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Evaluation of fermentation rate for the production of a protein based African seed condiment

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Abstract: *Parkia biglobosa* (African locust bean) seed was fermented aerobically to produce a vegetable protein based condiment using various temperature differences and ambient temperature. The rate of fermentation was monitored using three (3) different methods namely: weight loss, pH and Carbon dioxide release. Samples were inoculated using *Bacillus subtilis* and *Saccharomyces cerevisiae* as starter culture. During fermentation, several changes occur in the seeds of the African Locust bean. The difference in the weight loss (initial and final weight of the fermenting samples) were used to monitor the rate of fermentation of the African Locust bean (*parkia biglobosa*) seeds to vegetable protein called 'Iru'. Fermentation of this seed to 'Iru' is an alkaline fermentation, which was confirmed by this work. As means of monitoring the rate of fermentation, the evolution of CO_2 was also monitored.

Keywords: *Parkia biglobosa,* alkaline fermentation, vegetable protein, condiment, pH

1. Introduction

African Locust bean with botanical name *P. biglobosa* is a leguminous plant found in the Savannah region of Nigeria [1]. *P. biglobosa* (African locust bean) tree is a perennial deciduous tree that grows from 7 to 20 meters high [2]. The tree was listed as one of the plants having real wound healing properties in South Western Nigeria [3]. The pods are flat and have irregular cluster of up to 30 seeds [2-4]. African locust bean tree was named *Parkia biglobosa* by Robert Brown, a Scottish botanist in 1826 after Mongo Park, a Scottish surgeon who explored West Africa in 1790's. Mongo Park gave this tree a local name 'nitta' [4-6].

P. biglobosa is usually fermented to a protein rich and tasty food condiment known as dawadawa or Iru which serves as flavour intensifier for soups and stews [7-9].

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The biological conversion of complex substrate such as starch or sugar into simple compounds by microorganisms is known as fermentation. Fermentation can also be defined as the production of energy from food materials in the absence of oxygen. It is one of the oldest forms of food preservation known to man [6, 10-12].

P. biglobosa is known by different names in different countries - kinda in Sierra Leone and Iru or dawadawa in Nigeria and Ghana [13, 14], Afintin and sonru in Benin republic [13], while in Japan it is known as natto [15]. Fermented seeds is produced by either natural fermentation or by adding microorganism as an inoculum. The consumption increases in urban areas progressively, since the condiments are considered as a functional food which can regularize blood arterial pressure.

There seems to be a general agreement on the microorganism responsible for the fermentation of *P. biglobosa* as *bacillus* specie [10, 11, 14, 16-19]. During the fermentation of *P. biglobosa* seeds careful investigation showed that *B. subtillis* is the most dominant bacterium responsible for the fermentation [12, 16, 20, 21]. Previous work done revealed that some species of *bacillus* such as *Bacillus lichenioformis, Bacillus megaterium, L. mesenteriodes* and *Staphylococcus* are also found in the fermented condiment which is also known as Iru.

Fermentation of African locust bean seeds is a traditional art that is practiced in rural areas with rudimentary utensils which did not put hygiene into consideration. Since there is no standard method for the fermentation, every producer produces base on their culture and traditions which brings variation in the quality of Iru [22]. The length of production and also the methods of fermentation vary from one producer to another and with the final intended use, resulting to non uniformity in products [13]. Flavour is considered the quality index of fermented African locust bean and plays an important role in consumer acceptability [23]. Although fermented African oil bean seeds have typical flavour and appealing organoleptic quality, differences in flavour range and intensities exist. These vary perhaps as attributes of producer organisms which are due to the various volatile compounds produced by the fermenting population[24].

2. Materials and Methods

P. biglobosa seeds used were purchased from local market. All the chemicals used were of analytical grade from Sigma manufacturing industry. The starter culture used was prepared in Covenant University Microbiology laboratory, Sango-Ota, Nigeria.

2.1. Laboratory preparation of *P. biglobosa*

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The *P. biglobosa* purchased from the market were processed using methods of [11, 25] .

2.2. Preparation of *B. subtilis*

Preparation of the inoculum used was carried out using methods of [22, 26, 27].

2.3. pH determination

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

2.4. Inoculation of Seeds

400 g of processed seed was inoculated with freshly prepared *B. subtilis*. Fermentation was carried out using a round bottomed flask in a thermostatically controlled fermenter. Samples were removed every 24 hours from the fermenter and packed into an air tight container which was stored in the freezer until needed.

2.5. Carbon dioxide release Monitoring

6 mm hose was passed from the inoculated African locust bean seed in a flask into another flask that has 90 ml $Ba(OH)_2$. The two flask were tightly covered with a cork and a tape to avoid the escape of the gas. This was done for all the samples and monitored for the 5 days of fermentation. Initially the solution was clear but latter turned white at contact with the process. White precipitate of $BaCO_3$ was formed underneath the flasks. The $BaCO_3$ was filtered and the filtrate titrated with Hydrochloric acid. The volume of the Carbon dioxide released was calculated.

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Plate 1: **a** - The tree *Parkia biglobosa* with pod, **b** – The pod with the fruit, **c** – Fermented processed molded seeds, **d** – Fermented dried seeds (Ekum 2013).

3. Results and discussion

3.1. Substrate Weight Loss Monitoring

Figures 1 to 3 shows the rate at which fermentation process affects the weight of the substrate at various fermentation temperatures and duration.

Highest % weight loss was recorded at the lowest temperature (40ºC) followed by 50 and 60 ºC, while poor weight loss was recorded at 70ºC. It is speculated that the reason for the observed behaviour at 70 ºC was because the temperature was too high for the inoculum or starter culture to operate optimally [12], figure 1. In figure 2, high % substrate weight loss was also steadily favoured at lower temperatures using *S. cerevisiae* as starter culture under aerobic condition. The results confirmed the fact that the highest optimum fermentation temperature for the growth of *S. cerevisiae* is 32.3 ºC and a maximum of 45.4 °C for activity [28]. Temperature is an important non-living condition (abiotic) that influences the *S. cerevisiae* growth. At high temperatures, some microorganisms become stressed and their cellular content becomes damaged. The results observed in this study support other observations and hypothesis in literatures that the growth rate of microorganisms (*S. cerevisiae*) is reduced as it moves away from its optimal growth temperature [29], and dies at 50 °C.

The effect of mixing *S. cerevisiae* and *B. subtilis* was examined only with two temperature, 40 and 50 ºC. The mixture at 40 °C gave results that have the same trend as those found where *B. subtilis* only was used. This suggested a higher activity by *B.s subtilis* than *S. cerevisiae* under the same conditions. This is expected since *B. subtilis* tend to dominate in any environment they are found with other organisms. Lower temperature favours substrate weight loss in this mixture as shown in Figure 3. The weight loss increased with time for both temperatures during the early period of the fermentation process but as fermentation duration increased, it was observed that the weight loss at 50 °C decreased, probably because the process has gone beyond the maximum operating fermentation conditions for the two organisms.

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It was observed that in all the samples, end products (fermented samples) were not physicochemically acceptable at higher temperatures, probably because of the high production of ammonia which led to a poor characteristic unacceptable aroma.

Shown in Figure 4 is the effect of natural or spontaneous fermentation at ambient condition on substrate weight loss. This is the process of allowing natural bacteria already present on the processed seeds to start the fermentation. The right environment was created to promote bacterial growth. In spontaneous (natural) fermentation, the sequence in which different microorganisms grow is expedient. A little change in the temperature can change the activity of the microbial process and affect the quality of the final product. An increase in the percentage substrate weight loss was observed to occur with the number of days of fermentation.

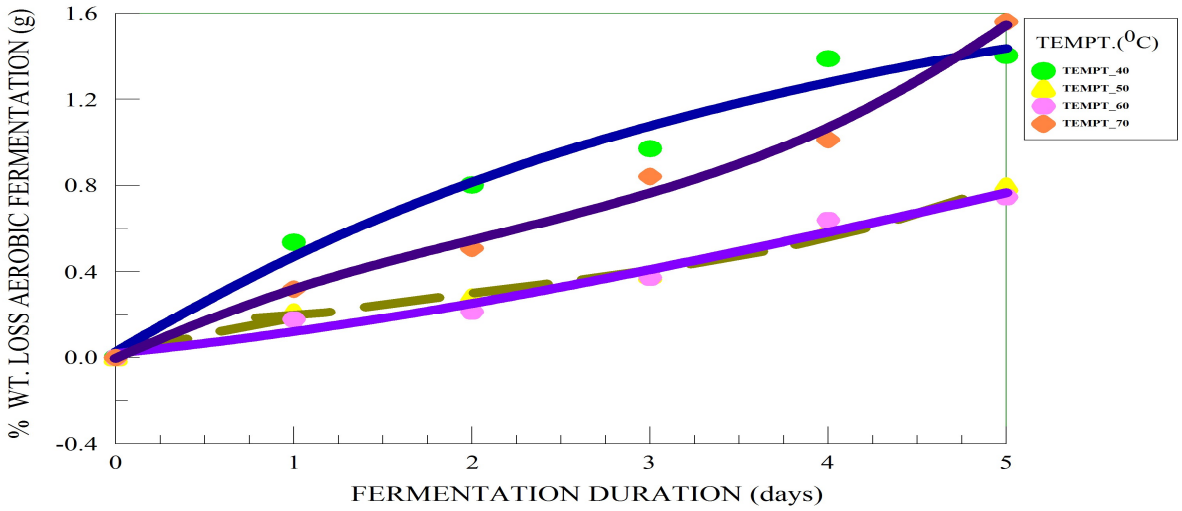

Figure 1. Effect of fermentation on substrate weight loss at different temperature using *B. subtillis* as starter culture

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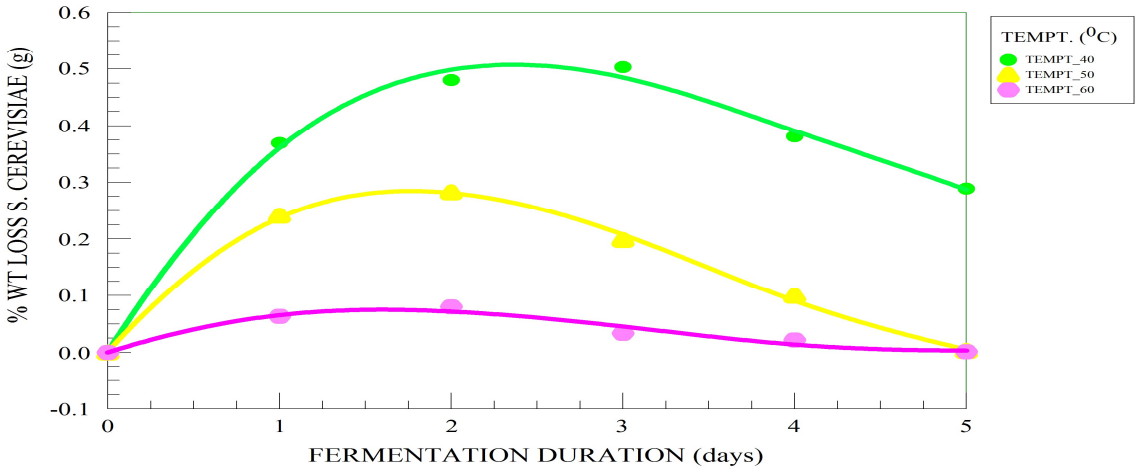

Figure 2. Effect of fermentation on substrate weight loss using *S. Cerevisiae* as starter culture

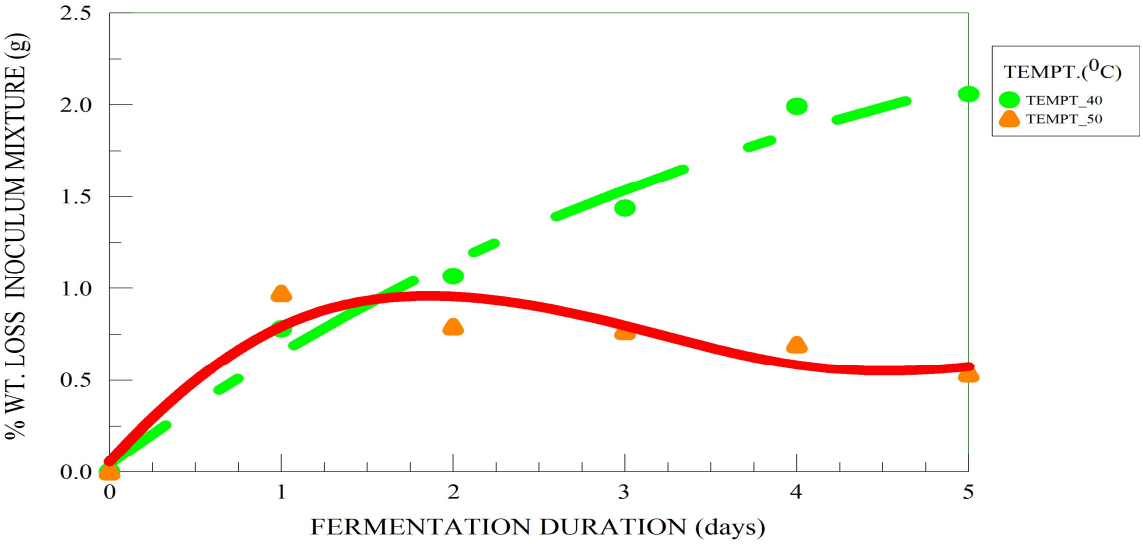

Figure 3. Effect of fermentation on substrate weight Loss (WT) using the mixture of *S. Cerevisiae* and *B. subtilis* as starter culture

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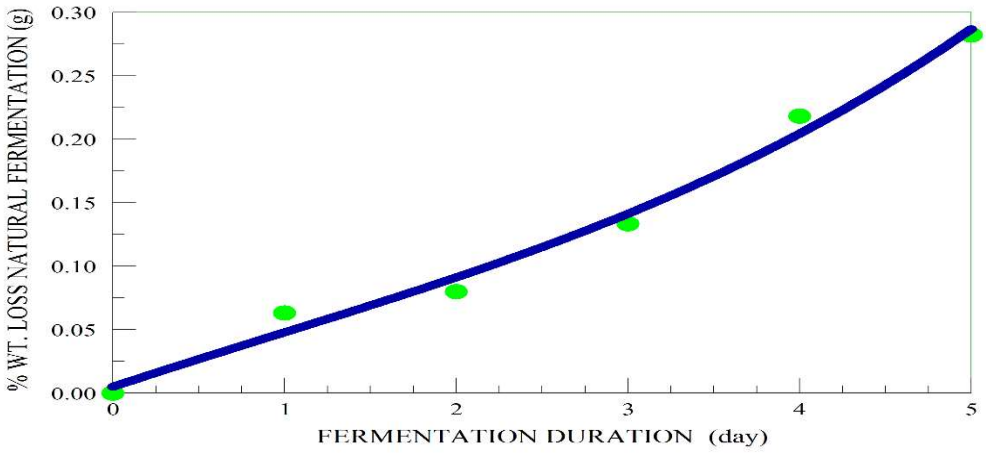

Figure 4. Effect of ambient temperature fermentation on substrate weight Loss

The pH Determination

Earlier studies have confirmed that the fermentation of African locust bean (*P. biglobosa)* seed to Iru is an alkaline fermentation which is a fermentation process during which the pH of the substrate increases to alkaline values that may be as high as pH 9 [11, 12]. The effect of temperature on the pH of the fermenting system were monitored as well as the rate of fermentation. The result obtained confirmed that the fermentation of *P. biglobosa* seeds to 'Iru' is an alkaline fermentation.

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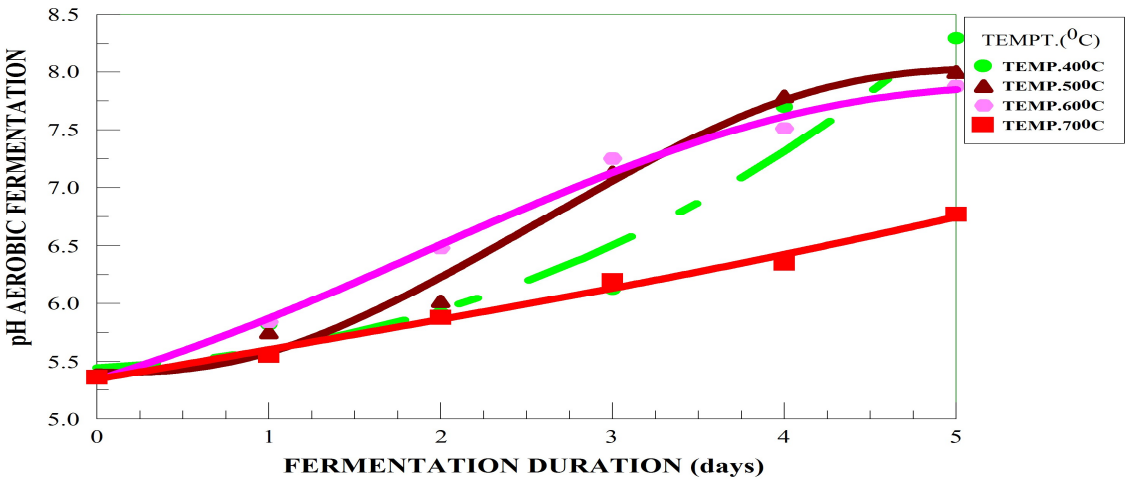

Figure 5. Effect of fermentation temperature on the pH value of the substrate using *B. subtilis*

Figure 5 shows that under aerobic fermentation, lower temperature favours increase in pH value towards alkalinity during fermentation. This is due to the ability of *B. subtilis* to hydrolyze proteins to produce proteases and obtain amino acids and ammonia as source of carbon and energy for growth, respectively. Increase in the alkalinity level of the media is caused by the dissolved ammonia. Increase in the fermentation temperature led to a corresponding increase in pH towards alkalinity at the initial fermentation stage until the optimum temperature of growth of each starter culture was reached where by any further increase in temperature led to a decrease in pH. Increase in pH was attributed to a rapid increase in the soluble nitrogen content during fermentation.

It is known that increase or decrease in the pH denatures the microorganism responsible for fermentation thereby decreasing the rate at which reaction takes place while optimal pH favours a faster rate of fermentation depending on the operating fermentation conditions and the organism i.e. the lower the pH the faster the rate of reaction. pH towards acidity affects the microorganism responsible for the breaking down of protein by denaturing them which can lead to low production.

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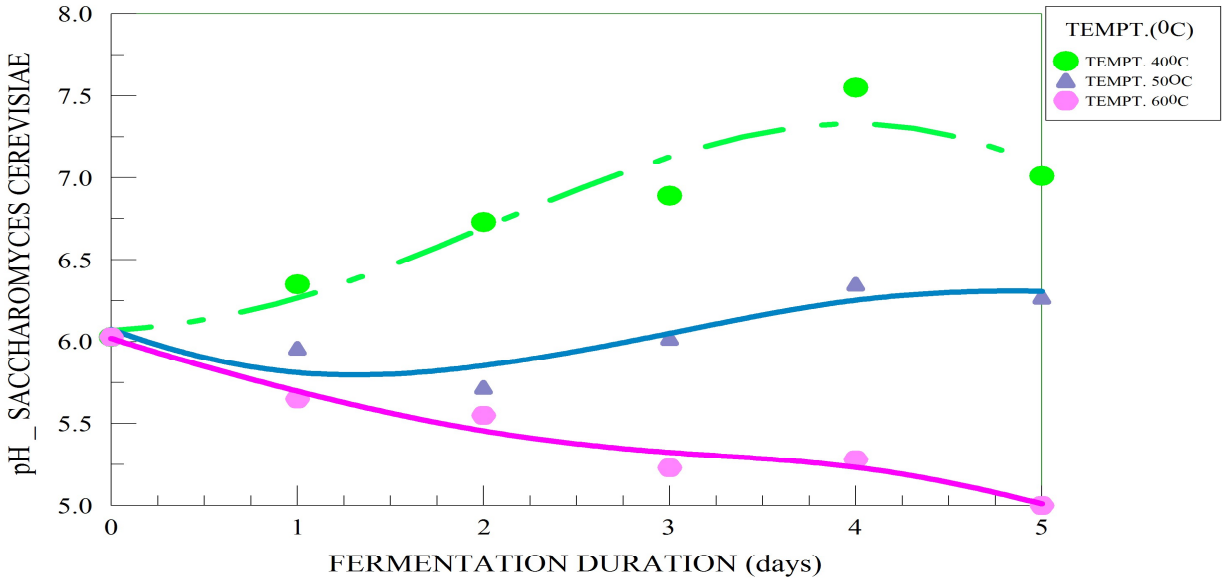

Figure 6. Effect of fermentation temperature on the pH value of the substrate using *S. cerevisiae* as starter culture

Since *S. cerevisiae* is a psychrophilic bacteria which operates at highest optimum temperature of 32.3 °C and maximum of 45.4 °C, pH values at 40 °C are found to be decreasing after the fourth day. Temperatures 50 and 60 °C are not favourable for the growth as any degree above 45 depresses fermentation with fungi, hence the unacceptable end product and decline in pH values since more lactic and acetic acids are produced. The temperature of the reaction being too high causes *S. cerevisiae* to be denatured and the liquid present in it to diffuse out in order to equalize the concentration outside the cell, this causes the yeast to dehydrate, shrink and eventually die. The results obtained at 50 and 60 ºC are in support of these observations (figure 6).

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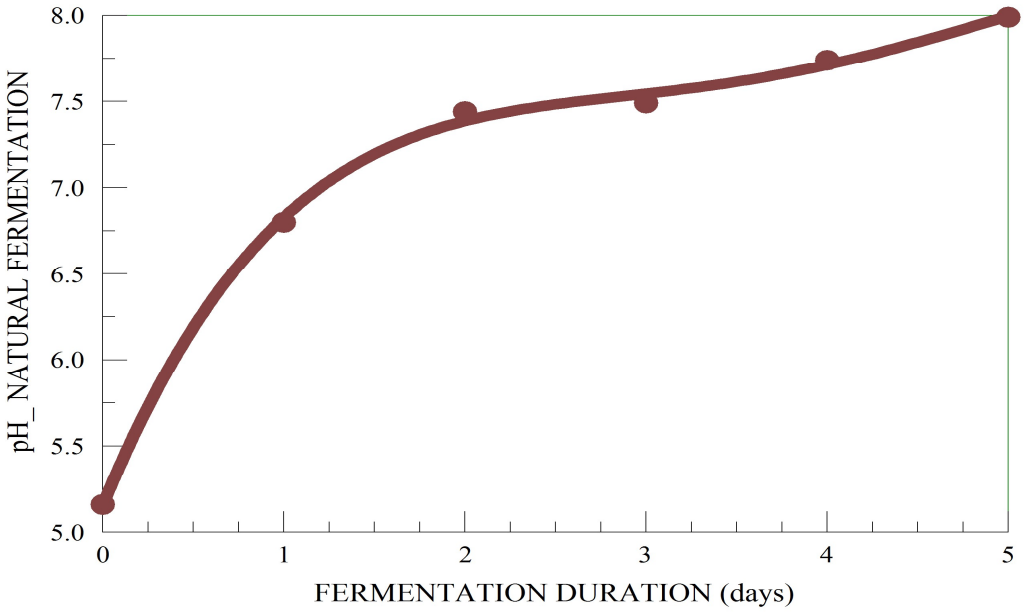

Figure 7. Effect of Natural Fermentation on the pH values of Substrate

Figure 7 represents the changes in pH of naturally fermented African locust bean seeds (No inoculum and it was fermented at room temperature). The increase in the pH value with fermentation duration suggests that metabolic activities that occurred may include proteolysis that allows liberation of amino acids and subsequent production of ammonia that leads to an increase in pH. Other metabolic activities which can also lead to an increase in pH are: Lipolysis and degradation of poly and oligosaccharides.

Carbon Dioxide release Monitoring

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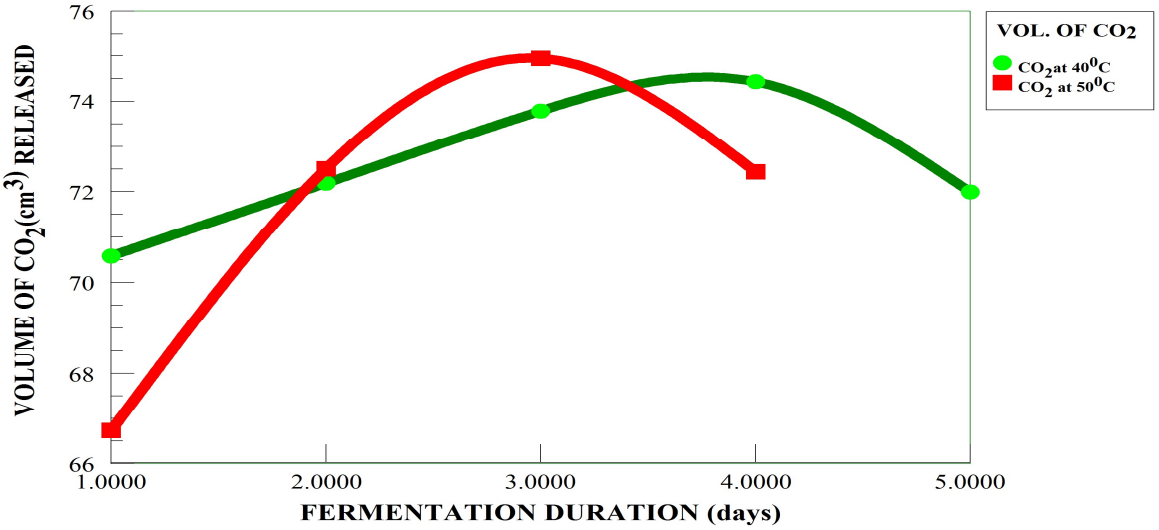

Figure 8. Effect of temperature on the volume of carbon dioxide released (*Bacillus subtilis* only)

Figure 8 shows the variation of evolved carbon dioxide during fermentation at two different fermentation temperatures. The figure shows that high temperature favours the release of carbon dioxide. On both temperature conditions tested, the evolved carbon dioxide peaked, although at different periods for the temperatures - before the 3rd day for the 50 ^{o}C and the 4th day at 40 ^{o}C.

A decrease in the volume of carbon dioxide released was noticed on the 3rd day of fermentation at 50 ^{o}C fermentation temperature**.**

The fact that evolved carbon dioxide was high at the initial period of fermentation and later decreased after 72 hrs (3 days), suggests that the microorganisms that initiate the fermentation process are most probably the gas-producing microorganisms.

Conclusion

This work revealed that reaction actually occur during the fermentation of African locust bean seed to a vegetable based Protein. Moderate temperature is required for the optimum growth of both microorganism used as inoculum. The art of fermenting naturally or spontaneously in an exclusively uncontrollable environmental conditions yields products with variation in quality and

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organoleptic properties, hence the reason for developing starter culture to initiate fermentation for the production of consistent products with acceptable qualities. A temperature rise of 20 °C (25 – 45 ºC) was observed between the first and fifth day of fermentation for ambient fermented process, this shows that fermentation process is an exothermic reaction. The heat generated during the fermentation process probably provided the required temperature conditions for the optimal activity of the proteolytic enzymes. The White precipitate of $BaCO_3$ formed underneath the flasks containing $Ba(OH)_2$ confirms the evolution of CO_2.

Conflict of interest

Authors declare no conflict of interest

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