Microbiological Quality of Some Yoghurt Sold in Uwelu-Community, Edo State, Nigeria

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Abstract: Yoghurt is a dairy product generally consumed around the globe due to its energy content and health advantages. An investigation on the microbiological quality of sixteen yoghurt samples from eight different brands, four registered by NAFDAC and four unregistered were randomly obtained The pH and microbiological parameters were evaluated using standard methods. The pH readings of the yoghurt samples ranged from 4.00- 5.60. The total viable bacteria and fungi counts of the two groups of yoghurt ranged from $3.0 \times 10^4 \pm 1.00$ to $28.0 \times 10^4 \pm 12.77$ and $4.9 \times 10^4 \pm 6.24$ to $10.9 \times 10^4 \pm 4.36$ respectively. There was a significant difference ($p<0.05$) in the levels of the total viable bacteria counts. However, there was no statistically significant difference ($P>0.05$) in the total fungi counts. Comparative assessment of pH with the total viable bacteria and fungi counts revealed a weak negative correlation with an R-value of -0.326 and -0.100. Consequently, the results obtained shows high levels of contamination by some medically important bacteria- *Bacillus* sp (100%), *Staphylococcus aureus* (63%), *Klebsiella* sp (50%), *Streptococcus* sp (38%) and *Pseudomonas* sp (25%) and fungi- *Aspergillus* sp (100%), *Saccharomyces cerevisiae* (63%), *Rhizopus* sp (50%) and *Mucor* sp (38%). These microbes may have contaminated the product during production, distribution and/or storage. It is therefore imperative to monitor the production process and sales of these products to protect the consumers from food-borne infection and intoxication.

Keywords: Yoghurt, Microbiological Quality, Contamination, Diary products, NAFDAC

INTRODUCTION

Yoghurt is one of the most famous fermented dairy products widely consumed around the globe. Lactic acid bacteria *Lactobacillus delbruckii* spp. *Bulgaricus* and *Streptococcus thermophilus* contained in the starter culture helps in the fermentation of milk to produce yoghurt. The role of these two bacterial genera in the production process can be put together as acidification of the milk and production of aromatic compounds (Serra *et al.,* 2009). The French named it 'la lait de la vieeternelle' – meaning the milk of timeless existence because they believed it increases the life span of the consumers (Metchnikoff, 1908). It was also believed to possess chemotherapeutic properties which enhance recovery from gastrointestinal disorders (Bhattarai and Das, 2016). It is a popular drink in Nigeria because of its probiotic, nutritional and organoleptic traits (De *et al*., 2014). Yoghurt contains high protein, phosphorus, and calcium content. Its carbohydrate can be easily digested by people with lactose intolerance deficiency. It is an effective reservoir of protein for the maintenance of good health (Cueva and Aryana, 2000). The bacteria *Lactobacillus delbruckii* spp. *Bulgaricus* and *Streptococcus thermophilus* commonly applied in the production yoghurt are termed as starter cultures (Tamine and Robinson, 2007). The anaerobic breakdown of milk sugar by these minute bacteria releases lactic acid which subsequently acts on the protein content of the milk to give yoghurt its specific traits and texture (McGee, 2006). *Lactobacillus delbrueckii* subsp. *bulgaricus* is mainly used in conjunction with *Streptococcus thermophilus* to kick start the fermentation process with the production of yoghurt as the end. The two species work together in synergy with *Lactobacillus delbrueckii* subsp. *bulgaricus* producing amino acids from the milk proteins which are subsequently utilized by *Streptococcus thermophilus* (Courtin and Rul, 2003). Both bacteria release lactic acid which functions as a preservative and also contribute to the sour flavour of the yoghurt.

The lactic acid production results in a decrease of pH which in turn cause clotting of the casein (milk protein) leading to the yoghurt thickness (Zourari *et al.,* 2003). Some strains of *Lactobacillus delbrueckii* subsp. *Bulgaricus* also produce antibacterial agent (bacteriocins) with a narrow spectrum of activity inhibiting the growth of closely related species (Simova *et al.,* 2008). Although these blends of yoghurt are consumed worldwide the majority is consumed in the Indian subcontinent (Tamine, 2004). Following the high request for yoghurt foods, machine-driven equipment to ease large scale production to meet the high demand and safety quality of products were put in place (Salinas, 1986). Also, an effective industrial process has not only improved the storage, distribution, and marketing of the finished product, the microbiological parameters according to (Tamine and Robinson, 2007) led to the maintenance of coliform free products. Yoghurt has also been found to be a good medium for the proliferation of spoilage and pathogenic microbes due to its high nutrient content, thus it is susceptible to contamination. Moulds and yeast are often implicated in the contamination of yoghurt. Fungi growing in yoghurt use part of the acid presence, leading to a decrease in the acidity level which triggers the growth of bacteria responsible for decay (Oyeleke, 2009) or other virulent organisms such as *Staphylococcus aureus* (Ifeanyi *et al.,* 2013; De *et al.,* 2014; Makut *et al.,* 2014). Fermented milk used in yoghurt production is apt to contamination. Like other dairy fermented products yoghurts are prone to bacterial contamination leading to food intoxication and/or poisoning of the finished products. So to ensure the safety of yoghurt offered for sale to consumers, there is a need for painstaking assessment of the methodologies for both production and vending in local and major streets markets. A practical oriented approach geared towards quality assurance of the yoghurts sold to the people should be taken into consideration (Karagul-Yuceer *et al.,* 2002). Some of the yoghurt products vended by mobile vendors are associated with improper handling and poor personal hygiene thus referred to as substandard (Kawo *et al.,* 2006). It is therefore pertinent that the evaluation of the microbiological quality of the yoghurt is done due to the health hazards associated with the consumption of badly prepared product containing pathogenic organisms. These health risks may range mild to serious infections which can become persistent to treat with the conventional antibacterial agents. This becomes clinically important if the bacteria isolated from the product under examination is resistant to routine antibiotics. Thus, it can bestow antibiotic resistance to the infected host while producing an alternative drug (Gould, 2004). An early observation of food pollutants contributes greatly to the safety thus leading to the improvement of public health of the consumers (Hove *et al.,* 2001). The initial microbial flora of raw milk utilized in the production of fermented products influences the bacteriological quality amongst other conditions of processing and post-heating treatment contamination. Gram-negative psychrotrophs, lactic acid bacteria and coliforms are some of the unwanted bacteria often implicated in spoilage of dairy products (Agu *et al.,* 2014). Coliforms enumeration allows for the assessment of raw milk standard and the efficiency of the processing (Rodrigues, 2010). Currently, there is a geometric increase in the rate of consumption of yoghurt in Nigeria, this has resulted in the proliferation of minute scale processing plant for yoghurt manufacturing (Nwagu and Amadi, 2010). This may be linked to the rise in awareness of the gains derived from the consumption of yoghurt. Bacteria genera of public health importance such as *Salmonella*, *Campylobacter, Listeria, Yersinia,* enterotoxigenic strains of *Staphyloccocus aureus* and pathogenic strains of *Escherichia coli* are often implicated in milk and dairy products(Agu *et al.,* 2014). In light of this, intense effort should be directed to the examination of the microbiological state of milk and dairy foods (Nduka, 2007).

Assessing the microbiological quality of yoghurts is important because of the health hazards associated with the consumption of poorly prepared yoghurt products containing pathogenic agents. There is a need to also create awareness among consumers to protect their health and rights. Therefore this research was undertaken with the aim to determine the microbiological load of some yoghurt brands vended in Uwelu-community and also to ascertain their conformity with acceptable microbiological standards and specifications for fermented dairy foods. The findings of this study will be beneficial to the consumers, governmental bodies saddle with the responsibility of maintaining public health of its citizenry and to the sellers themselves on the health issues these foods might cause.

MATERIALS AND METHODS

Sampling technique and Sample vicinity

This assessment was done in Uwelu community in Egor Local Government Area, one of the eighteen (18) Local Government Area of Edo State which lies on a geographical coordinate of latitude 05°44′N and 07°34′N and Longitude 05°04′E and 06°45′E. The area which has a total landmass of 93Km^2 and a population of 339,899 (National Population Census, 2006). A total of Sixteen (16) samples of sachets plain yoghurts were obtained from eight different brands with four registered by NAFDAC and four unregistered. The registered samples were designated as A1 A2, B1 B2, C1 C2, D1 and D2 while the unregistered samples were labelled E1 E2, F1 F2, G1 G2, H1 and H2 respectively. These products were randomly purchased from retails stores and mobile vendors within the study area and kept under refrigerated condition during the transport to the laboratory. Samples were properly labelled and promptly analyzed according to microbiological standard operating procedures.

pH Analyses

After sample collection organoleptic properties and colour were recorded. Then pH readings were obtained using a digital Jenway Model 3510 benchtop pH metre. For examination of pH, 70ml of each yoghurt sample was dispensed into 100ml beaker and the pH was thereafter obtained using the pH meter after calibrating with pH 7 buffer.

Microbiological Analyses

A 1000µl of 10^{-3} dilution point of each yoghurt samples were inoculated into sterile Petri dishes in triplicates before Potato dextrose agar (PDA) containing 20µg of chloramphenicol and Nutrient agar (NA) both of Oxoid products were dispensed into it to ascertain the total fungi counts (TFC) at 25^{o}C for 5-7days incubation and the total viable bacteria counts (TVBC) at 37^{o}C for 18 to 24h incubation. Enumeration of colonies was done with the aid of the illuminated colony counter (Gallenkamp, England). The mean and standard deviation of the colony counts from the triplicate Petri dishes were expressed as colony-forming unit per millilitre (cfu/ml).

Identification of bacteria and fungi isolates associated with the collected samples

Pure cultures of the isolated bacteria were identified using their cultural and morphological characteristics on media. This was subsequently followed by a microscopic investigation of the bacterial isolates under the microscope. The cultural features examined included shape, consistency, margins and elevation. Biochemical and physiological tests were engaged to confirm their identity (Cheesbrough, 2010). The fungal isolates were characterised and identified according to their microscopic outlook and colonial morphology and compared with already existing and known taxa as described by (Oyeleke and Manga 2008).

Statistical analysis

The mean and standard deviation of the laboratory values obtained for total viable bacteria counts and total fungi counts were obtained using Microsoft excel 2010 and also were analyzed using the IBM SPSS software version 25.

Student's t-test was applied to test for significance among the groups. Furthermore, correlation assessment of pH with total viable bacteria counts and total fungi counts were also tested to ascertain the relationship between the variables under examination.

RESULTS

The study was done to determine the microbiological status of some brands yoghurt sold in Uwelu community Egor Local Government Area of Edo State. Table 1 represents the number of bacteria and fungi colonies counted on Nutrient agar (NA) and Potato dextrose agar (PDA) for all the yoghurt samples within the vending sites. Table 2 represents the pH, Mean and Standard deviation of the total bacteria and fungi counts for the yoghurt samples. The 16 samples of yoghurt obtained were white, with D1 and D2 being solid in terms of its state, E1- H2 was paste-like, while A1- C2 was watery. The pH readings were in the range of 4.00- 5.60. Sample B2 registered yoghurt brand had the highest pH value of 5.60 while sample H2 unregistered yoghurt brand had the lowest pH value of 4.00. The results are presented in Table 2. The values of the total viable bacterial count of the registered samples ranged from $3.0 \times 10^4 \pm 1.00$ to $7.0 \times 10^4 \pm 8.19$, with sample B2 having the least value whereas sample D1 had the highest reading. The values for the unregistered samples ranged from of $15.0 \times 10^4 \pm 2.00$ to $28.0 \times 10^4 \pm 12.77$, with sample H1 having the highest value while sample E2 had the least value as presented in Table 2. Total fungi counts of the registered samples ranged $4.9 \times 10^4 \pm 6.24$ to $9.0 \times 10^4 \pm 9.85$ with sample D1 having the highest value and sample B2 had the lowest value. The values of the unregistered samples ranged $6.8 \times 10^4 \pm 1.00$ to $10.9 \times 10^4 \pm 4.36$ with sample G2 having the lowest value and F2 had the highest value. There were no fungi growths detected in the registered sample C1 and C2 and in the unregistered sample H1 and H2 after the prescribed period of incubation. A significant difference ($p<0.05$) was seen in the levels of the total viable bacteria counts amidst the registered and unregistered samples. However, there was statistically no significant difference ($P>0.05$) seen in the total fungi counts within the registered and unregistered samples as represented in Table 3. Comparative assessment of pH with the total viable bacteria and fungi counts were done using IBM SPSS version 25. The finding revealed a weak downhill negative correlation with an R-value of -0.326 and -0.100 and p-value of 0.217 and 0.712 respectively. Figure 1 shows the total percentage occurrence of the bacterial isolates from the yoghurt samples. *Bacillus* sp has the highest percentage occurrence of 100% followed by *Staphylococcus aureus* 63%, *Klebsiella* sp 50%, *Streptococcus* sp 38% and *Pseudomonas* sp 25%. Figure 2 depicts the total percentage occurrence of fungal isolates from the yoghurt samples. Six fungi genera were isolated and identified using morphological, cultural and microscopic features. *Aspergillus* sp was found to have the highest percentage occurrence of 100%, followed by *Saccharomyces cerevisiae* observed in sample B, D, F, G and H with 63%, *Rhizopus* sp isolated from samples C, E, F and H having 50% and *Mucor* sp implicated in samples D, F and G with 38% respectively.

DISCUSSION

The expiry, production date and NAFDAC registration number of the yoghurt samples were documented. The pH readings of the registered and unregistered yoghurt samples were in the range of 4.01-5.60 and 4.00 – 5.48 respectively. This pH values recorded is within the range of 4.73 – 5.11 also reported by Digbabul *et al.* (2014). This high pH and low acidity impedes the growth of coliforms but support the proliferation of acidophilic yoghurt associated bacteria as well as fungi hence their presence in the samples. The results of the correlation of pH values of the samples with those of the total viable bacteria and fungi counts revealed a weak negative correlation.

This correlation means that as pH decreases there is an increase in the proliferation of more putrefactive bacteria and fungi vice versa. The total viable bacteria and fungi counts of the entire samples ranged from 3.0 x 10^4 ± 1.00 to 28.0 x 10^4 ± 12.77 and 4.9 x 10^4 ± 6.24 to 10.9 x 10^4 ± 4.36 respectively. High bacteria count is expected because of the presence of starter cultures, which are mainly lactic acid bacteria. The values obtained for total bacteria and fungi counts were within the stipulated limit of 10^6-10^7 cfu/ml (Codex Alimentarius 2003). High counts of yeast and mould have also been reported in yoghurts by previous studies (Ifeanyi *et al.,* 2013; De *et al.,* 2014; Digbabul *et al.,* 2014). A significant difference ($p<0.05$) was detected in the levels of the total viable bacteria counts amidst the registered and unregistered samples. However, there was statistically no significant difference ($P>0.05$) seen in the total fungi counts within the registered and unregistered samples The presence of *Staphylococcus aureus*, a pathogenic organism in the yoghurt samples is of great public health concern. *Staphylococcus aureus* produces some enzymes which help in Staphylococcal invasiveness of the immune system and many heat-stable extracellular substances known as enterotoxins that render the product unsafe even though it appears pleasant to the eyes (Prescott *et al.* 2005). The appearance of *Staphylococcus aureus* is mainly as a result of human interface possibly from the skin, nasal passage and activities like talking, coughing and sneezing which produces droplets that settle on the yoghurts in the course of manufacturing, distribution and storage. It is resistant to heat drying and radiation, its presence in the samples examined may cause Staphylococcal food poisoning which is a major cause of food intoxication caused by the injection of improperly stored or cooked foods in which the organism has grown (Willey *et al*., 2008). The presence of Streptococci from this study is in agreement with the works of Bramley *et al.* (2004) who reported that organisms that contaminate the outer linings of the teats and udders of cows include Staphylococci, Streptococci (which can grow in refrigerated conditions), spore formers, coliforms and Gram-negative bacteria capable surviving pasteurization temperature. *Bacillus* sp was implicated in all the samples examined, it is a motile bacterium, Gram-positive with a rod shape. Its produces heat resistant spores found in dust, soil and raw foods. Their presence in yoghurt samples implies post-pasteurization contamination (Huck *et al.,* 2008). Coliforms are viewed as conventional flora of the intestinal tract of man and animals and their presence implies direct faecal contamination (Agu *et al.,* 2014). Their level and presence of other indicator organisms have become an index for food hygiene. *Klebsiella* sp is a coliform susceptible to pasteurization. However, its appearance in after pasteurization process of yoghurts may be attributed to unsanitary habits of the handlers, storage vicinity and water used during production. It has been implicated in bacterial pneumonia with a severity more than those caused by *Streptococcus pneumonia*. *Aspergillus* sp was found in all samples studied in this research with 100%. Aflatoxins are associated with some species of *Aspergillus* which is cancerous to man when ingested. This implies that most of the products investigated in this study were unsafe for human consumption based on the World Health Organisation, (2000) guidelines. The isolation of yeast and moulds in the yoghurts is indicative of poor sanitary during production, packaging, and handling by vendors. Yoghurts totted with sugar is particularly susceptible to increase proliferation of yeast cells which invariably leads to higher results (Lourens- Hattingh and Viljoen, 2001). Food management procedures by vendors are important to avoid contamination of the final products sold to consumers since no conventional training is done before commencing the business of vending yoghurts.

CONCLUSION

Microbiological assessment of some registered and unregistered yoghurt samples within Uwelu- community revealed the presence of some medically important bacteria and fungi. *Bacillus* sp and *Aspergillus* sp were seen in all the samples assessed. The significant difference seen in the levels of the total viable bacteria counts among the registered and unregistered samples may be linked to improper sanitary conditions and/or milk extraction from unhealthy cows during production processes. It is therefore imperative that enactment and implementation of standardised procedures for yoghurt production, handling and storage, especially those offer for sale to the public. Furthermore, a high level of cleanliness should be maintained to ensure that the products are of good quality.

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Conflicts of Interest

The authors declare no competing interest.

Table 1: Results of total viable bacteria and fungi counts for all the yoghurt samples

S/N	Sample code	No of Bacteria colonies counted on NA	No of Fungi colonies counted on PDA
1	A1	47, 46, 57	82, 79, 100
2	A2	35, 36. 31	58, 55, 52
3	B1	39, 33, 36	63, 59, 70
4	B2	30, 29, 31	44, 47, 56
5	C1	32, 31, 36	49, 53, 48
6	C2	42.45, 33	80, 75, 82
7	D1	68, 63,79	93, 98, 79
8	D2	35, 33,43	64, 69, 71
9	E1	200, 208, 222	80, 93, 100
10	E2	152, 148, 150	69, 73, 68
11	F1	260, 249, 241	94, 98, 93
12	F2	263, 217, 330	104, 112, 111
13	G1	215, 209, 206	91, 95, 93
14	G2	243, 249, 228	67, 69, 68
15	H1	277, 269, 294	118, 125, 120
16	H2	249, 263, 268	85, 94, 118

- NA- Nutrient agar PDA- Potato dextrose agar

Table 2: Results of pH, Mean and Standard deviation of the total bacteria and fungi counts for the yoghurt samples

S/N	Sample code	pH	Mean ± SD* (TVBC)	Mean ± SD* (TFC)
1	A1	5.45	$5.0 \times 10^4 \pm 6.08$	$8.7 \times 10^4 \pm 11.36$
2	A2	5.42	$3.4 \times 10^4 \pm 2.65$	$5.5 \times 10^4 \pm 3.00$
3	B1	5.58	$3.6 \times 10^4 \pm 3.00$	$6.4 \times 10^4 \pm 5.57$
4	B2	5.60	$3.0 \times 10^4 \pm 1.00$	$4.9 \times 10^4 \pm 6.24$
5	C1	5.45	$3.3 \times 10^4 \pm 2.65$	ND
6	C2	4.62	$4.0 \times 10^4 \pm 2.65$	ND
7	D1	4.57	$7.0 \times 10^4 \pm 8.19$	$9.0 \times 10^4 \pm 9.85$
8	D2	4.01	$3.7 \times 10^4 \pm 5.29$	$6.8 \times 10^4 \pm 3.61$
9	E1	4.06	$21.0 \times 10^4 \pm 11.4$	$9.1 \times 10^4 \pm 10.15$
10	E2	5.31	$15.0 \times 10^4 \pm 2.00$	$7.0 \times 10^4 \pm 2.65$
11	F1	5.28	$25.0 \times 10^4 \pm 9.54$	$9.5 \times 10^4 \pm 2.65$
12	F2	4.79	$27.0 \times 10^4 \pm 56.82$	$10.9 \times 10^4 \pm 4.36$
13	G1	4.63	$21.0 \times 10^4 \pm 4.58$	$9.3 \times 10^4 \pm 2.00$
14	G2	4.23	$24.0 \times 10^4 \pm 10.82$	$6.8 \times 10^4 \pm 1.00$
15	H1	5.48	$28.0 \times 10^4 \pm 12.77$	ND
16	H2	4.00	$26.0 \times 10^4 \pm 9.85$	ND

*SD- Standard Deviation *TVBC- Total Viable Bacteria Count *TFC- Total fungi count

Table 3: Student's t-test statistics

Parameter	F-Statistic	pValue	Remarks
Total Viable Bacteria Count	6.884	P= 0.020	S
Total Fungi Count	0.327	P= 0.576	NS

NS- not significant, S- Significant at p=0.05

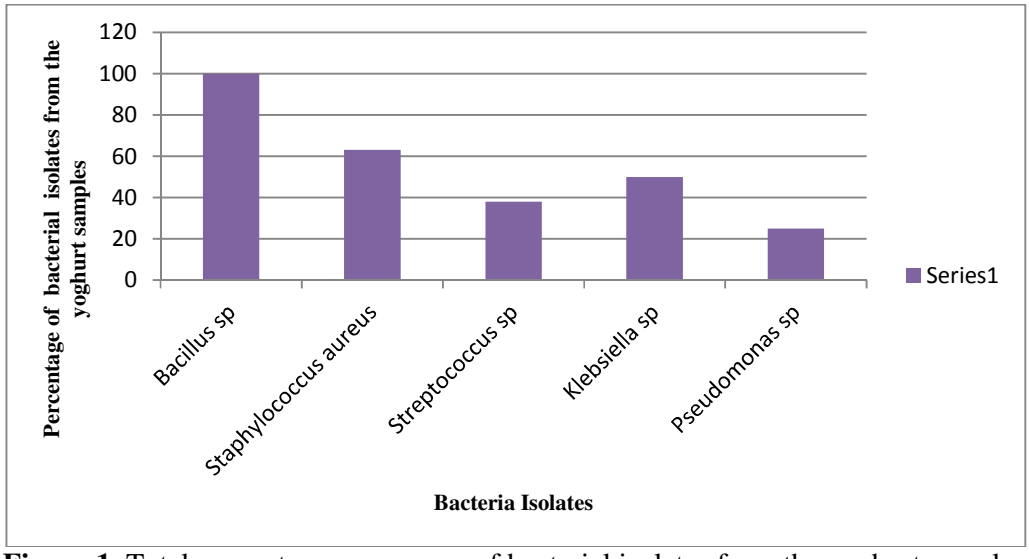

Figure 1. Total percentage occurrence of bacterial isolates from the yoghurt samples

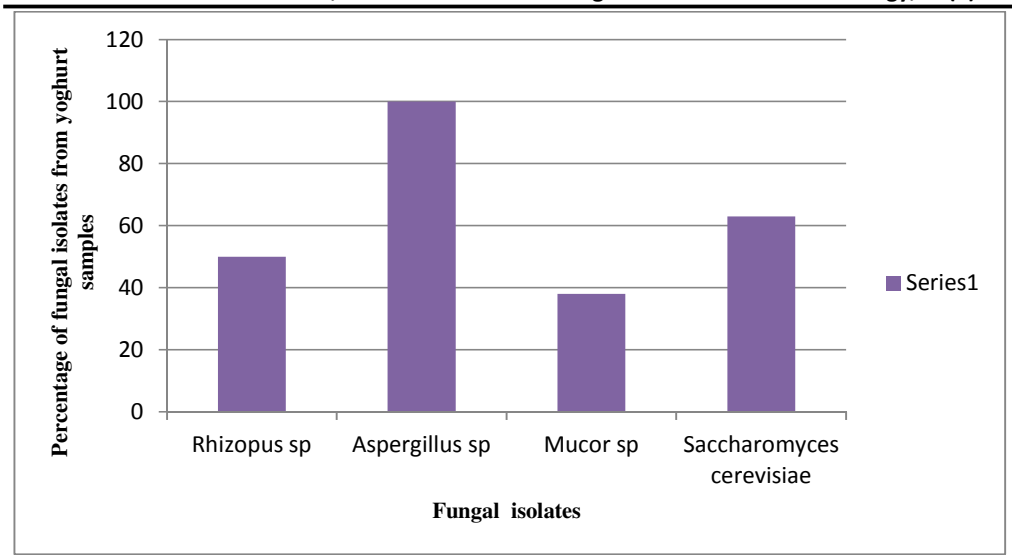

Figure 2. Total percentage occurrence of fungal isolates from the yoghurt samples

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