International Journal of Biological Chemistry

ISSN 1819-155X

Academic Journals Inc.

www.academicjournals.com

International Journal of Biological Chemistry 1 (4): 237-243, 2007
ISSN 1819-155X

Effect of Fermentation on the Microbiology and Mineral Composition of an Edible Mushroom *Termitomyces robustus* (Fries)

B.K. Bello and B.J. Akinyele
Department of Microbiology, Federal University of Technology,
P.M.B. 704, Akure, Ondo State, Nigeria

Abstract: *Termitomyces robustus* was subjected to natural fermentation at 30±2°C. The associated microorganisms, pH and mineral content were determined using standard methods. The bacteria isolates include *Aerococcus viridans*, *Bacillus licheniformis*, *Bacillus subtilis*, *Leuconostoc mesenteroides*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia* and *Staphylococcus aureus*. while the fungi are *Aspergillus fumigatus*, *Aspergilus flavus*, *Aspergillus niger*, *Brachysporum nigrum*, *Candida albican*, *Lemonniera aquatica*., *Paecelomyces* sp. *Penicillium italicum*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae*. increase in bacteria load was observed from 0 and 48 h while decrease was observed from 72 h of fermentation. The microbial counts ranged from 8.0×10^6 to 5.7×10^6 cfu g^{-1}. increase in fungi count ranged between 1.0×10^6 spore g^{-1}. the pH values decreased as fermentation progresses. Salted-fermented samples had the highest mineral content.

Key words: Leuconostoc mesenteroids, proteus vulgaris fermentation, edible, mushroom, *Terimitomyces robustus* (Fries)

INTRODUCTION

Mushrooms have been used as food supplement in various cultures. They can be cultivated, eaten for their edibility and delicacy and are considered as source of proteins, vitamins, fats, carbohydrates, amino acids and minerals (Jiskani, 2001). Essential amino acids such as lysine and cytosine are also present as well as water soluble vitamins and minerals in mushroom. The energy value of mushroom varies according to species (Buigut, 2002). Edible mushrooms are used extensively in cooking, because they are highly nutritious, they are rich in protein, vitamin as well as some mineral such as Calcium, Pottasium, Magnesium and Iron (Jiskani, 2001).

Termitomyces R. Heim is a paleotropical genus of agarics obligately symbiotic with termites belonging to the subfamily Macrotermitinae (Isoptera) (Rouland-Lefevre *et al*., 2002). The Termitomyces species are usually characterized by the termite association, pinkish spore print, prominent perforatorium on the pileus and the subterranean pseudorhiza connected to the comb in the termite nest. *Termitomyces* species were generally rich in minerals such as potassium, calcium, magnesium, iron and manganese (Mattila *et al*., 2001).

Fermented food constitute a large proportion of the diet of Nigerians, such foods are important because of their increased nutritional values as well as improved flavour and aroma characteristics. Many of fermented products result in new and desired products (Aderiye and Ogunjobi, 1998). Fermented foodstuffs remain key constituent of diet in many part of the world (Ogunshe *et al*., 2006).

Fermentation has some uses exclusive to foods. Fermentation can produce important nutrients or eliminate antinutrients. Food can be preserved by fermentation, since fermentation uses up food

Corresponding Author: B.K. Bello, Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Ondo State, Nigeria

energy and can make conditions unsuitable for undesirable microorganisms. For example, in pickling the acid produced by the dominant bacteria inhibit the growth of all other microorganisms. The increased nutritive values of fermented foods are due to the breakdown of digested sugars, free fatty acids, amino acids as well as synthesis of certain vitamins (Obizoba and Attai, 1991). However, this research is aiming at: evaluating the effect of salting and blanching on the microbial load and types of microorganisms present in the samples and the effect of fermentation on the mineral composition of the mushroom.

MATERIALS AND METHODS

Mushroom (*Termitomyces robustus*) were purchased from Igbatoro, a farm settlement near Akure, Ondo State, Nigeria.

Preparation of Samples

The purchased *Termitomyces robustus* were divided into three portion for different treatment. (blanched, salted and untreated (control).

The untreated sample were prepared by weighing fresh sample of the mushroom into four portion of 100 g each, also, the blanched sample was prepared by pouring boiled water that has been cool to a temperature of 60°C on 400 g of the fresh sample and allow to stay four 10 min after which it was then divided into four portion of 100 g each, The salt treated sample was prepared by soaking 400 g of the mushroom in 1000 mL of distilled water in which 100 g of NaCl has been dissolved, the mushroom was allow to stay in the salt solution for 20 min before divided into four portion of 100 g each. From the first portion of each treatment (unfermented samples),microbial load, isolation of microorganism, pH and mineral composition were carried out.

Solid substrate fermentation processing was employed 100 g of the clean untreated samples were properly wrapped in aluminum foil paper this was done in three places and labeled day 2, day 4 and day 6. All the sample were kept in a clean sterile beaker each and properly sealed in the laboratory at room temperature over a period of 6 days for natural fermentation to occur with the natural microflora in the sample. Similar procedure was done on the salt treated sample and the blanched samples. Eighty gram from the untreated samples were then taken, the salt treated sample as well as the blanched were also taken, microbial load, isolation of microorganism using serial dilution and pour plate methods. The fresh, blanched and salted samples were serially diluted and plated out on nutrient agar for bacteria and potato dextrose agar for fungi. The resultant colonies after incubation at 37°C for 24 h for and 27°C for 3-5 days for fungi were characterized and identified with the criteria of Holt (1994) and other convectional methods for bacterial identification. Isolated fungi were identified based on the observation of cultural and morphological characteristics, nature of growth rate, colour of colony and sporulation (Onion *et al*., 1981). pH was measured using pH meter and mineral analysis were investigated at 2 days interval for a period of 6 days. Atomic absorption spectrophotometer (Pye Unicam SP9 AAS) was used for the determination of calcium, iron, magnesium, potassium, manganese and zinc etc (Kine *et al*., 1991).

RESULTS

The change in microbial population during natural fermentation of *Termitomyces robustus* at different time interval (Table 1). The initial bacterial count obtained from unfermented fresh, blanched and salted sample at 0 day were 4.9×10^6, 1.5×10^6 and 8.0×10^6 cfu g^{-1}. The fresh sample record the highest bacterial load 4.9×10^6 cfu g^{-1}, while the salted record the least 8.0×10^6 cfu g^{-1} at days 2 of fermentation, the untreated sample still had the highest bacterial load, 5.7×10^6 cfu g^{-1} while that of

Table 1: Total bacterial count obtained during natural fermentation of *Termitomyces robustus*

	Total viable	Bacterial count (cfu g^{-1})	
Fermentation period (day)	Fresh (untreated sample) (cfu g^{-1})	Blanched	Salted
0	4.9×10^6	1.5×10^6	8.0×10^6
2	5.7×10^6	4.5×10^6	1.9×10^6
4	5.4×10^6	4.0×10^6	1.8×10^6
6	5.0×10^6	3.3×10^6	1.5×10^6

blanched is 4.5×10^6 and salted 1.9×10^6. At days 4 the bacteria load has record a decrease in values for the three samples 5.4×10^6 for untreated sample, 4.0×10^6 for the blanched sample and 1.8×10^6 for the salted sample. At days 6 the bacteria loads were 5.0×10^6 for the untreated, 3.3×10^6 for blanched and 1.5×10^7 for salted sample.

Generally, there was an increase in bacterial load between day 0 and days 2 of fermentation, between days 2 and 6 a decrease in value was observed.

The bacteria species isolated and identified from the unfermented fresh sample are *Bacillus licheniformis*, *Bacillus subtilis* and *Pseudomonas aeruginosa*. From the unfermented salt treated and blanched samples, *Staphylococcus aureus* and *Bacillus licheniformis* were identified, respectively. From untreated fermented sample; *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Proteus vulgaris* and *Bacillus subtilis* were isolated. Three isolates were identified from salt treated fermented samples and are *Bacillus licheniformis*, *Aerococcus viridians* and *Lenconostoc mesenteroides*. From blanched fermented sample *Staphylococcus aureus* and *Bacillus subtilis* were isolated..

The changes in fungal population during natural fermentation of *Termitomyces robustus* for 6 days is described in Table 2. Fungi count of the untreated sample ranged between 6.0×10^6 to 1.0×10^6 spore g^{-1}, while in the salted samples it ranged between 1.0×10^6 to 5.0×10^6 spore g^{-1}. The blanched sample fungal load ranged between 1.0×10^6 to 5.0×10^6 spore g^{-1}. between day 0-days 2 the fungi count of the fresh sample showed an increase from 6.0×10^6 to 7.0×10^6 spore g^{-1}, the count increase to 8.0×10^6 spore g^{-1} at days 4 and this remain till termination of the experiment. For the salted sample the count increased from 1.0×10^6 at day 0 to 3.0×10^6 spore g^{-1} at days 2, from day 3 to days 4, an increase was also observed from 3.0×10^6 to 4.0×10^6 spore g^{-1}. At days 6 the count increase to 5.0×10^6 spore g^{-1}. the blanched sample observed increase in fungal population alongside days of fermentation.

Ten species of fungi were isolated and identified from both the unfermented and fermented samples; four were isolated from unfermented fresh, salt treated and the blanched sample. (*Saccharomyces cerevisae*, *Aspergillus fumigatus*, *Rhizopus stolonifer* from fresh sample, *Lemonniera aquatica* from salted and blanched sample. Isolated from untreated fermented sample are *Penicillium italicum*, *Aspergillus flavus* and *Paecelomyces* species. From salt treated fermented samples are *Candida albicans*, *Brachysporum nigrum*, *Aspergillus niger*. and from the blanched fermented samples, are *Penicillum italicum* and *Aspergillus flavus*.

Table 3 shows the mineral content of both the fermented and the unfermented sample of *Termitomyces robustus*

Magnesium had the least content for blanched and salted samples on day 0, 309.78 for blanched and 338.75 for salted sample and the highest content on day 6. 462.30 for blanched and 488.42 for salted. Untreated sample had the least magnesium content on days 2, 294.49 and highest on days 6, 385.32. Zinc had the highest content for the untreated, blanched and salted sample on day 0, 575.90, 565.90 and 571.90 for the three samples. A drastic decrease was observed between days 2 and days 6. Calcium had the highest value for all the samples on days 6. 325.22 for untreated, 350.22 for blanched and 399.92 for the salted and the least value on day 0. 221.24 for fresh, 220.24 for blanched and 210.34 for salted sample. Iron had the least value on day 0, 168.04 for untreated, 158.04 for

Table 2: Total fungal count obtained during natural Fermentation of *Termitomyces robust*

	Total viable	Fungi count (spore mL^{-1})	
Fermentation period (day)	Fresh (untreated sample) (spore mL^{-1})	Blanched	Salted
0	6.0×10^6	1.0×10^6	1.0×10^6
2	7.0×10^6	3.0×10^6	3.0×10^6
4	8.0×10^6	3.0×10^6	4.0×10^6
6	8.0×10^6	5.0×10^6	5.0×10^6

Table 3: Minerals (mg/100g) and pH content of the fermented and the unfermented *Termitomyces robustus*

Days of fermentation	Samples	Mg	Zn	Ca	Fe	K	Mn	Na	pH
Day 0	Fresh (untreated)	348.75	75.90	221.24	168.04	405.33	131.07	335.28	5.43
	Fresh (Blanched)	309.78	65.90	220.25	158.04	205.33	121.07	337.28	5.42
	Fresh (salted)	338.75	71.90	210.34	160.04	305.33	129.07	335.28	6.45
Day 2	Untreated	294.49	65.23	311.98	170.36	256.13	140.85	322.15	4.63
	Blanched	318.75	56.94	336.79	160.47	410.54	130.66	338.30	5.05
	Salted	357.14	64.70	323.94	165.85	349.40	135.54	409.54	4.72
Day 4	Untreated	298.61	55.94	320.28	178.98	365.96	140.60	319.20	4.48
	Blanched	322.82	62.36	345.03	173.50	305.92	133.89	398.76	4.70
	Salted	437.04	63.08	325.93	180.68	401.38	142.60	415.28	4.32
Day 6	Untreated	385.32	45.95	325.22	195.17	300.72	153.48	238.24	4.22
	Blanched	462.30	65.07	350.22	185.10	343.62	150.72	423.83	4.60
	Salted	488.42	76.30	399.92	216.86	378.17	162.89	467.12	4.03

blanched and 160.04 for the salted sample. The highest value was however, observed on days 6, 195.17 for untreated, 185.10 for blanched and 216.86 for the salted sample. Potassium had the values of 405.33, 205.33 and 305.33 for the untreated, blanched and salted samples on day 0, on days 2, blanched and salted samples increased to 410.54 and 349.40, while untreated samples reduced to 365.96, on day 4, untreated and salted samples increased to 365.96 and 401.38 while the blanched reduce to 305.92. On day 6, the blanched sample increased to 343.62 while the untreated and salted reduced to 300.72 and 378.17. The manganese content ranged between 131.07 to 153.48 for the fresh sample, 121.07 to 150.72 for the blanched and 129.07 to 162.89 for the salted sample. Blanched and salted samples had the least sodium content on day 0, 337.28 for blanched and 335.28 for salted sample, there highest values was observed on days 6, 423.83 for blanched and 467.12 for salted sample. Untreated sample had the highest value on day 0. 335.28 and least value on days 6, 238.24. The pH value ranged between 4.03 to 6.45.

DISCUSSION

Mushrooms are usually consumed after processing either by salt treating or blanching which tends to increase their palatability, digestibility, keeping qualities and safety. During processing, some nutritive value may be reduced and some increased while in some cases antinutritional substances may be reduced depending upon the processing methods (Bradbury and Holloway, 1998).

It can be observed from the results that there was a decrease in bacterial load between days 2 and 6 of fermentation and this may be due to preferential conditions often manifested by many microorganisms. Hence, some microorganisms could be inhibited by the metabolic products of others, some are able to utilize these metabolic products for their growth. The decrease in bacteria load alongside days of fermentation could be due to the growth of Bacillus licheniformis which produces bactracin and polymycin antibiotics able to inhibited the growth of other bacteria (Jones, 1993). The increase in fungal count throughout the period of fermentation may also be due to the optimum pH obtained throughout the period of fermentation which was favourable for the growth of fungal because fungi are only tolerant to acid.

The result of this study however, showed that untreated fermented sample had more microbial load than the blanched and salt treated samples. This might be as a result of the blanching effect and high concentration of salt on some organism in the treated samples. In all the samples, the most predominant bacteria were *Bacillus* species and *Pseudomonas* species and were isolated from both fermented and unfermented samples. This may be due to the fact that these bacteria were proteolytic and normal flora of the sample. *Bacillus* species can thrive on various kind and vast number of food and food stuff thus showing the ability of their spores to withstand high temperature (Barber and Achinewu, 1992).

Many proteinaceous oil seeds have also been fermented by *Bacillus* and *Staphyloccocus* species and several fermented product rely on the participation of various *Bacillus* species (Beamont, 2002) in the fermentation of, dawadawa and ogiri. Therefore the isolation of *Bacillus subtilis* as one of the predominant bacterial flora in the fermenting samples of *Termitomyces robustus* is in accordance with previous works on fermented food.

Bacillus species could be proteolytic, lipolytic and amylolytic depending on the type involved in fermentation as in the production of Aisa, a food condiment from Albizia saman (Ogunshe *et al*., 2006). *Staphyloccocus* species which was isolated from the fermented blanched sample is an indicative of human contamination because *Staphylococcus* species are found to be present on human skin. Prescott *et al*. (1999) also suggested that some Bacillus species inhabit high temperature habitats. Jones (1993) reported that these bacteria produce spores, which are heat resistance thus making them to survive extremely high temperature. Isolation of Leuconostoc mesenteroides from salt treated fermented sample shows that the organism can survive in salt solution. This medium makes it a better fermentable substrate for the organism.

The moisture content in the sample activate the proteolytic enzyme of the microbes such as *Aerococcus* specie likely to be trapped from the air and *Proteus vulgaris*, *Leuconostoc* species, *Bacillus* species and *Pseudomonas* species which are natural microflora of the *Termitomyces robustus*, which in turn triggers the lipolytic enzymes and the hydrolytic enzymes which degrade starch to sugar thus the possible fermentation of the mushroom sample (Barber and Achinewhu, 1992).

It can also be observed that *Penicillium italicum* and *Aspergillus flavus* were predominant in all the samples. This shows that the fungi may be likely part of the normal flora of the mushroom sample and their involvement in the fermentation, since these fungi are capable of producing hydrolytic enzymes which can hydrolyze starch, proteinases which can break down protein and lipases which can breakdown lipid in the sample. Nouts and Rombouts (1995) reported that *Aspergillus* species and *Rhizopus* species are important in the fermentation of soyabean in the production of Tempe an Indonesian food. He also reported that vegetable is fermented predominantly with the spores of Rhizopus species.

The pH of the samples showed decreased in values as fermentation progressed. This shows that fermentation had caused the reduction in pH as a result of the production of more lactic acid by the activities of microorganism utilizing the sugar present in the samples to produce acid and ethanol. The acid produced, leads to decrease in pH which consequently, resulted in decreasing microbial load. Rainbault (1998) pH culture may change in response to metabolic activities. The most obvious reason is the secretion of organic acids such as citric, acetic or lactic which will cause pH to decrease.

The value of the various minerals obtained from both the unfermented and the fermented samples shows significant differences either increasingly or decreasingly. From the results it also shows that the sample may serve as a good source of Calcium, Magnesium, Iron and sodium because their values has significantly increased and this may satisfy the nutritional need for consumers because their values are reasonably high. These minerals if present in diet is important for the metabolic activities, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance among others.

The reduction in values of some mineral such Zn, Ca and Mg of the fresh fermented sample may be due to the fact that microorganisms utilize these mineral elements for their growth. Rainboult (1998) stated that during fungal fermentation, the fermenting fungi utilize mineral salt for metabolic activities. The increase in some of the mineral content such as Ca, Mg, K, Mn and Na of the blanched and salted fermented sample may be as a result of the reduction in the antinutrient content as a result of the processing method. This antinutritional content normally bind with some metal like Ca and Mg and prevent their bioavailability. Oboh and Akindahunsi reported that processing method such as fermentation and blanching greatly reduced antinutrient content such as phytate and oxalate that bind with some metal preventing their bioavailability.

It may also be due to the reduction in the microbial load which occur as a result of the decrease in pH as fermentation progresses which makes the environment unconducive for the proliferation of some microorganism to utilize the mineral availability.

However, products from natural fermentation had a comparative improvement in relation to mineral contents when compare with unfermented samples. Blanching or salting prior to fermentation significantly increases the mineral bioavailability of the fermented products.

CONCLUSIONS

The results of the analysis of the fermented and unfermented samples of *Termitomyces robustus* as reported in this project shows that the samples were very rich in some mineral such as calcium, magnesium, iron, manganesse and sodium, because their values had significantly increased after fermentation. These minerals in diet are important for metabolic reaction, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance among others as reported by Mattila *et al*. (2001).

It is important to establish the fact that fermentation of mushroom slightly soften the spongy texture without altering the colour and flavour. Mushroom fermented along with high concentration of salt could successfully be converted to sauce of desirable physicochemical characteristic and increase shelf life. It is however, important to conclude that salt treating of the mushroom sample along with fermentation will not only improved the nutrient content, but also serve as a valuable and economical means of preserving it for future use.

REFERENCES

Aderiye, B.I. and A.A. Ogunjobi, 1998. Fermentation of yam: Microbiology and sensory evaluation of cooked fermented yam tissues. Plant Food for Human Nutrition. Nig. Food J., 52: 49-54.

Barber, L.I. and S.C. Achinewhu, 1992. Microbiology of Ogiri production from melon seeds. (*Citrus vulgaris*). Nig. Food J., 10: 129-135.

Beamont, M., 2002. Flavouring composition prepared by fermentation with *Bacillus* sp. Int. J. Food Microbiol., 75: 189-196.

Bradbury, J.H. and W.D. Holloway, 1998. Chemistry of tropical root crops: Significant for nutrition. Agriculture in pacifies: ACIAR. Monograph No. 6, pp: 201.

Buigut, S.K., 2002. Mushroom production in sustainable small-scale farming system, opportunities and constraints: A survey of Uasin Gishu district. In: Proceedings of the Holticulture Seminar on Sustainable Hroticultural Production in the Tropics at Jomo Kenyatta University of Agriculture and Technology, Juja, Kenya, pp: 1-5.

Holt, G.J., 1994. The Shorter Bergey's Manual of Determinative Bacteriology. 8th Edn., The Williams Company. Wavenly Press. Int. Baltimore, Md, USA.

Jiskani, M.M., 2001. Energy potential of mushrooms. The Economic and Business Review, Oct. 15-21, P. IV.

Jones, D.G., 1993. Exploitation of Microorganisms. 1st Edn., Chapman and Hall. London, pp: 248-267.

Kine, B.B., O.U. Eka, C.Y. Aremu and E.U. Essien, 1991. Comparative studies on the food qualities of African yam bean and cowpea seeds. Trop. J. Applied Sci., 1: 65-70.

Mattila, P., K. Konko, M. Eurola, J.M. Pihlava, J. Astola, L. Vahteristo, V. HIetaniemi, J. Kumpulainen, M. Valtonen and V. Pironen, 2001. Contents of vitamins, mineral elements and some phenolic compounds in cultivated mushrooms. J. Agric. Food Chem., 49: 2343-2348.

Nouts, M.J.R. and F.M. Rombouts, 1995. Microbial fermentation in the production of plant foods. J. Applied Bacteriol. (Symposium Supplement), 79: 108.

Obizoba, I.C. and J.V. Attai, 1991. Effect of soaking, sprouting, fermentation and cooking on the nutrient composition and some antinutritional factors of sorghum (Guineabia) seeds. PI. fd. Hm Nutr., 42: 13-23.

Ogunshe, A.O., A.E. Ayodele and I.O. Okonko, 2006. Microbial studies on Aisa: A potential indigenous laboratory fermented food condiment from Aibizia saman (Jacq) F. mull. Pak. J. Nutr., 5: 51-58.

Onions, A.H.S., D. Allsopp and H.O.W. Edddins, 1981. Smith's Introduction of Industrial Mycology. 7th Edn., The Pitman Press, Bath., pp: 65-92.

Prescott, L.M., J.P. Hawley and A.D. Klein, 1999. Microbiology. 4th Edn., McGraw Hill, USA.

Rainbault, M., 1998. General and microbiological aspect of solid substrate fermentation. Electronic J. Bacteriol., 1: 3-14.

Rouland-Lefevre, C., M.N. Diouf, A. Brauman and M. Neyra, 2002. Phylogenetic relationships in *Termitomyces* of ITS: A first approach to elucidate the evolutionary history of the symbiosis between fungus-growing termites and their fungi. Molecular Phylogenetics and Evolution, 22: 423-429.