ORIGINAL ARTICLE

EFFECT OF LACTIC-ACID FERMENTATION ON THE SHELF LIFE OF VEGETABLES

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| Received 09 September 2019 | | Accepted 10 October 2019 | | Published 17 October 2019 | | ID Article| Ebah-Ref.1-ajira8-290919|

ABSTRACT

Background: Fermentation has been a crucial food processing technology for prolonging shelf life of food before refrigerators were invented and is still used but mostly in rural communities. It is however, mostly based on raw materials such as grains and starchy tubers. Not much is known about vegetable Fermentation in Nigeria. **Objective**: This study was aimed at investigating the effect of lactic acid fermentation in increasing the shelf life of vegetables. **Methods**: Fermented vegetables were cultured using the pore plate technique. **Results**: Lactic acid fermentation of vegetables most especially cabbages has been shown to be dominated by species of *Lactobacillus, Leuconostoc*, andin some cases *Pediococcus*. In this study, bacteria of the species *Lactobacillus, Leuconostoc, Pediococcus, Streptococcus, Pseudomonas, Staphylococcus* and *Gluconobacter*were isolated from cabbage, carrots and tomatoes which had been shredded and packed in air tight jars for fermentation. A rapid decrease in pH coincided with a rapid increase in number of lactic acid bacteria and decrease in aerobic bacteria. The microbial load was found to vary between 1.6 x 10^6 and 3.0 x 10^8 for cabbage, 5.0 x 10^6 and 1.4 x 10^8 for carrots, 7.0 x 10^5 and 6.8 x 10^7 for tomatoes. *Lactobacillus* spp. had a close range of occurrence on all the vegetables which constitute 42.4%, 42.9% and 46.5% on cabbage, carrots and tomatoes respectively while *Pediococcus* spp. frequency include 23.6% and 20.2% on cabbage and carrots respectively and *Staphylococcus* spp. on the same vegetables include 1.2% and 5.5% respectively. However, *Gluconobacter* spp. constituting 8.5% was isolated on tomatoes only. **Conclusion**: it was evident that Lactic acid fermentation is a cheap way of increasing shelf-life and reducing pathogenic organisms in vegetables.
Key words: *Fermentation, Shelf-life, Bacteria, Food, Microbiota, Lactic acid*

1. INTRODUCTION

Microbiology of fermentation of foods cannot be overemphasized as food fermentation has been used by humans to preserve foods and improve its aroma and digestibility for thousands of years. Therefore, fermented foods are part of diet of human beings all over the world [1,2]. It is apparent that before the invention of refrigerators, man had discovered fermentation as way of improving the shelf life of foods, improving its nutritional values and reducing its risk of food borne illnesses [3].

In many African countries, fermented foods are a major part of the daily diet. The investigation by Mathara and others revealed the importance of lactic acid fermentation in the traditional cultural diets of African people (Mathara *et al*., 2004). Aside lactic fermentation, other forms of fermentations include alkaline fermentations and mixed lactic acid and alcoholic fermentations [4]. Microorganisms allow fermentation of products when they consume the available organic substrate. But bacterial growth can bring about food spoilage as undesirable product while desirable products lead to fermentation which often used to improve food quality. Fruits and vegetables canhave alcoholic flavor while milk can assumed mildly acidic taste and before turning to became cheese; cabbage turned to sauerkraut [5].

Fruits are commonly processed for alcoholic fermentation of wine and beer as they are rich in sugars, vitamins and minerals. As juices are slightly acidic, they are therefore a suitable medium for the growth of yeasts, and fruit sugars are rapidly converted into ethanol. Vegetables on the other hand, have low sugar content but are rich in minerals, vitamins, have neutral pH and thus provide a natural medium for fermentation by LAB. Fermentation of fruits and vegetables can occur 'spontaneously' by the natural lactic acid bacterial surface microflora, i.e., *Lactobacillus*, *Leuconostoc*, *Pediococcus*, etc. However, the use of starter culture such as *lactobacillus plantarum*, *Lb. rhamnosus*, *Lb. gasseri* and *Lb. acidophilus* (all probiotic strains) provides consistency and reliability of performance [6].

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Vegetables can be preserved either when it still fresh or dry; but fresh vegetable has highest nutrient composition. In Nigeria, vegetables generally are available in open markets at a very cheap price especially during the rainy season and very scarce in dry season because farmers use artificial irrigation to grow the few vegetables for the rest of the year due to lack of water during the dry season [7, 5].

In addition, the high moisture in vegetables allows microorganisms to survive because high moisture in an environment increase the water activity which is the minimum amount of water require by microorganisms to thrive in an environment as a microbiota. Meanwhile, it has been seemingly impossible for the storage of vegetables for a long period due to the climatic conditions of many African countries [8]. As a result, there is a need for methods like fermentation after harvesting of vegetable; the method which will improve its processing for safety and stable supply in the markets throughout the year. Fermentation usually disrupts activities of microorganisms just as lactic acid fermentation of fruits and vegetables is very beneficial by the antimicrobial activity [9, 10, 5].

Lactic acid (LA) fermentation is considered a simple and useful form of biotechnology to keep and/or enhance the safety, nutritional, sensory and shelf life properties of vegetables and fruits [11]. Lactic acid bacteria (LAB) convertthe carbohydrate contents of the vegetables and fruits into Lactic acid, whichdecreases the pH of the fermented products to around 4.0 ensuring stability.Lower pH value restricts the growth of spoilage flora and pathogenicbacteria. These bacteria improve the human intestinal microbial balance andenhance health by inhibiting the growth of pathogens such as *Escherichiacoli*, *Salmonella* and *Staphylococcus* [12, 13].

Moreover, previous studies showed bacteria such as *Lactobacillus* do produce probiotic that are capable of prevention of human diarrhoea by temporary modification of the composition of the microbiota of the intestines and then improve the host immune system [14, 15, 1]. Lactic acid bacteria can produce organic acids to inhibit the growth of fungi therefore, they are important in safety of fermented foods, increasing the shelf life of foods and changing the composition of food by soften its texture, so that minimal amount of cooking time and energy is require to make it done [5, 15, 16, 17]. So this study was carried out with an aim to increase the shelf life of vegetables by means of lactic acid fermentation.

2. MATERIALS AND METHODS

2.1. AREA STUDY

This study was carried out in Makurdi, the Benue State Capital Nigeria. Vegetables were collected from Wurukum market and the University of Agriculture minimarket.

2.2. Sample Collection

Three different samples were used in this study: cabbage(*Cucumis sativus)*, tomatoes(*Lycopersicum esculentum)* and carrots (*Daucus carota)*. A head of cabbage and carrots were collect from Wurukum market, Makurdi and fresh tomatoes were collected from minimarket in University of Agriculture, Makurdi.

2.3.Fermentation Samples Preparation

Dry-Salted Fermented Vegetables**:**All the samples were washed and shredded evenly. Carrots and the cabbage were thinly cut to enable compact packing and air exclusion. Tomatoes were cut into larger chunks due to the possibility of the succulent vegetable dissolving in the brine if cut thinly. In a large bowl, for 25kg of vegetables 0.75 kg of salt was added. Salt extracts the juice from the vegetables and creates the brine, weight(stones) is placed to compress the vegetables and assists the formation ofbrine, which takes about 24 hrs. As soon as brine is formed, fermentationstarts and bubbles of CO_2 begin to appear.The cabbage was transferred into a jar and covered to allow it ferment in its juice. Brine-Salted Fermented fruits and vegetables: To the carrot and tomato, however, a brine solution of 0.75kg salt was dissolved in a cup of water and was added to completely cover the chopped vegetables respectively and each was left to ferment in a closed jar. 1ml of each sample was taken on a two day interval for serial dilution [18]. Note: Salting is an important step in vegetable fermentation. Sodium chloride concentration can range from 20 to 80 g/l during fermentation. LAB can tolerate high salt concentrations. This salt tolerance gives them an advantage over less tolerant species and allows LA fermentation that inhibits growth of non-desirable organisms [19]. Salt induces plasmolysis in plant cells which releases mineral salts and nutrients from the vacuole and creates anaerobic conditions for proper growth of LAB around the submerged product.

2.4.Media Preparation

All media used in the course of work were prepared in compliance with directives from the manufacturers. Nutrient agar, MRS agar, and MacConkey agar were the three media used in carrying out the study.

2.5. pH Determination

pH of all samples was determined using a pH meter after standardization with appropriate buffers.

2.6.Serial Dilution

A 5-fold serial dilution was carried out. 1ml of fermentation juice was taken from each of the three samples and diluted serially along test tubes labeled 10^0-10^5 for each sample respectively. 1ml of the 10^5 test tube was taken for inoculation for all samples.

2.7. Media Inoculation

Inoculation was by pour plate method. 1ml of the 10^5 test tube was transferred into three Petri dishes for each sample respectively. Prepared media which had all been allowed to cool to a low temperature without coagulating were poured over the inocula and swirled gently for proper distribution of inocula in Petri dishes. Inoculated Petri dishes were incubated for 24 hours.

2.8. Microbial LoadDetermination

The standard plate count method which is a direct way of counting was used to determine the number of bacterial cells on an agar plateas describe by Prescott *et al.*, 2002 and Stuart (2005) [20, 21]. Colonies appearing on the agar plates after incubation were counted and recorded. Total microbial count per milliliter was determined using the formula below [22].

$$\text{Number of bacterial cells in a sample} = \frac{\text{Number of colonies}}{\text{Volume plated x dilution factor}} \qquad \textbf{(1)}$$

2.9. Isolation of Bacteria

After 24 hoursof incubation at 37°C, distinct colonies with different morphological characteristics were taken and sub-cultured on nutrient agar for further identification.

2.10Characterization and Identification of Isolates

Bacterial isolates were identified using Gram staining and biochemical tests such as indole test, citrate test, urease test, oxidase test, catalase and sugar hydrolysis tests.

2. RESULTS

Table 1 shows the colony forming unit per ml cfu/ml of all the ferments taken on a two day interval. It shows an increase in the viable bacterial counts with increase in fermentation time.

A decrease in pH was observed in each sample with the progress of fermentation except in the tomatoes (Table 2).
In table 3, the most probable isolates from the three samples following morphological characteristics and results from biochemical tests performed on all isolates.

Table 1: The table presents total Bacterial Viable Counts (cfu/ml)of Fermenting Carrots, Cabbage, and Tomatoes on Nutrient Agar.

Day	Cabbage	Carrots	Tomatoes
2	$1.6x10^6$	$5.0x10^6$	$7.0x10^5$
4	$1.3x10^8$	$8.8x10^7$	$6.8x10^7$
6	$3.0x10^8$	$1.4x10^8$	$2.8x10^7$

Table 2: The table presents pH of Sauerkraut, Fermented Tomatoes and Carrots.

Fermentation Time (days)	Cabbage	Carrots	Tomatoes
1	5.2	6.0	5.0
2	3.78	3.42	3.4
3	3.4	3.40	3.38
4	3.35	3.34	3.37
5	3.34	3.16	3.37
6	3.33	3.14	3.38

Table 3: The table presents the frequency Percentage of Isolates from Each Sample

Isolate	Cabbage (%)	Carrots (%)	Tomatoes (%)
Lactobacillus spp.	42.4	42.9	46.5
Leuconostoc spp.	27.1	31.5	39.5
Pseudomonas spp.	2.5	4.2	-
Streptococcus spp.	4.4	-	-
Pediococcus spp.	23.6	20.2	-
Staphylococcus spp.	-	1.2	5.5
Gluconobacter spp.	-	-	8.5

- : Absent

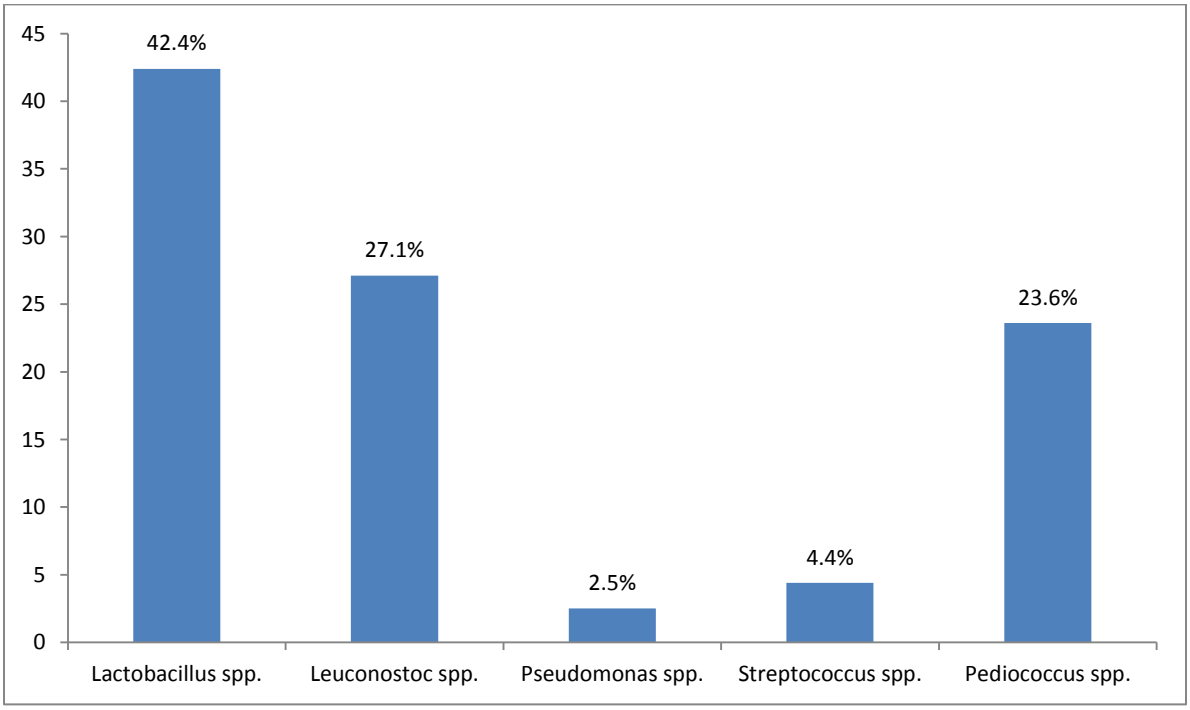

Figure 1: The figure presents the percentage of Fermentation Microbes Isolated from Cabbage.

Lactobacillus spp. are the most probable microbes on Cabbage which consist of 42.4%, followed by *Leuconostoc* spp. which consist of 27.1% and *Pediococcus* spp. which also consist of 23.6% while the least probable organisms include *Streptococcus* spp. and *Pseudomonas* spp. which consist of 4.4% and 2.5% respectively.

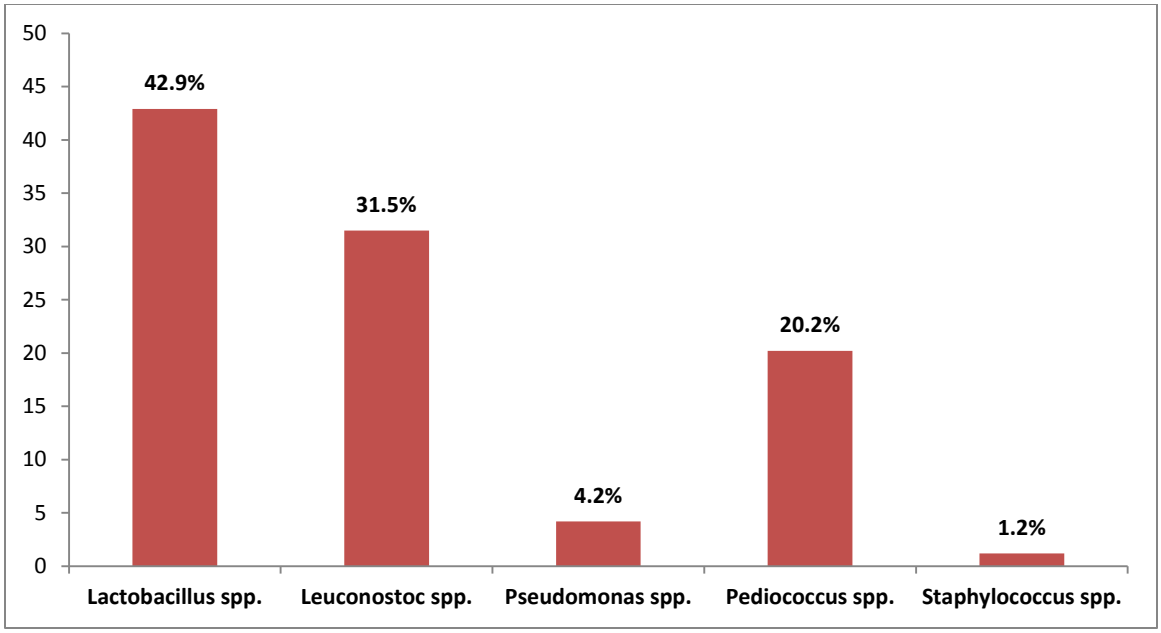

Figure 2: The figure presents the percentage of Fermentation Microbes Isolated from Carrot.

The Percentage of probable microbes on carrot in Figure 2 shows *Lactobacillus* spp. (42.9%), as the most probable organism followed by *Leuconostoc* spp. (31.5%), *Pediococcus* spp. (20.2%) *Pseudomonas* spp. (4.2%) and the least organism which comprises*Staphylococcus* spp. (1.2%).

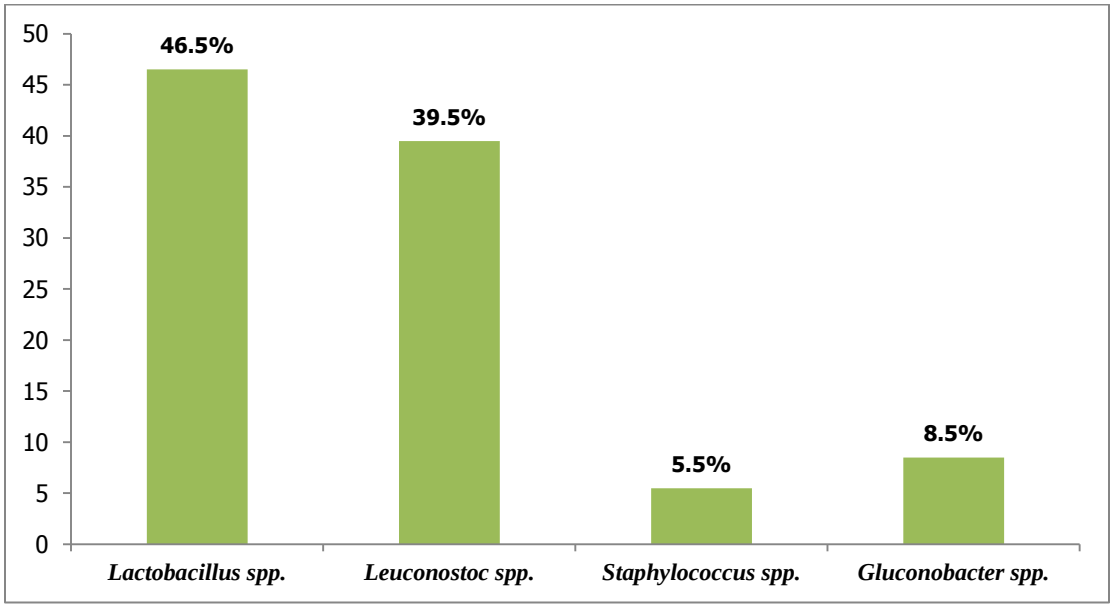

Figure 3: The figure presents the percentage of Fermentation Microbes Isolated from Tomatoes.

Figure 3 is the Percentage of probable microbes on tomatoes which include *Lactobacillus* spp. (46.9%), as the most probable organism, *Leuconostoc* spp. (39.5%), *Gluconobacter* spp. and the least probable microbe includes *Staphylococcus* spp. (5.5%).

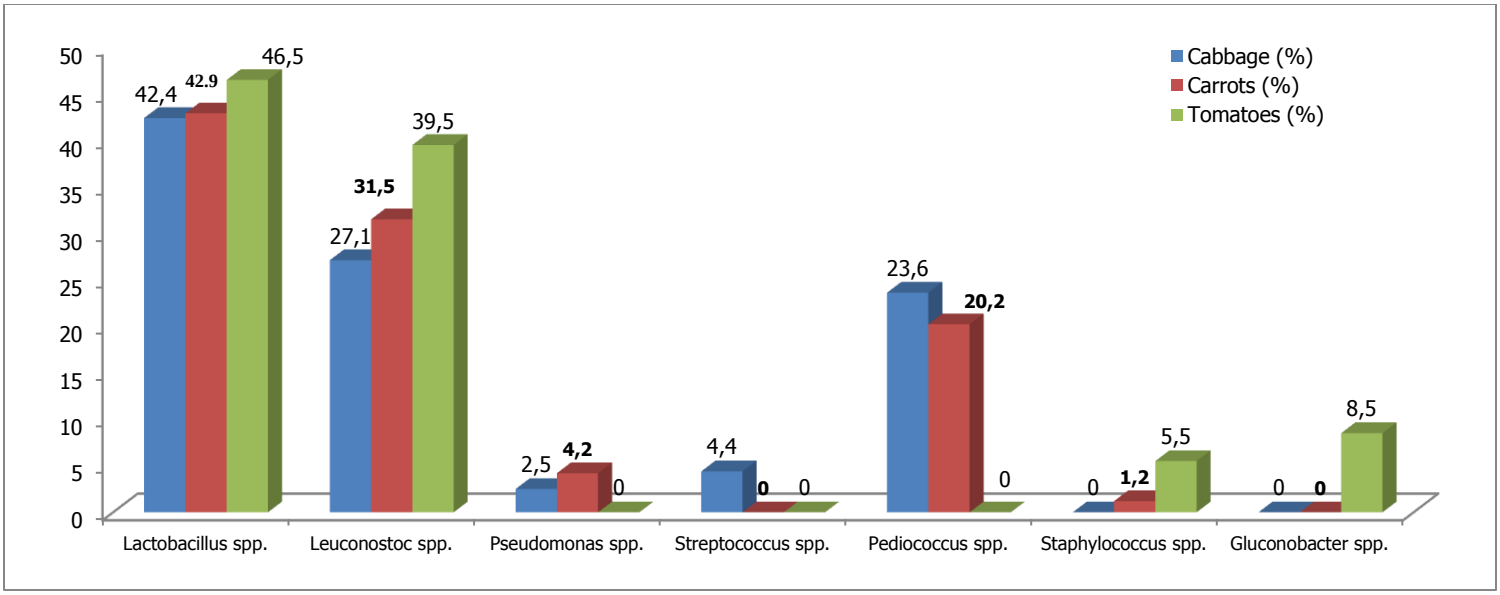

Figure 4: The figure presents the profile of Fermentation Microbes Isolated from Cabbage, Carrot and Tomatoes.

Lactobacillus spp. had a close range of occurrence on all the vegetables which constitute 42.4%, 42.9% and 46.5% on cabbage, carrots and tomatoes respectively. Also, cabbage, carrots and tomatoes, *Leuconostoc* spp. had frequency of 27.1%, 31.5% and 39.5% respectively. *Pseudomonas* spp. which consists of 2.5% and 4.2%was isolated only on cabbage and carrots respectively while *Streptococcus* spp. (4.4%) only isolated on cabbage. More also, *Pediococcus* spp. frequency include 23.6% and 20.2% on cabbage carrots respectively and *Staphylococcus* spp. on the same vegetables include 1.2% and 5.5% respectively. However, *Gluconobacter* spp. constituting 8.5% was isolated on tomatoes only.

4. DISCUSSION

In this study, the Lactic acid bacteria genera isolated include *Lactobacillus* spp., *Pediococcus* spp., *Leuconostoc* spp and *Streptococcus* spp which is similar to the ones described by Di Cagno *et al*. (2013) and Paramithiotis *et al*. (2010) [5, 23].The naturally occurring microbial load was found to vary between $1.6x10^7$ and $3.0x10^8$Cfu/mlfor cabbage, $5.0x10^6$ and $1.4x10^8$Cfu/ml for carrots and $7.0x10^6$ and $6.8x10^7$Cfu/ml for tomatoes. The lactic acid bacterial counts were found to increase from the start of fermentation and increasedup to the sixth day of fermentation. This was in agreement with the work of Doyle *et al*. (2001) who reported an increase in lactic acid bacterial counts favoured by complete lack of oxygen, lowered pH and elevated salt content [24].

Ray and Panda (2007) reported that certain bacteria are acid tolerant (i.e.*Lactobacillus* and *Streptococcus*) and can survive at reduced pH levels (3.0–4.0) [25]. Lactic acid bacterial counts of all samples increased constantly up to the sixth day except in the tomatoes. Rate of lactic acid bacterial growth was slower in tomatoes than in other vegetables with a decline in number of lactic acid bacteria on the sixth day. Decrease in number of lactic acid bacteria was followed by a slight rise in pH though an increase in number of aerobic bacteria was not readily detected. Rapid decline in pH shows there was an increase in acidity of fermenting samples due to lactic acid production by lactic acid bacteria.

Final pH of fermented cabbage (sauerkraut) was 3.33 in line with Jay *et al*. (2005) who suggested that the final pH of sauerkraut lies in the range of 3.1 and 3.7 [26]. Fermentation was dominated by *Lactobacillus* spp. closely followed by *Leuconostoc*spp. but highest was *Lactobacillus* spp. In agreement with Jay*et al.* (2005) and Doyle *et al.* (2001) who reported an immediate decrease in number of strictly aerobic bacteria due to increase in facultative anaerobic lactic acid bacteria and lack of oxygen, number of aerobic bacteria failed to increase in the course of fermentation because their decline [26, 24]. Further studies by some researchers had shown that lactic acid bacteria fermentation is a means of eradicating Gram-negative bacteria from food products [27, 28].

Bubbles seen in the fermentation jars were sign of fermentation. An increase in bubbles and a rise in volume of the fermentation juice to almost over flowing the jars especially in the fermenting cabbage was a good sign of vigorous fermentation which is in accordance with Liu *et al*. (2011) [17].

5.CONCLUSION

Vegetable fermentation is a way of preserving vegetables, so that leftover and surplus vegetables can be preserved in a cheap and economic way to stop their deterioration by microorganisms. *Pseudomonas* sppand *Staphylococcus* spp. are

normally get to vegetables from soil or contaminate vegetables as a result of human contact with food. Therefore, preservative method is necessary to get rid of these contaminants by fermentation which can be done by lactic acid bacteria which are beneficial bacteria. These bacteria are also consumed as probiotics. This study is timely as local communities need to be giving orientation on the important of fermented vegetables. In addition, government and non-governmental organizations should promote the development of the bio-fermentation technology to boost food safety and availability. In a nut shell, this study affirms that lactic acid fermentation is efficient to hinder the growth of contaminants which are often pathogenic or spoilage organisms.

6. REFERENCES

1. Barbara,P., Francesca, P., Sofia, C. M., Elisabetta, G., Charles, F. and Wilhelm, H. (2011). Diversity and functional properties of Lactobacillus plantarum-group strains isolated from Italian cheese products. *Dairy Science Technology, EDP sciences/Springer.* 91(1):65-76.
2. Franz, C.M.A.P., Cho, G.S., and Holzapfel, W.H. (2011). Probiotics: taxonomy and technological features. Chapter 1. In: Shah, Nagendra P., Gomes da Cruz,Adriano, de Assis Fonseca Faria, Jose (Eds.), Probiotic and Prebiotic Foods: Technology, Stability and Benefits to Human Health. Nova Science Publishers, Hauppauge, New York.pp1-24.
3. Mathara, J.M., Schillinger, U., Kutima, P.M., Mbugua, S.K. and Holzapfel W.H. (2004). Isolation, identification and characterization of the dominant microorganisms of kulenaoto: The Maasai traditional fermented milk in Kenya. *International Journal of Food Microbiology* **94**: 269–278.
4. Franz, C.M.A.P and Schillinger, U. (2010).Tea Coffee and Cocoa.*In: J. Tamang* (ed.) Fermented Foods and Beverages of the World.
5. Zabat, M.A.; Sano, W.H.; Wurster, J.I.; Cabral, D.J.; Belenky, P. (2018). Microbial Community Analysis of Sauerkraut Fermentation Reveals a Stable and Rapidly Established Community Foods. *7*:77.
6. Di Cagno, R., Coda, R., De Angelis, M. andGobbetti, M. (2013). Exploitation of vegetables and fruits through lactic acid fermentation.*Food Microbiology* 33:1–10.
7. Oyewole, O.B. (1997). Lactic fermented foods in Africa and their benefits. *Food Control*. 8:289-297.
8. Caplice, E. and Fitzgerald, G. F. (1999). Food fermentations: role of microorganisms in food production and preservation. *International Journal of Food Microbiology*.**73**:230-233.
9. Joshi, V.K. and Shiwali, T. (2000). Lactic acid fermented Beverages. IN: Postharvest Technology of Fruit Vegetables. Verma \lR and Joshi VK (/eds), Indus Publishing Co., New Delhi. 1102-1127.
10. Joshi, V.K., Sharma, S. and Neerja, R. (2006).Production, purification, stability and efficiency of bacteriocin from the isolate of natural lactic acid fermentation of vegetables.*Food Technol Biotechnol.***44** (3): 435-439.
11. Demir, N., Bahceci, K.S. and Acar, J. (2006). The effects of differential initial *Lactobacillus plantarum* concentrations on some properties of fermented carrot juice.*Journal of Food Processingand Preservation.* 30(3):352–363.
12. Ohmomo, S., Murata, S., Katayama, N., Nitisinprasart, S., Kobayashi, M., Nakajima, T., Yajima, M. and Nakanishi, K. (2000). Purification and some characteristics of enterocin ON-157, a bacteriocin produced by *Enterococcus faecium* NIAI 157.*Journal of Applied Microbiology.* 88:81–89.
13. Ross, R.P., Morgan, S. and Hill, C. (2002). Preservation and fermentation: past, present and future. International Journal of Food Microbiology 79(1-2): 3–16.
14. Holzapfel, W.H., Haberer, P., Snel, J., Schillinger, U., Huisin't, eld, J.H. (1998).Overview of Gutflora and Probiotics.*International Journal of Food Microbiology* 41:85–101.
15. Micheal, D. V., and Marteau, P. R. (2007). Probiotics and prebiotics: Effects on diarrhea. *Journal of Nutrition*, 137(3):803 -811.
16. Sauer, M., Porro, D., Mattanovich, D., andBranduardi, P. (2008). Microbial production of organic acids: expanding the markets. *Trends in Biotechnology* **26**:100-108.
17. Pot, B.and Tsakalidou, E. (2009). Taxonomy and metabolism of Lactobacillus, in Lactobacillus Molecular Biology: From Genomics to Probiotics, A. Ljungh and T.Wadstrom, Eds., Caister Academic Press. 3–58.
18. Liu, S.N., Han, Y. and Zhou, Z. J. (2011).Lactic acid bacteria in traditional fermented Chinese food.*Food Research International* 44:643–651.
19. Rao, M.S., Pintado, J., Stevens, W.F. and Guyot, J.P. (2004). Kinetic growth parameters of different amylolytic and non-amylolytic *Lactobacillus* strains under various salt and pH conditions.*Bioresource Technology*. 94(3):331–337.
20. Prescott, L. M., Harley, J. P. and Klein, D. A. (2002).Microbial Growth.General Microbiology (5th edition). McGraw-Hill, New York; 111-130.
21. Stuart, Hogg (2005). Essential Microbiology.*Microbial Genetics.The University of Glamorgan, UK.John Willey and Sons Limited.* 290
22. Harrigan,W.F. and McCance, M.E.(1976). Laboratory method in food and dairy Microbiology. Acadmic press INC. NewYork.U.S.A
23. Paramithiotis, S., Hondrodimou, O.L. andDrosinos, E.H. (2010). Development of the microbial community during spontaneous cauliflower fermentation.*Food Research International.* 43:1098–1103.
24. Doyle, M.P., Beuchat L.R. and Montville, T.J. (2001).*Food Microbiology*: Fundamentals and Frontiers, 2nd ed. ASM Press, Washington DC.
25. Ray, R.C. and Panda, S.H. (2007). Lactic acid fermented fruits and vegetables: an overview. *In*: M.V. Palino (ed.). Food Microbiology Research Trends. Nova Science Publishers Inc., Hauppauge, New York, USA. pp. 155–188.
26. Jay, J.M., Loessner, M.J., and Golden, D.A. (2005).Modern Food Microbiology. 7th ed. Springer, USA., 790.
27. Mensah, P. (1997). Fermentation: the key to food safety assurance in Africa. *Food Control*.**8**:271-278.
28. Motarjemi, Y. (2002). Impact of small scale fermentation technology on food safety in developing countries.*International Journal of Food Microbiology*.**75**:213-229.

Cite this article: Ebah, Esther Eneyi, Wusuum, Brenda, Akande, Titilayomi, Emmanuel, Olumuyiwa Onifade, Ikala, Ruth Ohie, and Ode, Tosin Adabola. EFFECT OF LACTIC-ACID FERMENTATION ON THE SHELF LIFE OF VEGETABLES. *Am. J. innov. res. appl. sci. 2019; 9(4):* 328-334