Journal of Microbiology and Pathology

Research Article Open Access

# Natural Fermentation of *Pyrus communis* (Pear) Mesocarp by Associated Consortium Fungal Species

**Oluwatosin Adewusi Adedeji* and Temitope Mulikat Audu**

*Institute of Ecology and Environmental Studies, Obafemi Awolowo University, Ife, Nigeria*

## Abstract

This study was conducted to evaluate the effect of associated consortium fungal species on the natural fermentation of the mesocarp of *Pyrus communis* (Pear). The combined role played by the fungi (moulds and yeasts) involve in the natural fermentation of the Pear mesocarp were also investigated.

Fresh and ripe pears were washed and surface sterilized with ethanol. The mesocarp was then scraped into a bowl and covered with a net to allow the action of aerobic fermentation for 50 days. Fungi were isolated from the samples at interval of 5 days. The morphological, microscopic and biochemical characteristics as well as the fungal count were also carried out according to standard methods. The physicochemical parameters of the pear's mesocarp such as pH, total titrable acidity (TTA), moisture contents and total reducing sugar were also determined at every 5 days according to standard methods.

The results showed that there were eight strains of mould namely: *Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Aspergillus fumigatus, Mucor mucedo, Penicillum frequentas, Penicillum chrysogemun and Fusarium solani;* and three strains of yeasts namely *Saccharomyces cerivisae, Schizoaccharomyces pombe and Saccharomyces ludwigii* were identified to be involved in the fermentation process for the fifty days. The physico-chemical parameters results indicated progressive increase in pH from 3.73-6.73 with all values within the acidic range permitting the growth of microbial species; the TTA exhibited a rise and fall trend with range from 1.3-2.75 cm· the moisture content was found to be highest at 73.4% while the total reducing sugar exhibited a rise and fall in its values. The results of the microbial counts showed that the growth of mould species had prevalence as fermentation increases. The frequency of occurrence of mould present showed *Aspergillus flavus* to have the highest occurrence at 63.63% and for the yeast *Schizosaccharomyces pombe* at 72.27%.

The study concluded that a consortium of fungal species which are naturally associated with pear mesocarp can be effectively used in its fermentation.

**Keywords:** *Pyrus communis* (Pear); Mesocarp; Fermentation; Fungal isolates

## Introduction

*Pyrus communis* (Pear), also known as European or Common pear is pear species that is native to central and Eastern Europe and southwest Asia. This fruit belongs to the rose family of plants (Rosaceae) and have been identified to possess several nutritional and health benefits to man as both the flesh and the skin contain phytonutrients such as antioxidant, anti-inflammatory flavonoids and potentially anti-cancer phytonutrients like cinnamic acids [1].

The carbohydrate in the fruit is low on the glycaemic index, they are slow to convert to sugar and enter into the blood stream and as such a healthy carbohydrate. It is also known to provide a good source of fibre with a significant amount of pectin and is effective in lowering the cholesterols levels in the body and toning the intestines [2,3]. Apart from containing anti-oxidant and anti-carcinogen glutathione which help prevent high blood pressure, stroke and cancer, it is a good source of vitamin, calcium and potassium as well as immune booster [2].

*Pyrus communis* is consumed fresh, canned as juice or dried and could also be fermented. When fermented, it is known as Perry or pear cider [4].

Fermentation refers to the slow decomposition process of organic substances induced by microorganisms, or by complex nitrogenous substances (enzymes) of plant or animal origin [5]. In this form of energy-yielding metabolism, an organic substrate, usually a carbohydrate is incompletely oxidised while an organic carbohydrate acts as an electron acceptor [6]. Fermentation may be classified based on the commodity fermented, the functions of food fermented and the microorganisms involved in the fermentation. The changes caused by fermentation may be desirable or disadvantageous.

Fruits fermentation may present several benefits to the people especially in the developing countries such as helping in improving food security by the removal of ant-nutritional factors from food, enhancement of nutritional value through increase in vitamin levels and improved digestibility and also increasing the lifespan of fruits [7]. The production of fermented food would also increase income, create employment opportunities as well as improve the health, cultural and social well-being of man [8]. This study therefore evaluated the effect of associated consortium fungal species on the natural fermentation of the mesocarp of *Pyrus communis* (Pear) which could be effective in harnessing the nutritional and health benefits of pear.

***Corresponding author:** Oluwatosin Adewusi Adedeji, Institute of Ecology and Environmental Studies, Obafemi Awolowo University, Ife, Nigeria, Tel: +2347030262067; E-mail: oluwatosinadedeji92@gmail.com

**Received** November 07, 2017; **Accepted** January 04, 2018; **Published** January 12, 2018

**Citation:** Adedeji OA, Audu TM (2018) Natural Fermentation of *Pyrus communis* (Pear) Mesocarp by Associated Consortium Fungal Species. J Microbiol Pathol 2: 102.

## Materials and Methods

### Sample collection

The pears were gotten from Ibaka market at Akungba Akoko, Ondo State. The pears were disinfected with ethanol and the peels were removed. The mesocarp was then aseptically removed using a spatula into a clean beaker to allow for aerobic degradation.

### Preparation and sterilization of media

Thirty nine (39) g of Potato Dextrose Agar (PDA) were weighed into conical flask and 100 ml of distilled water was dispensed into the flask to form a homogenized solution. It was then sterilized in an autoclave for 15 mins at 121°C, it was allowed to cool. 500 mg of antibiotic (Streptomycin) was added to inhibit bacterial growth and maintain acidity.

### Sample preparation, inoculation and isolation

Five sterile test tubes containing 9 ml of distilled water was set up on the test tube rack. Five (5) g of the fermented sample was measured and mixed into a beaker containing 50 ml of sterile distilled water, thoroughly mixed and covered with a foil paper. Isolation of fungi was done by serial dilution method [9]. Two sterilized petri dishes were removed from the autoclave and allowed to cool. It was labelled with the dilution number assigned for each assay with 2 and 5 for fungi identification (mould and yeast respectively). The already prepared PDA media was poured into each plate, swirled carefully to allow even distribution, allowed to gel, taped and inverted. The plates were incubated for 3 days at room temperature. The fungi species were identified according to their morphological and cultural characteristics [10]. All isolates were sub-cultured on new sterile plates of PDA to get pure fungus.

### Identification and characterization of isolates

**Lacto phenol staining:** The fungi isolates were identified by placing a drop of lacto-phenol cotton blue on a clean microscopic slide. With the aid of a pair of mounted inoculating needles, a small portion of the mycelium was removed from the fungal culture and placed in a drop of lacto-phenol cotton blue. A cover slip was gently placed on it and excess lacto-phenol blue was wiped by putting the slide between two filters and gentle pressure was applied around the cover slip. The slide was placed under the microscope and was viewed using 10x and 40x objective lens. This process as repeated for all isolates and they were all identified [11]. All the isolates were stored on PDA slant and kept at 4°C in the refrigerator for preservation.

**Gram staining:** Gram's staining was used to determine the morphological characteristics of the isolate. A heat fixed smear of 18-24 hr old culture of each fungal isolate was prepared on grease free microscopic slide. The smear was stained with crystal violet for 60 s and then rinsed off with Gram's iodine solution and allowed to react for 60 s. The smear was then washed with 95% ethanol to decolourize the smear until no violet colour was noticed. Another round of washing off with distilled water was done and the smear was counter stained with safranin for 60 s. The slide was then rinsed off with distilled water, air dried and observed under oil immersion objective lens of a microscope. Gram positive appeared purple while gram negative appeared pinkish [12].

**Biochemical test:** The pure culture of fungal isolates from the fermented pear mesocarp were sub-cultured to get young cultures between 18-24 hours old required for the biochemical test and were subsequently identified and characterized through standard biochemical tests with the aid of API 20E tests strips.

## Physical-Chemical Analysis

At every 5 days for the whole 50 days, samples were taken during the natural fermentation process and analysed for pH, titrable acidity, moisture content and reducing sugar.

### PH determination

The pH of the samples was determined according to the method of Five (5) grams of the sample were taken a put in a beaker containing 25 ml of distilled water and mixed thoroughly a filtered with what man No 14 filter paper. The pH of the filtrate was measured using Hanna pH meter.

### Total titrable acidity (TTA)

0.4 g of Na0H solution was measured and dissolved in 500 ml of distilled water. It was mixed evenly and allowed to settle 2.5 g of the mashed sample was suspended in 25 ml of distilled water and 2-3 drops of phenolphthalein was indicator was added. The content was titrated against 0.1 M of Na OH in a burette until there was a slight change in the colour. The result at which this change occurred was read on the burette and recorded. It can be measured using the formula,

$$TTA = \frac{Titre\ value\ x\ 0.02\ x\ 40}{2}$$

### Moisture content

A clean and well labelled Petri dish that has been dried in an oven was measured ($W_1$) 10 g of the pear sample was added to the dish and weighed ($W_2$). The dish was placed into the thermosetting oven at about 105°C for 24 hours. The dish was then transferred to the desiccator, cooled for about an hour and weighed ($W_3$). The weight loss after calculated in percentage was recorded as the moisture content (AOAC, 2000).

$$\%\ Moisture\ Content = \frac{Loss\ in\ Weight}{Weight\ of\ Sample\ before\ drying} x\ 100$$

$$= \frac{W_2 - W_3}{W_2 - W_1} x\ 100$$

### Total reducing sugar

Fifty (50) ml of 100% ethanol was mixed thoroughly with 50% distilled water and allowed to settle. Two (2) g of the mashed sample was measured into a sterile beaker containing 20 ml of the mixture (ethanol and water) and left to dissolve. The suspension was then sieved using what man no 1 filter paper into a sterile Mar Cartney bottles and kept to cool. This was done for each of degradation. The ethanol filtrate was diluted into $10^4$using 10-fld dilution method. Out of the dilution, 0.5 ml each was pipette into 11 test tubes. A 5% phenol solution was prepared by adding 95 ml of water to 5 g of phenol. 0.5 ml of the 5% phenol was added to each of the test tubes and allowed to stand for 10 minutes, and then, 2.5 ml of the concentrated sulphuric acid ($H_2SO_4$) was added from the burette. It was shaken thoroughly an allowed to cool. The optical density readings were taken at 490 nm using UV-visible spectrophotometer. The blank was prepared by adding 0.5 ml distilled water and 0.5 ml of 5% phenol to 2.5 ml of concentrated $H_2SO_4$ acid. This was used to standardize the spectrophotometer. The quantities of sugar were read from the standard curve of a known concentration of glucose [13].

## Results and Discussion

The fungal isolates found the mould species to have the greatest prevalence during the natural fermentation process with the mould count showing a gradual increment up to day 40 without any declination. The isolated moulds included *Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Aspergillus oryzae, Fusarium solani, Penicillium frequentas, Penicillium chrysogenum* and *Mucor mucedo* (Table 1). This is corroborated in the submission of as the ability of these fungal species to produce pectinase, an enzyme responsible for the fermentation of pectin a component of pear mesocarp. The isolated *Aspergillus* and *Penicillium* species are also common soil microflora capable of fermenting organic matter saprophytically. In this study, there were only three species of yeast present throughout the fermentation and are identified as *Saccharomyces cerivisae, Schizoaccharomyces pombe and Saccharomyces ludwigii* (Table 2) [14].

According to the results obtained from the naturally fermented pear mesocarp, the pH values remained at acidic range (Figure 1). The optimum pH for most microorganisms is near the neutral pH 7.0. Moulds and yeast are usually acid tolerant and are therefore associated with the spoilage of acidic food and fruits. Yeast can grow in a pH range of 4 to 4.5 and moulds can grow from pH 2 to 8.5 but favour an acid pH [15]. This is also in accordance with the submission of on the influence of pH on the growth of some toxigenic species of *Aspergillus, Penicillium* and *Fusarium* species which evaluated the optimum growth pH of *Aspergillus* and *Penicillium* to be around 4.5 to 5.0 [16].

Also, the percentage moisture content was found to be the highest at 73.4% and lowest at 1.5% (Figure 2). This is due to the weight loss in the pear mesocarp from the first day of natural fermentation to the last day when the sample has fully dried up.

Also, the rise and fall in the values of the total titratable acidity during the process of fermentation also permitted the growth of microbial species (Figure 3).

The frequency of occurrence of mould present showed *Aspergillus flavus* to have the highest occurrence at 63.63% and for the yeast *Schizosaccharomyces pombe* at 72.27% (Table 3). Their occurrence can be justified in the natural fermentation of pear mesocarp due to the presence of fermentable sugar, which is required as their basic substrate for growth [17,18].

The total reducing sugar measured, evaluated based on degree of absorbency, was observed to progressively decrease (Figure 4). There was however, a rise and fall in its value to the last day of fermentation. This observable trend could be due to the activities of the fermenting micro flora which have the ability to utilize the oil content of the

| Isolates | Cultural Characteristics | | Cellular Characteristics | | Probable Organism |
|---|---|---|---|---|---|
| | Hyphae | Edges | Septate | Spore and Conidia Arrangement | |
| A | Branched True Hyphae | Rough | Septate | Conidia is large radiating head, mostly globose and unnucleated | Aspergillus niger |
| B | Branched True Hyphae | Rough | Septate | Conidia head radiating on large conidiophores | Aspergillus flavus |
| C | Branched True Hyphae | Rough | Septate | Strictly columnar conidia heads | Aspergillus fumigatus |
| D | Branched True Hyphae | Rough | Septate | Slightly roughened or finely echinulate | Aspergillus oryzae |
| E | Branched True Hyphae | Rough | Septate | Smooth walled ellipsoidal or globose to sub-globose | Penicillium chrysogenum |
| F | Branched True Hyphae | Rough | Septate | Usually abundant, produced on elongated conidiophores | Fusarium solani |
| G | Branched True Hyphae | Rough | Non-Septate | Spores are either simple or branched. A densed layer of short repeatedly branched sporangiosphores | Mucor mucedo |
| H | Branched True Hyphae | Rough | Septate | Conidia is long, compact columns, globose to sub-globose | Penicillium frequentas |

**Table 1:** Morphology and microscopic characteristics of mold isolated from the naturally fermented *Pyrus communis* (Pear) mesocarp.

| Isolates Code | | I | J | K |
|---|---|---|---|---|
| Cultural Characteristics | Shape | Circular | Irregular | Circular |
| | Elevation | Raised | Raised | Flat |
| | Edge | Lobate | Rough | Lobate |
| | Consistency | Opaque | Opaque | Opaque |
| | Surface | Smooth | Dried | Slightly Dried |
| Cellular Characteristics | Spore | No Spore | No Spore | No Spore |
| | Gram Reaction | Positive | Positive | Positive |
| Biochemical Characteristics | Catalase Test | Positive | Positive | Negative |
| | Sugar Utilization | | | |
| | Glucose | AG | AG | AG |
| | Mannitol | Ag | Ag | AG |
| | Inositol | Ag | AG | Ag |
| | Sorbitol | Ag | AG | Ag |
| | Rhamnose | Ag | AG | AG |
| | Saccharose | AG | Ag | AG |
| | Melibiose | An | Ag | AG |
| | Amygdalin | An | An | An |
| | Arabinose | An | AG | An |
| Probable Organism | | Saccharomyces cerivisae | Schizosaccharomyces pombe | Saccharomyces ludwigii |
| AG: Full Colour Change, Full Gas and ¼ Gas Production, Ag: Colour Change, Acid and Partial Gas Production, An: Acid Production but No Gas. | | | | |

**Table 2:** Morphology and microscopic characteristics of yeast isolated from the naturally fermented *Pyrus communis* (Pear) mesocarp.

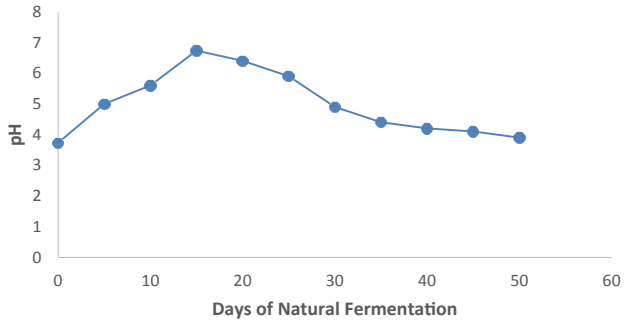


**Figure 1:** pH reading of the naturally fermented pear mesocarp.

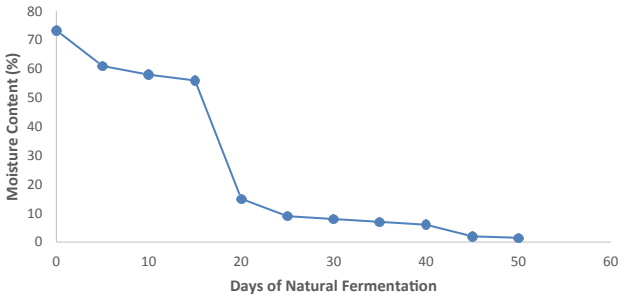


**Figure 2:** Change in moisture content of the naturally fermented pear mesocarp.

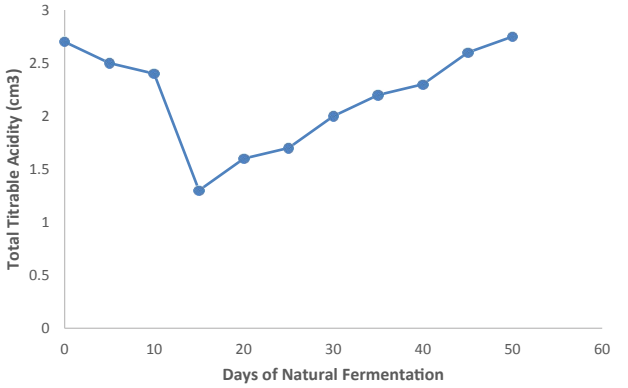


**Figure 3:** Change in total titratable acidity of the naturally fermented pear mesocarp.

substances as a soured of organic carbon, thereby leading to the gradual depletion of the sugar content present in the pear mesocarp [17].

The change in mould and yeast count during the natural fermentation generally shows that the mould had the greatest prevalence during the period of natural fermentation of the Pear with a mould count showing gradual increment up to the 40 day over the period of the 50 days than the yeast (Figure 5) [19,20].

## Conclusion

In conclusion, fungal species have been found to be closely associated with foods of plant origin and exhibit the ability to naturally ferment per mesocarp under favourable laboratory conditions. As a

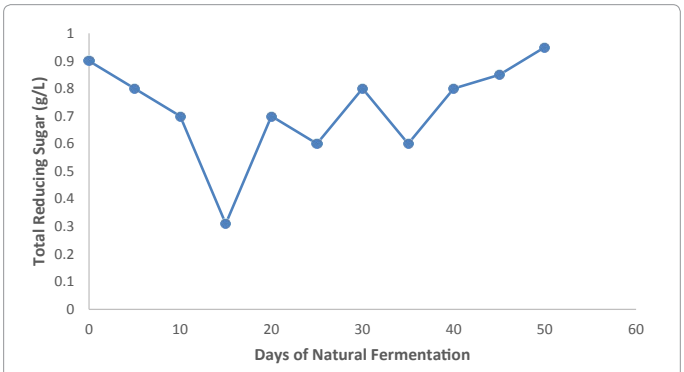


**Figure 4:** Change in total reducing sugar values of the naturally fermented pear mesocarp.

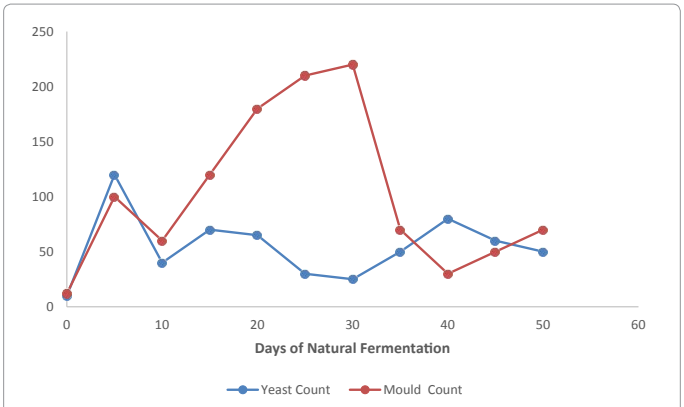


**Figure 5:** Change in mould and yeast count of the naturally fermented pear mesocarp.

| | Mould Isolates | | | | | Yeasts Isolates | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Days** | **A** | **B** | **C** | **D** | **E** | **F** | **G** | **H** | **I** | **J** | **K** |
| 0 | Present | Absent | Absent | Present | Absent | Present | Absent | Absent | Absent | Present | Present |
| 5 | Present | Absent | Absent | Absent | Present | Present | Absent | Absent | Absent | Present | Absent |
| 10 | Present | Present | Absent | Absent | Absent | Absent | Absent | Present | Absent | Absent | Absent |
| 15 | Present | Present | Present | Absent | Absent | Present | Absent | Present | Present | Present | Absent |
| 20 | Absent | Absent | Present | Present | Absent | Present | Present | Present | Absent | Absent | Absent |
| 25 | Absent | Absent | Present | Absent | Present | Absent | Absent | Absent | Absent | Absent | Absent |
| 30 | Absent | Absent | Present | Present | Absent | Present | Absent | Present | Absent | Present | Absent |
| 35 | Absent | Absent | Present | Absent | Absent | Present | Present | Absent | Present | Absent | Absent |
| 40 | Present | Absent | Absent | Absent | Absent | Present | Absent | Absent | Absent | Absent | Absent |
| 45 | Absent | Present | Present | Present | Present | Present | Absent | Present | Absent | Absent | Present |
| 50 | Absent | Present | Present | Present | Absent | Absent | Absent | Absent | Absent | Absent | Absent |
| % Frequency | 45.5 | 36.36 | 27.3 | 63.6 | 45.5 | 18.18 | 18.18 | 36.3 | 27.3 | 72.7 | 36.4 |

**Table 3:** Frequency of occurrence of fungi isolates from the naturally fermented *Pyrus communis* (Pear) mesocarp.

result of this, environmental pollution caused by this can be countered by the introduction of the specific fungal species.

The use of fermentation processes to produce microbial biomass has several advantages over other unconventional process and thus, instead of causing wastage and pollution around the environment, fermented fruit can be employed in the:

- Single cell protein
- Production of organic acids
- Wine production (Pear wine)
- Artificial production of vinegars.

### References

1. Colaric M, Stampar F, Hudina M (2007) Content levels of various fruit metabolites in the Conference pear response to branch bending. Scientia Horticulture 113: 261-266.
2. Phyllis AB, James FB (2000) Prescription for Nutritional Healing (3rdedn).
3. Phyllis AB (2003) Prescription for Dietary Wellness. Penguin 67: 14-97.
4. Tanrioven D, Eksi A (2005) Phenolic Compounds in Pear Juice from Different Cultivars. Food Chemistry 93: 89-93.
5. Walker PMB (1988) Chambers Science and technology Dictionary chambers Cambridge University Press UK.
6. Adams MR (1990) Tropical Aspects of Fermented Foods. Trends Food Science and Technology 141-144.
7. Parades-Lopez O (1992) Nutrition and safety Considerations in Application of Biotechnology to Traditional fermented Foods report of an Adhoc Panel of the Board on Science and Technology for International Development National Academy Press, Washington DC USA.
8. Battock MJ (1992) Street Foods and Development in Bangladesh Food Laboratory News Volume 8: 2 Germany.
9. Anega KR (2005) Experiment in Microbiology Plant pathology and biotechnology. New Age International Publishers New Delhi.
10. Rhode B, Hartman G (1980) Introducing Mycology by Example Schering Akti-Engesellschaft, Hamburg 111.
11. Domsch KH, Anderson T (1980) Compendium for Soil.
12. Olutiola PO, Famurewa O, Sotang HG (1991) an Introduction to General Microbiology. A Practical Approach. Cab Heidelberg Verilog Santali Dreukerec Gmbh, Heidebersemone 1-17.
13. AOAC (2000) Official method of analysis. Association of Official Analytical Chemist Seventh Edition Virginia USA 12-98.
14. Vibha (2010) Effect of Fungal Metabolites and Amendments on Mycelia Growth of Rhizoctonia solani. J Plant Prot Res 50: 93-97.
15. Mountey GJ, Gould WA, Weiser HH (1988) Practical Food Microbiology and Technology. AVI Books Van No strand Reinhold Company New York USA.
16. Wheeler KA, Hurdman BF, Pitt JI (1991) Influence of pH on the Growth of Some Toxigenic Species of Aspergillum, Penicillium and Fusarium. International Journal of Food Microbiology 12: 114-150.
17. Ofodile LM, Doherty VF, Oyetola OT, Oguntuyi BC, Kolawole JO (2011) Biodegradation of Orange and Pineapple Peels for Production of Single Cell Protein. Journal of Environmental Issues 1: 31-36. Yaba College of Technology Lagos Nigeria.
18. Mike B, Sue A (1998) Fermented Fruits and Vegetables - A Global Perspective. FAO Agricultural Services 134.
19. Kovac B (1997) The Use of the mould Rhizopus oligosporus in Food technology and Biology.
20. Michael M, Joseph P (2005) The Encyclopedia of Healing Foods.