6) from refrigeration temperature after four weeks. All samples incorporated with moringa seed flour increased in total count with increase concentration of *Moringa oleifera* seed powder after the first 24 h with (3.70×10^6 –1.15×10^6) from refrigeration and (3.70×10^6–2.25×10^6) from ambient temperature, but drastically reduced with advanced storage period. This may be due to antibacterial effect of the moringa seed flour. This result is in agreement with the report of Kurosaki and Nishi (1998); they reported that the effect of antimicrobial agent usually increased with doses. The result of this study is also in agreement with research findings of Saadabi and Abu Zaid (2011); Onsare *et al*. (2013); Vinoth *et al*. (2013). They reported that the biological active compound isolated from both seeds and leaves of the plant exhibited bactericidal effect against Gram positive and Gram negetive bacteria, and

moringa *seeds* contained a wide range of these phytochemicals there by inhibiting the growth of the bacterial cells in the yoghurt samples. Marrufo et al. (2013) reported that, the antimicrobial activity of *Moringa oleifera* seed is due to the presence of a significant phytochemical of a short polypeptide called 4(ά – L – rhamnosyloxy) benzyl-isothiocyanate. This peptide act directly on bacteria and result in growth inhibition by disrupting cell membrane synthesis and/or synthesis of essential enzymes (Marrufo et al., 2013). It was also observed that 2.5% yoghurt moringa seeds flour sample from refrigeration temperature had the highest inhibitory effect with total count of (1.15×10^6) followed by 2.5% sample from ambient temperature with (2.25×10^6). Sample with 0.5% moringa seed flour from ambient temperature showed lower inhibitory effect (2.25×10^6) after four weeks. All samples incorporated with *Moringa oleifera* seed flour from refrigeration temperature showed high inhibitory effect than samples from the ambient temperature. This result is in agreement with that reported by (Badomas *et al*., 2014) they indicated that, the biological active compound from seeds of *Moringa oleiferaand stenopetala* plant when used as preservative in cheese production at 2 and 2.5% concentration exhibited bactericidial effect against *S. aureus*, *S.typhi*, *Shigella* and *Candida albicans*, *E. coli*, *S. aureus*, *P. aeruginosa* and these organisms range from pathogenic and toxigenic organism liable to cause food borne illnesses and food spoilage due to bacteria presence (Ali, 2014). They also reported that *labneh* manufactured with 0.5 - 2.5% *Moringa oleifera* seeds and leave flour showed best concentrations and had the highest inhibitory potential against bacteria population at refrigeration temperature than ambient temperature. They concluded that, the use of 0.5 - 2.5% *moringa* seeds flour as a *lebneh* preservative led to improved microbial stability and nutritional content, as well as higher sensory acceptability.

Mold and yeast were not observed in samples for fresh, after one week of storage (both refrigeration and ambient storage) and after two weeks from the refrigeration temperature. However, molds and yeasts were detected in samples of control after 3 and 4 weeks from refrigeration temperature with total count of (1.224×10^6) and (1.532×10^6) and after 2, 3 and 4 weeks from ambient temperature with total count of (1.204×10^6), (1.313×10^6) and (2.591×10^6). Also mold and yeast were observed in sample of yoghurt with 2% and 2.5% moringa seed flour from ambient storage after 4 weeks with total count of (1.235×10^6) and (1.835×10^6). These result obtained from this study are in line with those reported by (Salem *et al.*, 2013) they indicated that yeast and mold were not detected in sour cream fortified with *Moringa oleifera* seeds and leaves extract and seed oil at 0.5 - 2% concentration, also that *Moringa oleifera* seeds had antimicrobial activities against Gram positive and Gram negative fungal species. Pinto *et al.* (2015) also reported that *Moringa oleifera* seeds contained small peptides which could play an important role in the plant's antimicrobial defense system. The proteins/peptides are believed to be involved in a defense mechanism against phytopathogenic fungi by inhibiting the growth of microorganisms through diverse molecular modes, such as binding to chitin or increasing the permeability of the fungal membranes or cell wall (Saad *et al.*, 2015). Pinto *et al*., (2015) reported that *Moringa oleifera* seeds contained cationic water-soluble (M_o-CBP3) antifungal protein which served as potential candidate for developing transgenic crops (Saad *et al.*, 2015) reported that M_o-CBP3 is a chitin-binding protein that inhibit the germination and mycelia growth of pythopathogenic fungi and are highly thermo stable and resistance to pH changes, and therefore may be useful in the development of antifungal preservatives. Notably, coliforms were not detected in all samples for fresh and during storage period. This corresponds with the statement of Sreelatha and Padma (2009) who remarked that processed milk should contain no trace of coliform. However, Bukar and Oyeyi (2010) recommended that yoghurt should contain less than 0.1x10 cfu/g. The absence of coliform is a good indication of the Good manufacturing practice, pasteurization and hygienic condition followed in its production. Todar (2008) reported that the absence of coliform bacteria in water was due to the acidic condition generated by *Moringa oleifera* seed flour when used in water treatment. This is because most bacteria grow well in an alkaline condition and coliform is a facultative an aerobicmicroorganisms that grow in aerobic environment and in fermentation that produce lactic acid. This bacteria can grow well at low pH environment but cannot survive acidic condition, therefore addition of *Moringa oleifera* seed flour prevent increase in pH and in turn prevent their growth.

Conclusion

From this study, it can be concluded that *Moringa oleifera* seed flour can be used in yoghurt as a preservative since it led to retarded microbial growth of (bacterial and yeast) and can also help to maintained the quality of the yoghurt there by prolonging the shelf life of the product in storage.

Conflict of Interest

Authors declare that there is no conflict of interest.

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