Fermentation rate monitoring in the production of African protein based condiments

Fermentation rate monitoring in the production of African protein based condiments

M. E. Ojewumi*[1], J.A. Omoleye [1], A.A. Ajayi[2], G.P. Ekanem[1]
[1]Covenant University, Km 10, Idiiroko Road, Canaan land, P.M.B. 1023, Sango Ota, Ogun state, Nigeria.
[2] Augustine University, P.M.B. 1010, Epe, Lagos.
*[1]Orcid: 0000-0002-9254-2450

Corresponding email: modupe.ojewumi@covenantuniversity.edu.ng

Abstract. Fermented African locust bean seeds can be used as substitute to chemically based food spices. It improves sensory properties of foods which includes the organoleptic characteristics. *P. biglobosa* (African locust bean) seeds were processed and fermented to a vegetable protein based African condiment known as 'Iru'. Fermentation process was carried out at four different temperatures which are 40, 50, 60 and 70℃. The monitoring of the fermentation rate was based on three parameters namely: Carbon dioxide production, pH and reduction in substrate weight. Substrates were inoculated with *Bacillus subtilis* and *Saccharomyces cerevisiae* as starter culture while fermentation process took place for the duration of five days under anaerobic condition. The result obtained from the pH of fermented seeds confirmed the fermentation process to be alkaline in nature.

Keywords: *Parkia biglobosa*, fermentation, condiment, vegetable protein, starter culture

1. Introduction

P. biglobosa tree is a leguminous plant found in the Savannah area of African [1].This tree grows from 7 to 20 meters in height as perennial deciduous plant [2]. [3] reported the plant to consist of numerous healing characteristics. [2-4] described the pods to have irregular and flat clusters of seeds in a row. This plant got its name as *P. biglobosa* by a Scottish botanist named Robert Brown in 1826 [4-6].
P. biglobosa is fermented to a protein rich and tasty food condiment which serves as flavor intensifier for soups and stews in most parts in Africa [7-9].
The conversion of complex substances such as starch to smaller compounds by microorganism is known as fermentation. It is one of the oldest means of food preservation known to man [6, 10-12].
Fermented *P. biglobosa* seed is giving diverse names in different countries - it is knows as Kinda in Sierra Leone, [13, 14] reported it as Iru or dawadawa in Ghana and Nigeria, Sonru and Afintin in Benin republic [13], while [15] reported it as Natto in Japan. *Bacillus* specie has been reported as the brain (microorganism) behind the fermentation of *P. biglobosa* seed [10, 11, 14, 16-19]. During the fermentation of *P. biglobosa* seeds careful observation revealed *B. subtillis* to be the most abundant microorganism available for the process of fermentation [12, 16, 20, 21].
The fermentation of *P. biglobosa* seeds into 'Iru' is a local and traditional art that is mostly carried out in the rural regions which involves the use of rudiment utensils where hygiene is not considered. Since standard methods for the processing of *P. biglobosa* seeds to condiments is not yet in place, therefore every producers produces based on traditions and culture, as a result of these variations in quality of the end products have been recorded [22].

The final intended use of product informs the fermentation methods and length of fermentation, these bring variation to the products resulting to non-uniformity in products [13]. Good flavor is quality index of 'Iru', this plays a vital role in consumer acceptability [23]. Processed *P. biglobosa* condiments is associated with a specific aroma and inviting organoleptic property, however differences in flavor range and intensities still exist [24].
This research work studied the method of fermentation rate monitoring which includes, Carbon-dioxide, pH and substrate weight. The final product (condiment) is a vegetable protein which serves as spice for stew and soup.

Figure 1: **a** - *P. biglobosa tree,* **b** – Seed Pod, **c** – Processed molded condiment, **d** – Dried condiment (Source: Ekum 2013).

2. Material and Methods

2.1 Laboratory preparation of seeds
Purchased raw seeds were handpicked and washed with distilled water. The seeds were boiled for 6 hours using pressure pot to soften the seed. The seed were later hand washed to remove the cotyledon and recooked for another 3 hours. The cooked seeds were packed inside polythene bag to keep the temperature for unset of fermentation [11, 24].

2.2 Preparation of Inoculum
200 g of processed seed sample was inoculated using 20ml inoculum. Samples were placed in a thermostatically controlled fermenter. Samples were picked every day and kept in a freezer for further analysis [22, 25, 26].

2.3 pH determination
Method [11] was used for the determination of pH.

2.4 Inoculation of Seeds

Freshly prepared *B. subtilis* was used to inoculate 400 g of processed seed. Five round bottomed flask were used using thermostatically controlled fermenter. Samples were removed every 24 hours from the reactor and stored in an air tight container which was also kept in the freezer until needed.

2.5. Carbon dioxide release Monitoring

Carbon dioxide was captured using method of [12, 27].

3. Results and Discussion

3.1. Substrate Weight Loss Monitoring

Temperature, time and its resulting effect on rate of fermentation and substrate weight were observed and reported in figures 2-4.

Figure 2, shows that at 40^{o}C, the highest percentage of weight loss was recorded followed by 50 and 60℃. The lowest weight loss was recorded at a temperature of 70^{o}C. An explanation for this could be that the high temperature inhibited the inoculum from optimal operation, hence poor yield. Figure 3 revealed that *S. cerevisiae* used under anaerobic condition and weight loss of the substrate was enhanced at low range temperatures. The results supported the fact that *S. cerevisiae* grows optimally between 32.3^{o}C and 45.4^{o}C [28]. Temperature is one of the major factors which affect the growth of *S. cerevisiae*. Under intense temperature, cells of some microorganism damages and may eventually die. The data obtainedin this work supports postulations in earlier works that the rate of growth of microorganism (*S. cerevisiae*) reduces as it deviates from optimum temperature [29, 30], and eventually dies out at 50°C.

Fermentation using *Saccharomyces* and *Bacillus* as inoculum under anaerobic conditions yielded similar results while higher weight loss was recorded with lower temperature. However, *S. cerevisiae* showed a better performance under anaerobic fermentation than *B. subtilis* since it is facultative in nature which can survive in an oxygen deficient environment. The end products for all samples done at temperature above 60°C were not organoleptically acceptable as the microorganisms have exceeded the optimum operating temperature. Poor aroma was also observed which can be due to the production of ammonium.

The effects of temperature on the substrate weight loss at various degrees were show in figures 2 – 4.

The combination of *S. cerevisiae* and *B. subtilis* were considered at 40 and 50 ^{o}C i.e the two microorganisms were added together. The result obtained with the combination of the two microorganisms at 40 °C is very close to the one obtained when only *B. subtilis* was used. This suggests that*B. subtilis*have a high activity than *S. Cerevisiae*when subjected to the same fermentation conditions. This was expected as *B. subtilis* usually dominates in any environment they are found. Figure 4 also revealed that low temperature favours substrate weight loss. During the early period of fermentation process, weight loss increased with time for the two temperatures considered. Since the process have gone beyond optimum operating temperature for the microorganisms used, weight loss decrease at temperature 50℃ indicating slow rate of fermentation.

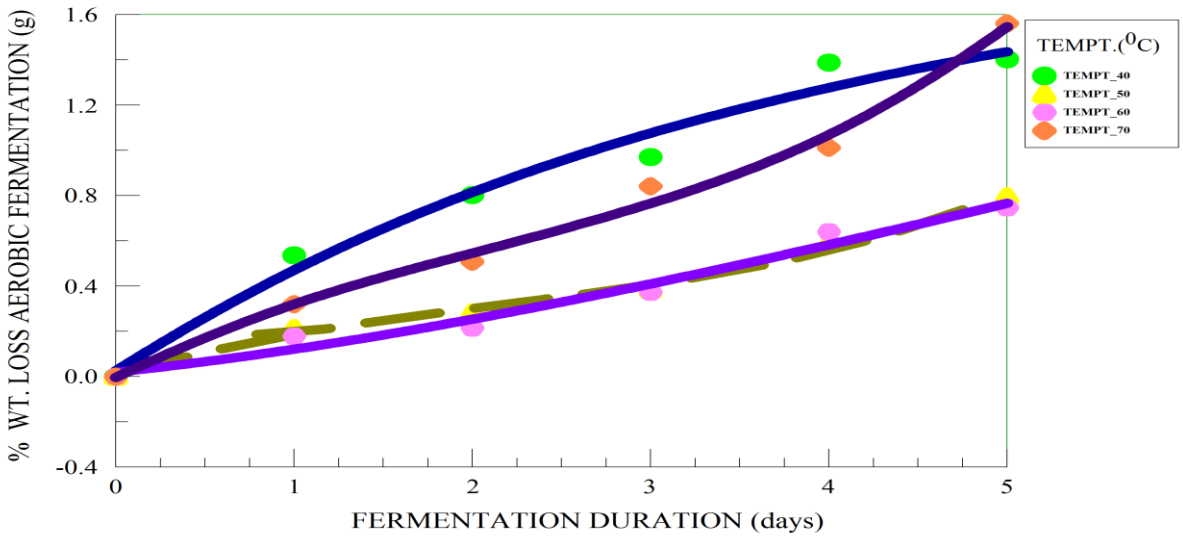

Figure 2. Substrate weight loss at different temperature with*B. subtillis*

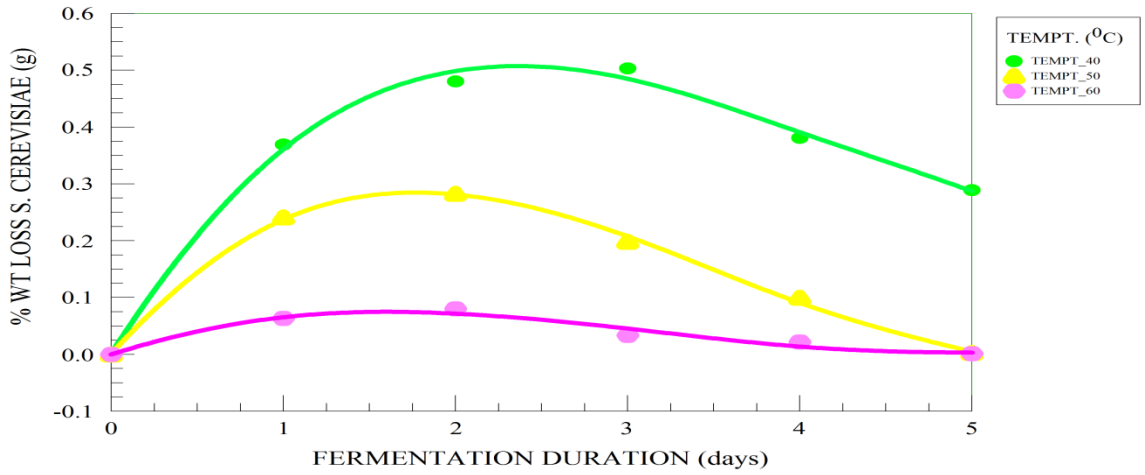

Figure 3. Substrate weight loss with*S. Cerevisiae* as starter culture

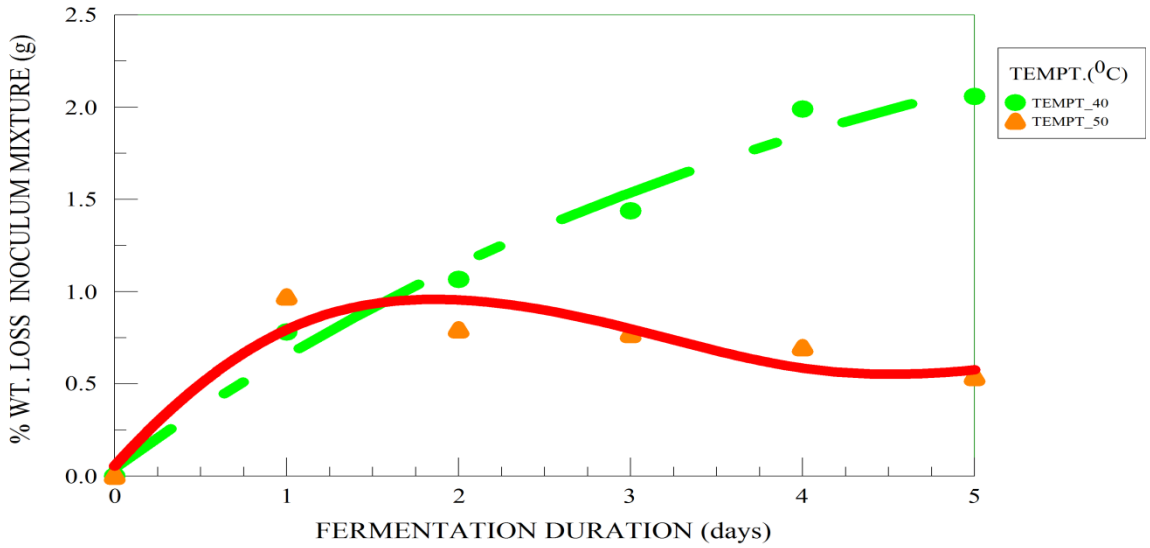

Figure4. Substrate weight Loss (WT) with the combination of *S. Cerevisiae* and *B. subtilis*

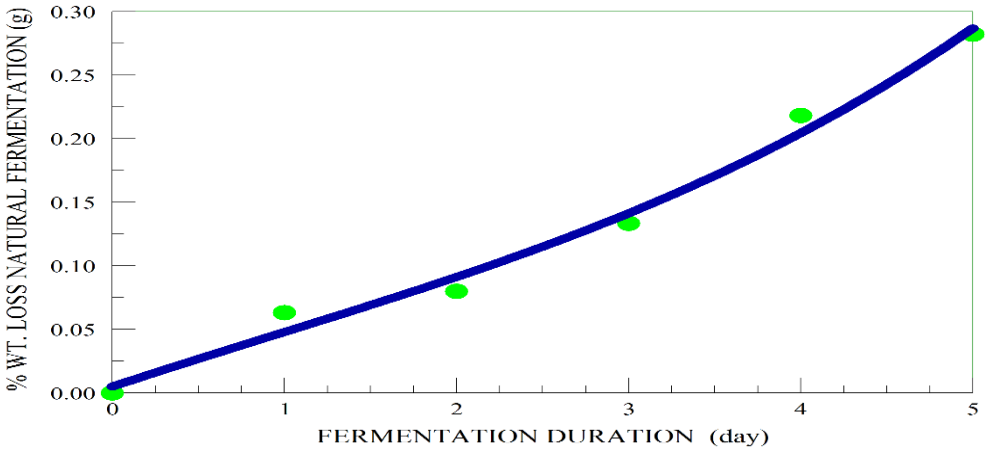

Fig. 5. Effect of Fermentation at room temperature on substrate weight Loss

Figure 5 presents the effect of natural fermentation process atroom temperature. In spontaneous/natural fermentation bacteria that are already present on the processed substrate is allowed to start the fermentation. To promote growth of bacterial, a conducive environment for reaction has to be provided.

*B. subtilis*is a probiotic bacterium which is capable of producing lactic acid; this organism acts by breaking down compounds of sugar present in the seeds. During natural fermentation

increment in temperature was monitored using thermometer.The bacterium that grows fastest during fermentation is determined by the change in temperature during the process. The sequence at which this microorganism grows is very vital in the process.Slight temperature deviation can alter microbial activity pathway and also affect the end products.

3.2. The pH Determination

The result obtained here confirmed fermentation of *P. biglobosa* to be Alkaline in nature. This work also confirmed that change in temperature during fermentation may also affect the pH.

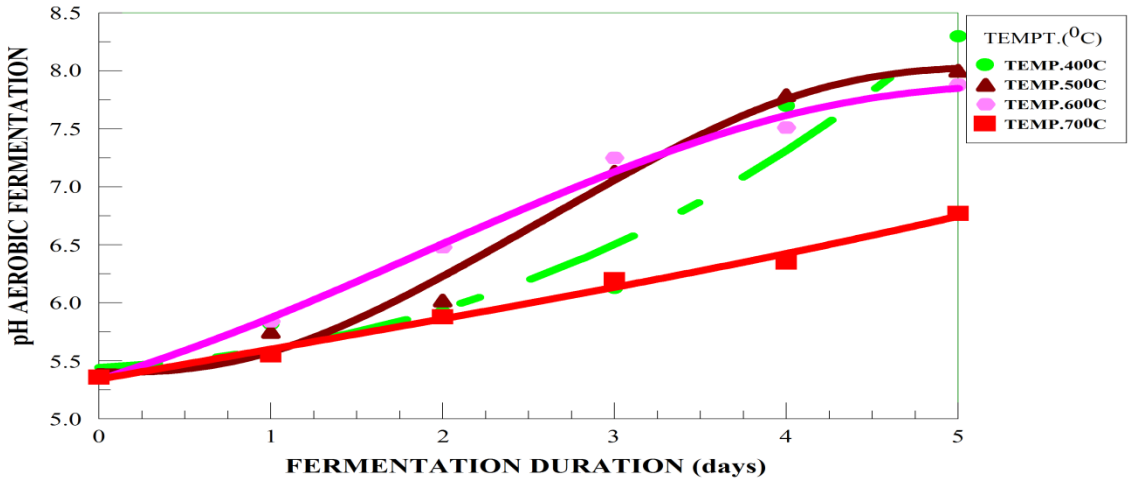

Figure 6. pH with*B. subtilis* as inoculum

Figure 6above shows the effect of temperature on fermentation under anaerobic condition. Increment in pH value is favoured by low temperature. *B. subtilis* possesses ability to hydrolyze protein to give proteases, amino acid and ammonia. This acts as sources of energy and carbon for growth. Dissolved ammonia caused an increment in the level of alkalinity in the media. At the initial stage, an increment in the temperature resulted in a corresponding increment in pH till the optimum temperature was reached. Beyond this temperature, a decrease in pH was obtained. During the fermentation, increment in pH resulted in increased soluble nitrogen content. Denaturing of a microorganism is affected positively or negatively either by the increase or decrease of pH. This results in a decrease of rate of reaction while a faster rate of reaction is favoured by optimum fermentation conditions. Microorganisms are adversely affected by acidic pH leading to denaturing of cells responsible for the degradation of protein.

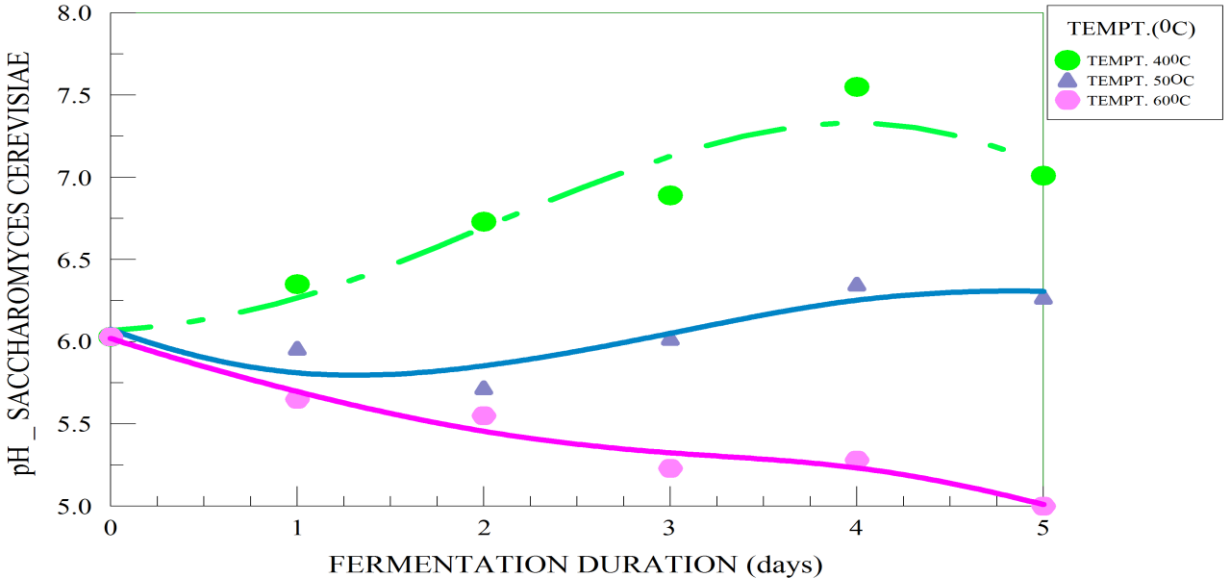

Figure 7. pH with*S. cerevisiae* as starter culture

An effect of temperature on the pH value of the substrate using S. cerevisiae thrives in low temperatures and operates between 32.3^{o}C and 45.4^{o}C optimally. At 50^{o}C and 60^{o}C, a redundancy in growth was observed. This is attributed to the fact that temperature above 45^{o}C inhibits fungi induced fermentation. This ultimately results in an undesirable end product and a reduction in pH as more acetic and lactic acid are formed.Excessively high reaction temperature results in denaturing of the *S. cerevisiae;* due to the high temperature the liquid present in the cell diffuses in order to balance the external concentration of the cell. This eventually leads to dehydration shrinkage and death of cell. Figure 6 shows the pH results obtained at 50^{o}C and 60^{o}C.

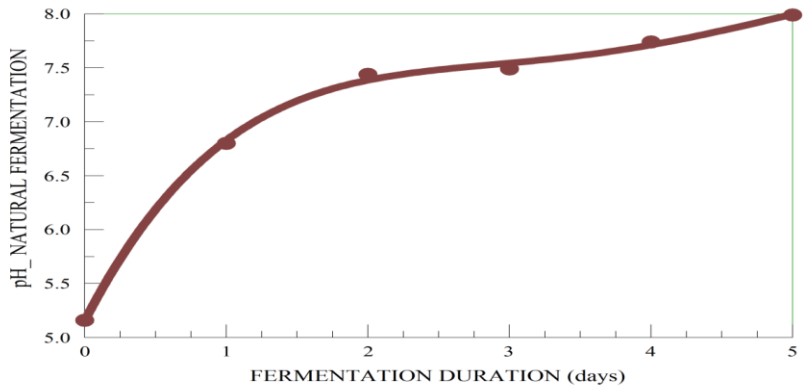

Figure 8.pH values of Substrate

Figure 8shows the effects of natural fermentation on the pH of processed*P. biglobosa* seeds without adding starter culture. Increment in pH during fermentation indicates a metabolic activity which usually occurs during proteolysis that liberates amino acids, which later produced ammonia that increase pH.

3.3. Carbon Dioxide release Monitoring

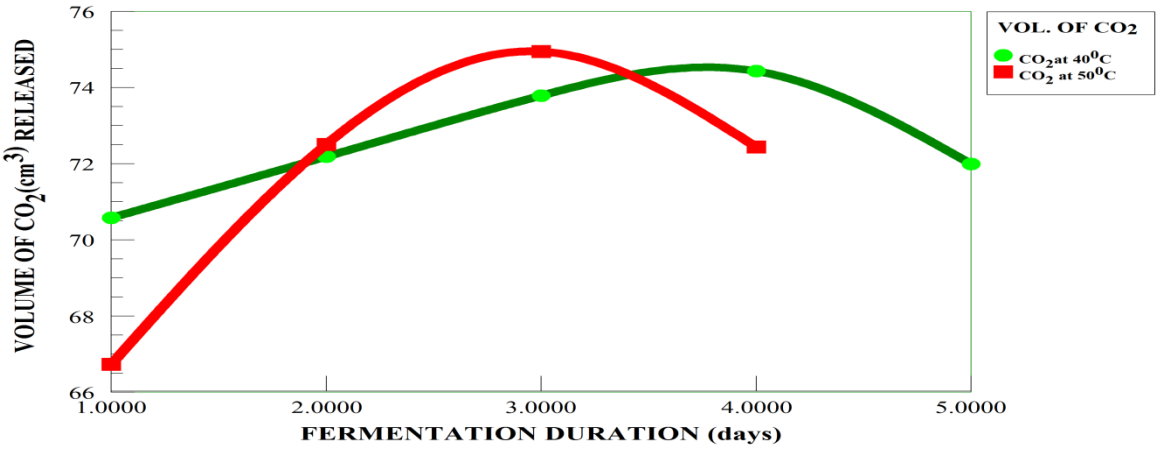

Figure 8. Carbon dioxide volume released with B. subtilis only

Figure 8represents the varying quantitiesof carbon dioxide gas released during fermentation. The data was obtained at 40 and 50℃. High temperature favours the release of carbon dioxide.The study revealed the peak of carbon dioxide evolved for 40 and 50℃. At 50^{o}C, the carbon dioxide released reached peak before the third day while the peak release was recorded on the 4th day for temperature at 40^{o}C. On the third day of fermentation, a reduction in the amount of carbon dioxide released was recorded at a fermentation temperature of 50^{o}C**.** The initial volume of carbon dioxide released when compared to the volume released after 72 hours showed a decrease. This suggests that the gas producing microorganisms are also linked with the fermentation process.

4. Conclusion

This study presents valuable insights into fermentation reaction of *P. biglobosa* seed which converts it to a vegetable-based Protein. This research concluded that for optimal growth of microorganisms used as inoculum, a moderate temperature is required. Spontaneous fermentation in an uncontrolled environment gives products with varying organoleptic properties and variant quality. This made it necessary for the development of a starter culture to kick start the reaction as it will ensure consistency in end result and quality. From the first to the fifth day of fermentation, increase in temperature between 25 – 45^{o}C was recorded for

ambient fermentation process; this shows that fermentation process is an exothermic reaction. The temperature required for optimal activities of proteolytic enzymes were fuelled by the heat given off during the fermentation process. The White precipitate of $BaCO_3$ formed at the bottom of the flasks containing$Ba(OH)_2$ confirmed the liberation of CO_2.

Acknowledgment

The authors wish to thank the management of Covenant University for the platform made available for this research.

Conflict of interest: Authors declare no conflict of interest

REFERENCES

[1]. Ojewumi M.E., Ayomide A.A.,Obanla O.M., OjewumiE.O. (2014). Pozzolanic properties of Waste Agicultural Biomass-African Locust Bean Pod Waste. World Journal of Environmental Biosciences, 6(3): 1-7.
[2]. Ojewumi M.E., EluagwuleB., AyoolaA.A., OgunbiyiA.T., AdeoyeJ.A., Emetere M.E., JosephO.O. (2017). Termiticidal Effects of African Locust Bean (*Parkia Biglobosa*) Seed Oil Extracts. International Journal of Current Research, 9(7): 53929-53934.
[3]. Ojewumi M.E., Omoleye J.A., Ajayi A.A. (2016). The Study of the Effect of Moisture Content on the Biochemical Deterioration of Stored Fermented *Parkia Biglobosa* Seeds. Open Journal of Engineering Research and Technology, 1(1): 14-22
[4]. Ojewumi M.E., Omoleye J.A., AjayiA.A. (2017). Optimization of Fermentation Conditions for the Production of Protein Composition in *Parkia biglobosa* Seeds using Response Surface Methodology. International Journal of Applied Engineering Research, 12(22): 12852-12859.
[5]. Ojewumi M.E., Omoleye J.A.,AyoolaA.A., Ajayi A.A.,AdekeyeB.T., AdeyemiA.O. (2018). Effects of Salting and Drying on the Deterioration Rate of Fermented *Parkia biglobosa* Seed. Journal of Nutritional Health & Food Engineering, 8(1): 1-5.
[6]. Omafuvbe B.O., Falade, O.S., Osuntogun, B.A., Adewusi, S.R. (2004). Chemical and biochemical changes in African locust bean (*Parkia biglobosa*) and melon (*Citrullus vulgaris*) seeds during fermentation to condiments. Pakistan Journal of Nutrition, 3(3): 140-145.
[7]. Ikenebomeh M., Kok R. (1984). Mass balance of the processing and fermentation of the African locust bean (*Parkia filicoidea* Welw.). Canadian Institute of Food Science and Technology Journal, 17(1): 48-50
[8]. Reddy N. (2018).Legume Based Fermented Foods: CRC Press.
[9]. Dajanta K., Apichartsrangkoon A.,Chukeatirote E. (2011). Volatile profiles of thua nao, a Thai fermented soy product. Food chemistry, 125(2): 464-470
[10]. Omafuvbe B., Abiose S., Shonukan O. (2002). Fermentation of soybean (*Glycine max*) for soy-daddawa production by starter cultures of*Bacillus*. Food Microbiology, 19(6): 561-566.
[11]. Ojewumi M.E., Omoleye J.A., Ajayi, A.A. (2016). Optimum fermentation temperature for the protein yield of *parkia biglobosa* seeds (Iyere). 3rd International Conference on African Development Issues (CU-ICADI 2016)

[12]. Ojewumi M.E. (2016). Optimizing the Conditions and Processes for the Production of Protein Nutrient from *Parkia biglobosa* Seeds. Thesis submitted for the award of Ph.D in Chemical Engineering, Covenant University, Nigeria.
[13]. Azokpota P., Hounhouigan D., Nago, M. (2006). Microbiological and chemical changes during the fermentation of African locust bean (*Parkia biglobosa*) to produce afitin, iru and sonru, three traditional condiments produced in Benin. International journal of food microbiology, 107(3): 304-309.
[14]. Odunfa S.A. Microbiology and amino acid composition of Ogiri—a food condiment from fermented melon seeds (1981). Molecular Nutrition & Food Research, 25(9): 811-816.
[15]. Beaumont M.(2002). Flavouring composition prepared by fermentation with *Bacillus* spp. International journal of food microbiology, 75(3): p. 189-196.
[16]. Sanni A. (1993). Biochemical changes during production of okpehe, a Nigerian fermented soup condiment. Advances in Food Sciences, 15(3): 97-100.
[17]. Odunfa S. (1985). African Fermented Foods In: Microbiology of fermented foods. Vol.: BJB Wood pp. 155-191, Elsevier Applied Science Publication, London.
[18]. Ouoba L., Rechinger, K., Barkholt V.,DiawaraB., Traore A.,Jakobsen M. (2003). Degradation of proteins during the fermentation of African locust bean (*Parkia biglobosa*) by strains of *Bacillus subtilis* and *Bacillus pumilus* for production of Soumbala. Journal of applied microbiology, 94(3): 396-402.
[19]. Ouoba L.I.I., Diawara B.,kofi Amoa-Awua W., Traoré A.S., Møller P.L. (2004). Genotyping of starter cultures of *Bacillus subtilis* and *Bacillus pumilus* for fermentation of African locust bean (*Parkia biglobosa*) to produce Soumbala. International journal of food microbiology, 90(2): 197-205.
[20]. Ojewumi M.E., Obielue B.I.,Emetere M.E., Awolu O.O.,Ojewumi E.O. (2018). Alkaline Pre-Treatment and Enzymatic Hydrolysis of Waste Papers to Fermentable Sugar. Journal of Ecological Engineering, 19(1): 211-217. https://doi.org/10.12911/22998993/79404
[21]. Ojewumi M.E., AdedokunE.O., Omodara O.J.,Oyeniyi E.A., Taiwo O.S., Ojewumi E.O. (2017). Phytochemical and Antimicrobial Activities of the Leaf Oil Extract of *Mentha Spicata* and its Efficacy in Repelling Mosquito. International Journal of Pharmaceutical Research & Allied Sciences, 6(4): 17-27.
[22]. Ojewumi M.E., Omoleye J.A., Ajayi A.A. (2016). The Effect of Different Starter Cultures on the Protein Content in Fermented African Locust Bean (*Parkia Biglobosa*) Seeds. International Journal of Engineering Research & Technology (IJERT), 5(4): 249-255.
[23]. Odebunmi E., Oluwaniyi O.,Bashiru M. (2010). Comparative proximate analysis of some food condiments. Journal Applied Science Research, 6(3): 272-274.
[24]. Odunfa S.,Adesomoju A. (1986). Fatty acid composition of African locust bean (*Parkia biglobosa*). Chemie, Mikrobiologie, Technologie der Lebensmittel, 10(3-4): 125-127.
[25]. Ojewumi M.E., Omoleye J.A., Emetere M.E., Ayoola A.A., Obanla O.R., Babatunde E.D., Ogunbiyi A.T., Awolu O.O., Ojewumi E.O. (2018). Effect of various temperatures on the nutritional compositions of fermented African locust bean (*Parkia biglobosa*) seed. International Journal of Food Science and Nutrition, 3(1): 117-122.

[26]. Ojewumi M.E., Omoleye J.A., Nyingifa A.S. (2018). Biological and chemical changes during the aerobic and anaerobic fermentation of African locust bean. International Journal of Chemistry Studies, 2(2): 25-30.

[27]. Ojewumi M.E., Anenih E.V., Taiwo O.S., Adekeye B.T., Awolu O.O., Ojewumi E.O. (2018). A Bioremediation Study of Raw and Treated Crude Petroleum Oil Polluted Soil with *Aspergillus niger* and *Pseudomonas aeruginosa*. Journal of Ecological Engineering, 19(2): 226-235. https://doi.org/10.12911/22998993/83564

[28]. Salvadó Z., Arroyo-López, J. Guillamón F., Salazar G., Querol A., Barrio E. (2011). Temperature adaptation markedly determines evolution within the genus Saccharomyces. Applied and environmental microbiology, 77(7): 2292-2302.

[29]. Ojewumi M.E., Okeniyi J.O., Okeniyi E.T., Ikotun J.O., Ejemen V.E., Akinlabi E.T. (2018). Bioremediation: Data on Biologically-Mediated Remediation of Crude Oil (Escravos Light) Polluted Soil using *Aspergillus niger*. Chemical Data Collections, 17- 18(2018), 196-204.

[30]. Ratkowsky D., Olley J., McMeekin T., Ball A. (1982). Relationship between temperature and growth rate of bacterial cultures. Journal of Bacteriology, 149(1): 1-5.