GLOBAL JOURNAL OF BIOLOGY, AGRICULTURE & HEALTH SCIENCES
(**Published By**: Global Institute for Research & Education)
www.gifre.org

Microbiological analysis of some locally produced fruit wine in Nigeria

[1]Oladipo I.C., [1]Akinsola O.E. & [1]Ojerinde B.O.
[1]Department of Science Laboratory Technology, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria

Abstract

This study was aimed at evaluating the level of bacterial contamination in some fruit wine produced in Nigeria and to determine the antibiotic susceptibility and physiological profile of the bacterial contaminants. Thirteen bacteria isolates were recovered from the different local fruit wine. The total bacteria colony count of the samples ranged from 12 x 10^7 to 90 x 10^7. The antibiotic susceptibility profile of the isolates was determined and 97% sensitivity to the clinically relevant antibiotics was noted while 3% resistance was found. Physiological parameters including temperature, pH, benzoic acid and sodium chloride were found to have profound effect on the growth rate of isolates. The results of this study showed that most of the fruit wine samples examined did not meet bacteriological quality standards. Hence, it is recommended that regular monitoring of fruit wine production quality should be carried out by putting in place appropriate agency.

Keywords: Wine, benzoic acid, pH, temperature, bacterial pathogens.

1.0 Introduction

Fruits are very essential for our health and from ancient time fruits are used for the production of different alcoholic beverages like wine (Joseph, 2010). The term wine is applied to the product made by alcoholic fermentation from fruits or fruit juices using yeast as inoculums (Joseph, 2010). Wine is considered to be one of the oldest alcoholic beverages produced by the process of fermentation. Fermentation is relatively low energy preservation process which increases the shelf life and decreases the need of refrigeration or any other forms of food preservation technology (Kourkoutas *et al.*, 2005).

Wine has as long history of use in the World of medicine and health. Wine and health is an issue of considerable discussion and research. Wine has a long history of use as an early form of medication, being recommended variously as a safe alternative to drinking water, an antiseptic for treating wounds and a digestive aid, as well as a cure for a wide range of ailments from lethargy and diarrhea to easing the pain of childbirth (Harding, 2005). Heavy wine consumption has been shown to have a damaging effect on the cellular processes that create bone tissue and long term consumption at high level increases the frequency of fractures (Walker-Bone, 2012). Wine benefit regarding cancer has been centered on the anti-oxidant properties of resveratrol found in grapes; in some laboratory result showing a protective quality that inhibit cancerous changes in cells. Some studies suggest that moderate wine consumption may lower the risk of lung (Wilbert, 2008), ovarian and prostate cancer (Robinson, 2006).

Fruits wine generally have relatively high levels of sugar and a low pH and this favors growth of yeasts, molds and some acid-tolerant bacteria. Spoilage may be manifested as surface pellicles or fibrous mats of molds, cloudiness, and off-flavors. Lack of oxygen in bottled and canned drinks limits mold growth. *Saccharomyces* and *Zygosaccharomyces* are resistant to thermal processing and are found in some spoiled wines (Silva and Gibbs, 2004). *Alicyclobacillus* species, an acidophilic and thermophilic spore-forming bacteria, has emerged as an important spoilage microbe, causing a smoky taint and other off-flavors in pasteurized wines (Chang and Kang, 2004; Chen *et al.*, 2006; Siegmund and Pollinger, 2006). *Propionibacterium cyclohexanicum*, an acid-tolerant non-spore forming bacterium also survives heating and grows in a variety of fruit wines (Walker and Phillips, 2007).

Unhygienic water for wine preparation, dressing with ice, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust can be major sources of microbial contaminants. Changes in pH may also promote the growth of pathogens (FDA, 2001). Presence of undesired microbes during different stages of winemaking can produce volatile acidity, off-flavors and polysaccharide hazes, all of which can diminish the quality and acceptability of the final product (Sponholz, 1993). And if released to the market can be deleterious to consumer's health. Therefore, this research is targeted towards microbial analysis of some wines produced in Nigeria in order to gain a better understanding of the sources of microbial contaminant in wines and the contaminants physiological properties.

2.0 Materials and Methods

2.1 Sample collection

Six different local fruit wines were purchased in Ogbomoso town, Oyo State, Nigeria. The samples were taken to the laboratory immediately for analysis.

2.2 Culture media

The media used in this study included MacConkey Agar, Nutrient Agar and Nutrient Broth. These were prepared according to the manufacturer's specification and sterilized at 121^{0}C for 15 minutes except otherwise stated.

2.3 Isolation procedures

One milliliter of each wine sample was serially diluted, 1ml of an appropriate dilution was inoculated on nutrient and MacConkey agar plates and the plates were incubated for 24 hours at 30^{O}C. After 24 hours sterile wire loop was used to pick the isolate from the plate and was streaked on a freshly prepared sterile nutrient agar and MacConkey agar plates, then incubate for 24 hours at 30^{O}C in order to get pure colonies. Pure cultures were then stored in a refrigerator at 4^{O}C. The routine laboratory method of Cruickshank *et al*. (1975) was used to characterize different isolates. The isolates were identified using their macroscopic, cultural, physiological and biochemical characteristics.

2.4 Isolation of microorganism

A seven-fold serial dilution was made for the fruit wine samples in appropriate dilution tubes. From the seven-fold dilutions of the homogenates; 1ml each from each 10^{-7} homogenates was pipette and introduced into the Petri dishes containing prepared solidified agar which included Nutrient agar and MacConkey agar. The plates were rocked gently to allow even distribution and the plates were then incubated at 37^{0}C for 24hours (Oladipo *et al*., 2014).

2.5 Total Colony Count

One milliliter of each sample was dissolved in sterile de-ionized water and serially diluted. One milliliter of appropriate dilutions was seeded on plate count agar using spread plate method, and the medium was then incubated at 30°C for 24 hours. The plate count agar was examined and colonies present were counted and recorded after incubation at 30°C for 24 hours, to get the total colony count in CFU/mL.

2.6 Determination of antibiotic susceptibility profile

The antibiotic susceptibility of the bacterial species isolated was performed on Müller-Hinton agar. Then, 0.1 mL of each bacterial isolates (10^{5}-10^{6} cells/mL**)** was seeded into each of the Petri dishes containing sterile Müller-Hinton agar and were allowed to stand for 30 minutes to enable the inoculated organisms to pre-diffuse. The commercially available discs containing the following antibiotics were aseptically placed on the surfaces of the sensitivity agar plates with a sterile forceps and were incubated at 30°C for 24 h. Zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimeters (NCCLS, 2001).

2.7 Physiological studies

2.7.1 ***Effects of different pH range on the isolates***

Nutrient Broth was prepared and the pH was adjusted using 0.5M sodium hydroxide (NaOH) and hydrochloric acid (HCl) to 9.0, 7.0, 3.0, and 1.0. It was then dispensed into screw-capped bottles and then sterilized at 121^{0} C for 15minutes. After cooling the selected test isolates were inoculated into it and incubated at 30^{0}C for 24hours. Growth was detected by increased turbidity using cecil 2031 automatic spectrophotometer. Uninnoculated tube served as control (Oladipo *et al*., 2009).

2.7.2 ***Survival of the isolates at different temperature ranges***

Nutrient Broth was prepared and dispensed into series of screw-capped bottles each containing 9ml and sterilized. After cooling the selected test isolates were inoculated into it and incubated at 40^{0}C, 50^{0}C, 60^{0}C, 70^{0}C for 24hours after which cecil 2031 automatic spectrophotometer was used to detect increase or decrease in turbidity. Un-innoculated tubes served as control (Oladipo *et al*., 2009).

2.7.3 ***Growth of the isolates in different concentration of sodium chloride***

Nutrient broth was prepared containing different concentration of (4, 8, 12 and 16% w/v) sodium chloride. It was then dispensed into screw-capped bottles and sterilized in an autoclave for 15minutes at 121^{0}C. After cooling, the selected test isolates were inoculated into it and incubated for 24 hours at 30^{0}C. Growth was detected using cecil 2031 automatic spectrophotometer. Un-inoculated tube served as control (Oladipo *et al*., 2009).

2.7.4 ***Survival of isolates in different concentration of benzoic acid***

Nutrient broth was prepared containing different concentration of (500mg, 1000mg, 1500mg and 2000mg) benzoic acid. It was then dispensed into screw-capped bottles and sterilized in an autoclave for 15minutes at 121^{0}C. After cooling, the selected test isolates were inoculated into it and incubated for 24hours at 30^{0}C. Growth was detected using cecil 2031 automatic spectrophotometer. Uninoculated tube served as control (Oladipo *et al*., 2010).

3.0 Results

The total bacteria count for various wine samples is as shown in Table 1; SGD showed the lowest colony count of 12×10^{7} while VRG showed the highest colony count of 90×10^{7}. The distribution of the bacterial isolates is shown in Table 2 and the most predominant organism was found to be *Aeromonas hydrophila*. A total number of 13 organisms were isolated from six local fruit wine samples. The isolates were initially differentiated on the basis of the cultural and morphological studies after which they were subjected to various biochemical tests such as oxidase test, oxidase test Gram staining, Gelatin hydrolysis, catalase test e.t.c. and were identified with the aid of Bergey's Manual of Systematic Bacteriology. The isolates were identified to be *Pseudomonas fluorescens* (2), *Bacillus cereus* (1), *Enterobacter aerogenes* (1), *Aeromonas hydrophila* (4), *Pseudomonas putida* (3), *Flavobacterium aquatile* (1) and *Bacillus pumilus* (1).

Table 1: Total bacteria colony Counts of the Isolates

Sample	Total colony count (CFU/mL)
SGD	12×10^7
SLN	38×10^7
VWG	45×10^7
CLA	58×10^7
CHS	62×10^7
VRG	90×10^7

CFU/mL- Colony forming unit per milliliter

Table 2: Distribution of the bacteria isolates in different samples

Isolates	Samples					
	SGD	SLN	VWG	CLA	CHS	VRG
Bacillus cereus	-	-	+	-	-	-
Aeromonas hydrophila	+	-	+	+	-	+
Bacillus pumilus	-	-	+	-	-	-
Flavobacterium aquatile	-	+	-	-	-	-
Enterobacter aerogenes	+	-	-	-	-	-
Pseudomonas putida	+	-	+	+	-	-
Pseudomonas fluorescens	-	-	-	-	+	+

The antibiotic susceptibility pattern of Gram-positive isolates shows that both *Bacillus cereus* and *Bacillus pumilus* were susceptible to all the antibiotics (Table 3). The antibiotic susceptibility test pattern of Gram-negative isolates shows that *Enterobacter aerogenes, Pseudomonas putida* and *Flavobacterium aquatile* were all susceptible to all the antibiotics. *Pseudomonas fluorescens* was resistant to only streptomycin but sensitive to other antibiotics. *Aeromonas hydrophila* was resistant to only septrin but susceptible to other antibiotics (Table 4).

Table 3: Antibiotic sensitivity profile for Gram-positive isolates

Isolates	**Antibiotics**									
	S	**CPX**	**R**	**AM**	**Z**	**APX**	**CN**	**PEF**	**E**	**SXT**
Bacillus cereus	24.0	24.0	24.0	21.0	24.0	20.5	24.0	24.0	24.0	24.0
Bacillus pumilus	24.0	24.0	24.0	24.0	24.0	21.0	24.0	24.0	24.0	23.5

KEY: S – Streptomycin (30µg), CPX - Ciprofloxacin (10µg), R – Rocephin (25µg), AM – Amoxicillin (30µg), Z - Zinnacef (20µg), APX – Ampiclox (30µg), CN – Gentamycin (10µg), PEF – Pefloxacin (10µg), E – Erythromycin (10µg), SXT – Septrin (30µg), R - Resistant. Diameter of zones of inhibition was measured in millimeter

Table 4: Antibiotic sensitivity profile for Gram-negative isolates

Isolates	**Antibiotics**									
	S	**OFX**	**PEF**	**CN**	**AU**	**AM**	**CPX**	**SP**	**CH**	**SXT**
Pseudomonas fluorescens	R	24.0	24.0	24.0	22.0	19.5	23.0	23.0	22.0	22.5
Enterobacter aerogenes	21.0	21.5	21.5	20.5	21.0	20.0	23.5	22.0	22.0	20.5
Pseudomonas putida	24.0	24.0	24.0	23.0	22.5	16.5	24.0	24.0	21.5	20.5
Flavobacterium aquatile	22.5	24.0	24.0	24.0	24.0	21.5	24.0	24.0	24.0	24
Aeromonas hydrophila	22.0	21.5	21.5	21.0	17.5	21.0	22.0	21.5	22.0	R

KEY: S – Streptomycin (30µg), OFX – Tarivid (10µg), PEF – Pefloxacin (30µg), CN – Gentamycin (10µg), AU – Augmentin (30µg), AM – Amoxacillin (30µg), CPX – Ciprofloxacin (10µg), SP – Sparfloxacin (10µg), CH – Chloramphenicol (30µg), SXT – Septrin (30µg), R - Resistant . Diameter of zones of inhibition was measured in millimeter

The effect of the different range of temperature on the growth of isolates was determined and it was observed that as temperature increased from 40^0C to 70^0C, the rate of growth of isolates decreased. *Bacillus cereus* decreased from 0.513 to 0.069 and *Enterobacter aerogenes* decreased from 0.753 to 0.161 (Figure 1). The effect of the different pH ranges on the growth of the isolates is shown in Table 5 and it was noted that as the pH of the medium increased from 1 to 9, the rate of growth of the isolates also increased. *Bacillus pumilus* increased from 0.138 to 0.762 and *Pseudomonas putida* increased from 0.118 to 0.444.

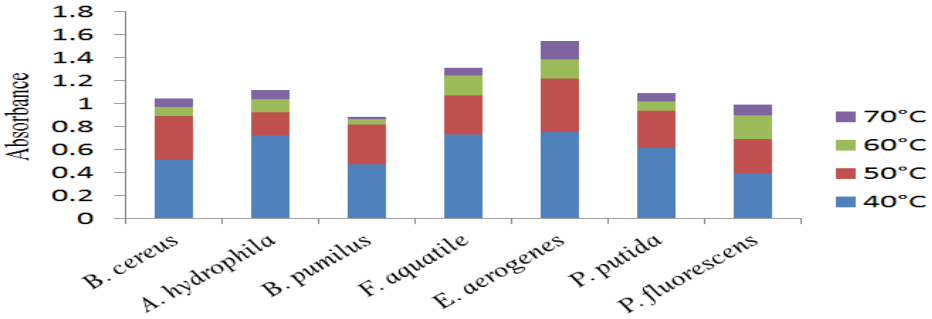

Figure 1: Rate of growth of isolates at different temperature (OD at 580 nm)

Table 5: Growth rate of isolates at different pH ranges (OD at 580nm)

Isolate	pH range			
	1.0	**3.0**	**7.0**	**9.0**
Bacillus cereus	0.112	0.126	0.160	0.621
Aeromonas hydrophila	0.148	0.191	0.339	0.636
Bacillus pumilus	0.138	0.157	0.177	0.762
Flavobacterium aquatile	0.103	0.157	0.164	0.189
Enterobacter aerogenes	0.110	0.140	0.160	0.653
Pseudomonas putida	0.118	0.140	0.165	0.444
Pseudomonas fluorescens	0.118	0.136	0.158	0.693

The optical density reading of the growth of isolates in different concentration of sodium chloride decreased as the concentration of sodium chloride increased. *Flavobacterium aquatile* decreased from 0.674 to 0.180 with increased concentration of sodium chloride from 4% to 16% (Figure 2). The effect of the different concentrations of benzoic acid on the growth of the isolates is shown in Table 6 and it was noted that as the concentration of benzoic acid increased from 500 to 2000mg/mL, the rate of growth of the isolates decreased. *Aeromonas hydrophila* decreased from 0.182 to 0.139.

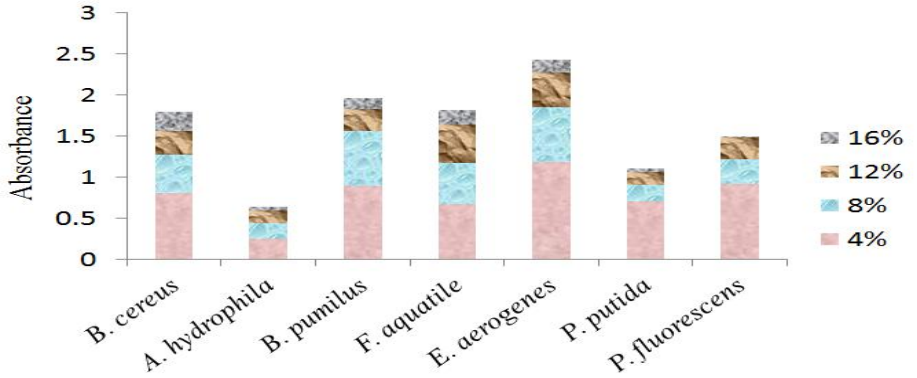

Figure 2: Rate of growth of isolates at different concentration of sodium chloride (OD at 580nm)

Table 6: Effect of different concentration of benzoic acid on isolates (OD at 580nm)

Isolate	Benzoic acid (mg/L)			
	500	**1000**	**1500**	**2000**
Bacillus cereus	1.315	0.137	0.109	0.107
Aeromonas hydrophila	0.182	0.159	0.152	0.139
Bacillus pumilus	0.163	0.144	0.117	0.102
Flavobacterium aquatile	0.426	0.155	0.154	0.141
Enterobacter aerogenes	0.425	0.338	0.247	0.212
Pseudomonas putida	0.299	0.223	0.127	0.110
Pseudomonas fluorescens	0.306	0.170	0.125	0.103

4.0 Discussion

The samples collected (fruit wine) were contaminated with *Bacillus cereus*, *Aeromonas hydrophila*, *Bacillus pumilus*, *Flavobacterium aquatile*, *Enterobacter aerogenes*, *Pseudomonas putida* and *Pseudomonas fluorescens*. All these were present in different proportion. The distribution of the bacterial isolates in the samples shows that *Aeromonas hydrophila* was the most predominant. *Bacillus cereus* is an opportunistic human pathogen and is associated with infections, causing periodontal diseases and other more serious infections (Wijnands *et al.*, 2006) through ingestion of food. *Aeromonas hydrophila* is also an opportunistic pathogen, meaning they rarely infect healthy individuals and its pathogenicity in humans has been recognized for decades (Hayes, 2006). It can cause gastroenteritis in human, myonecrosis and eczema in people with compromised or suppressed immune systems (FDA, 2006). The pathogenicity of *Flavobacterium aquatile* is not well understood; in humans, however, they cause neonatal meningitis, catheter-associated bacteremia, and pneumonia and have also been associated with some cases of advanced human immunodeficiency virus

disease (Manfredi *et al.*, 1999; Siegman-Igra *et al.*, 1987). *Enterobacter aerogenes* is a nosocomial and pathogenic bacterium that causes opportunistic infections including most types of infections. It has been found to live in various wastes, hygienic chemicals and soil (FPA, 2007). *Bacillus pumilus* causes food intoxications to humans which have toxic effects on epithelial cells. The symptoms that resulted from infection included dizziness, headache, chills, back pain, stomach cramps and diarrhea (Hormazabal and Granum, 2007). *Pseudomonas putida* is found in most soil and water habitat where there is oxygen. This bacterium is associated with pneumonia, enteritis, vaginitis and mastitis (Marcus, 2003; Kowalski, 2002). *Pseudomonas fluorescens* can be found in the soil and in water. *Pseudomonas fluorescens* is an unusual cause of disease in humans, and usually affects patients with compromised immune systems (e.g. patients on cancer treatment).

Contamination of fruit wines with these bacteria may develop from soil during cultivation of fruit, mechanical damage caused by methods used during harvesting, packaging and inadequate transportation which can lead to tissue wounds, abrasion, breakage, squeezing and escape of fruits which may increase susceptibility to decay and growth of microorganisms (Holdsworth, 1989). It could also be as a result of using unhygienic water for dilution, prolonged preservation, unhygienic surroundings often with swarming houseflies and fruit flies and airborne dust (Sandeep *et al.*, 2004).

The antibiotic susceptibility test shows that most of the isolates were sensitive to all antibiotics used, the two gram positive isolates were both sensitive to clinically relevant antibiotics used while Gram negative isolates were 96% sensitive and 4% resistant; this indicates that infection arising from the different samples of wine can be treated with the all the antibiotics used.

The result obtained from the test of the effect of temperature on the isolates indicates that as the temperature increased, the growth of isolates decreased. Adams and Moss (2008) reported that *Bacillus cereus* grows at 10^{o}C to 50^{0}C with an optimum between 28^{0}C and 35^{0}C (Johnson, 1984). This might be the reason for great reduction of the optical density reading as the temperature increased from 40^{0}C to 70^{0}C. Significant decrease in growth rate with increase in temperature implies that the microorganisms are mesophiles because they all grow and metabolize best at 40^{0}C (Madigan *et al.*, 1997). At extreme temperature the native structure of the organisms were compromised and became inactive (Breaker and Ronald, 2000).

The survival rate of the isolates at different pH ranges shows that the growth of the organisms increased as the pH of the medium changed from acidity to alkalinity. The pH values however, fell within the alkaline range for the observed maximum growth of the organisms. This indicates that organisms thrive best in alkaline medium than in acidic medium (Frazier and Westhoff, 1986). This result supports the findings of Ryu and Beuchat (1998) who reported increase in the rate of survival of the isolates at alkaline pH.

Growth rate of all the isolates was found to decrease as the concentration of sodium chloride increased, this result is in accordance with the previous work of Boles *et al*. (2000), who reported that the concentration of sodium chloride reduced microbial growth rate. Increase in concentration of sodium chloride reduced the available water present tying up some of the water and thereby reduced the rate of microbial growth.

The growth of the organisms decreased with increased concentration of benzoic acid and this is supported by (Ihekoronye and Ngoddy, 1995), who reported that preservatives are used to store food substances and they act by inhibiting microbial growth. Preservative may be bactericidal and kill target organisms or bacteriostatic which may prevent their growth and thus improve shelf-life of the product (Fawole and Osho, 2002).

5.0 Conclusion

From the data presented in the current study, it can be concluded that the microbiological quality of fruit wine samples made from local fruits were not satisfactory as *Pseudomonas* species, *Enterobacter aerogenes*, *Bacillus* species, *Aeromonas hydrophila* and *Flavobacterium aquatile* were detected from the samples. The lack of knowledge on safe fruit wine preparation as well as the contamination source can contribute to the elevation of pathogens in prepared wine. It is therefore essential for the people who handle and prepare wines, to be properly trained on safe fruit handling techniques. Regular monitoring of the quality of fruit wines for human consumption is recommended to avoid any bacterial pathogen outbreak.

References

Adams, M.R. & Moss, M.O. (2008). Bacterial agents of food borne illness in food microbiology. 3rd edition, RSC, Surrey, UK, pp. 182 -269.

Boles, J. A., Rathgether, B. M. & Shand, P. J. (2000). Staphylococcus in salted meat product. Journal of meat science. 55, 222-231.

Chang, S.S. & Kang, D.H. (2004). *Alicyclobacillus* spp. in the fruit wine industry, history, characteristics and current isolation/detection procedures. *Crit Rev Micobiol* 30, pp. 55-74.

Chen, S.Q., Tang, Q.Y., Zhang, X.D., Zhao, G.H., Hu, X.S., Liao, X.J., Chen, F., Wu, J.H. & Xiang, H. (2006). Isolation and characterization of thermo-acidophilic endospore-forming bacteria from the concentrated apple wine processing environment. *Food Microbiol* 23, pp. 439-445.

Cruickshank, R., Duguid, J. P., Marmion, B. P., & Swainr, H. A. (1975). Medical Microbiology, vol. 2, The Practice of Medical Microbiology. Edinburgh, London and New York: Churchill Livingst one.

Fawole, M.O. & Osho, B.A. (2002). Laboratory Manual of Microbiology. Spectrum Books LTD, Nigeria, pp6 – 45.

Food and Drug Administration. (2001). In Bacteriological Analytical Manual online, USA. Chapter 12 pp. 1-6.

Food and Drug Administration. (2006). Statement on causes of *Aeromonas hydrophila* in people with compromised immune systems

Food Protection Agency. (2007). Pathogenicity of *Enterobacter aerogene* ,FPA. pp. 24-43.

Frazier, W.C. & Westhoff, D.C. (1986). Food infections and intoxications from Microbiology, 3rd Edn. Zara goza Editorial Acribia S.A., pp 413.

Harding, G. (2005). "A Wine Miscellany" Clarkson potter publishing, New York ISBN 0-307-34635-8. pp. 66-70 & 108.

Hayes, J. (2006). "*Aeromonas hydrophila*". Oregon State University.

Hormazabal, V. & Granum, P. (2007.)"Food poisoning associated with pumilacidin-producing Bacillus pumilus in rice" International Journal of Microbiology 115, pp. 319-324.

Holdsworth, S.D. (1989). The preservation of fruit and vegetable food products. Science in Horticulture Products. Macmillian Press, London, pp. 109-110.

Ihekoroye, A.I. & Ngoddy, P.O. (1985). Integrated Food Science and Technology for the Tropics. Macmillan Educational LTD, pp. 121-147.

Johnson, K.M. (1984). Bacillus cereus food borne illness - An update. *Journal of food protection*. 47, 145 -153

Joseph, A.U. (2010) Comparative studies of Wine produced by spontaneous and controlled Fermentation of preserved Cashew (*Anarcardium occidentale*) juice.

Kowalski, H. (2002). "U.S.-German Research Consortium Sequences Genome of Versatile Soil Microbe". J. Craig Venter Archive.

Kourkoutas, Y., Kanellaki, M., Koutinas, A.A., & Tzia, C. (2005). Fermentation as a relatively low energy preservation *Journal Food Eng*. 69, pp.115-123.

Madigan, M. T., Martinko, J.M. & Parker, J. (1997). Brock Biology of Microorganisms. Prentice Hall, 8th edition, New Jersey, USA, pp. 968

Malik, A. & Ahamed, M. (1994). Evidence of drug and metal resistance in Escherichia coli strains from sewage water and soil. *Chem.*

Environ. Res. 3, pp.3-11

Marcus, A. (2003). "Versatile soil-dwelling microbe is mapped". Genome News Network.

Manfredi, R., Nanetti, A., Ferri, M., Mastroianni, A., Coronado, O. V., & Chiodo, F. (1999). *Flavobacterium* spp. organisms as opportunistic bacterial pathogens during advanced HIV disease. *J. Infect.* 39, pp.146-152.

National Committee for Clinical Laboratory Standards. 2001. Performance standard for antimicrobial susceptibility testing; 12th *Informational Supplement. NCCLS document M100-S12*. National Committee for Clinical Lab. Standards, Wayne, Pa.

Oladipo, I.C, Onyenike, I. C. & Adebiyi, A.O. (2009). Microbiological analysis of some vended sachet water in Ogbomoso, Nigeria.

African Journal of Food Science 3(12), pp. 406-412.

Oladipo, I.C, Adeleke, D.T. & Adebiyi, A.O. (2010). The Effect of pH and chemical preservatives on the growth of Bacterial

isolates from some Nigerian Packaged fruit Juices. *Pakistan Journal of Biological Science* 13(1), pp. 16- 21.

Oladipo, I.C., Atolagbe, O.O. & Adetiba, T.M. (2014). Nutritional evaluation and microbiological analysis of yoghurt produced from full cream milk, tiger-nut milk, skimmed milk and fresh cow milk. *Pensee Journal* 76 (4), pp. 30-38

Robinson, J. (2006). "The Oxford Companion to Wine". Oxford University Press ISBN 0-19-860990-6. Third Edition pp. 433

Ryu, J. H. and Beuchat, L. R. (1998). Influence of acid tolerance responses on survival, growth and thermal cross-protection of

Escherichia coli O157:H7 in acidified media and fruit juices. *Int. J. Food Microbiol*. 45, pp. 185 -193

Sandeep, P.V., Micalizzi, B. & Stefanini de Guzman, A.M. (2004). Some bacteria associated with fruit wine. *Journal of Food*

Production 53(12), pp. 1069-1072.

Siegman-Igra, Y., Schwartz, D., Soferman, G. & Konforti, N. (1987) *Flavobacterium* group IIb bacteremia: report of a case and review of Flavobacterium infections. *Med Microbiol Immunol.* 176, pp. 103-111.

Siegmund, B. & Pollinger-Zierler, B. (2006). Odor thresholds of microbially induced off-flavor compound in apple wine. *J. Agr. Food Chem* 54, pp. 5984-5989.

Silva, F.V.M. & Gibbs, P. (2004). Target selection in designing pasteurization processes of shelf-stable high-acid fruit product. *Crit. Rev. Food Sci. Nutr.* 44, pp. 353-360.

Sponholz, W. (1993). Spoilage by wine microorganisms. In Fleet, G.H. (ed), Wine Microbiology and Biotechnology, Harwood Academic Publishers, Switzerland, pp. 395-420.

Walker-Bone, K. (2012). Recognizing and treating secondary osteoporosis. *Nat Rev Rheumatol* 8(8), pp. 480-92.

Walker, M. & Phillips, C.A. (2007). The growth of propionibacterium cyclohexanicum in fruit wines and its survival following elevated temperature treatments. *Food Microbiol* 24, pp. 313-318.

Wilbert, C. (2008). "Red Wine may cut risk of lung cancer" WebMD.

Wijnands, L., Dufrenne, J., Zwietering, M.H., & Leusden, F. (2006). "Spores from mesophilic *Bacillus cereus* strains germinate better and grow faster in simulated gastrointestinal conditions than spores from psychotropic strains". *International Journal of Food Microbiology*. 112 (2), pp.120-128.