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Characterization of A FIIRO-Fabricated Stainless Steel, Solid State Fermentor by the Fermentation of Boiled Melon Seeds (*Cucumis melo*) into Ogiri.

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ABSTRACT

Results of the physico-chemical and biochemical parameters obtained from the fermentation of boiled melon seeds into ogiri in the FIIRO-fabricated stainless steel, solid state fermentor was observed to conform to observations in previous experiments on fermentation of boiled melon seeds and other leguminous seeds by various authors. Being a non-heated, double walled, and lagged piece of incubating chamber designed to enable a hygienic and controlled fermentation and to conserve the heat generated during fermentation, heat generated during fermentation was observed to be conserved leading to maintenance of a steady temperature of fermentation within the chamber. Invasion by rodents, flies, cockroaches and pathogens during fermentation was eliminated, thus leading to a hygienic fermentation. Thus, the fermentor can be said to meet the desired requirements for fermentation of melon seeds in the solid state and may also be used for the solid state fermentation of other leguminous seeds. This fermentor may thus be utilized to achieve a hygienic fermentation and also production of an acceptable ogiri of non-obnoxious odour and flavor and one that will not pose health hazard to the consumer.

Key words: controlled fermentation, solid state fermentation, boiled melon seeds, ogiri, stainless steel fermentor.

1.0 Introduction

Ogiri is a product of the fermentation of boiled melon seeds (family, *Cucurbitaceae)*. It is an important food condiment in the Eastern, South-Western and Middle belt regions of Nigeria and it is used as a flavouring agent in sauces and stews that serve as accompaniment to starchy root and vegetable diets. It is also added to other preparations as seasoning e.g. in staple foods such as "ikokore" – a Nigerian local pottage. The traditional preparation of ogiri from melon seeds is by the method of uncontrolled solid state fermentation, Achi, (2005) and it involves boiling the raw seeds after which they are dehulled, and then boiled again to soften seeds for fermentation. The softened seeds are wrapped in leaves, kept in sacks and incubated near earthen pots, to allow for fermentation for a period of three to five days or longer after which the mash is dried and milled to a smooth paste, the 'ogiri'. This method of fermentation is uncontrolled as it is without proper protection from invasion by rodents, cockroaches, flies and pathogenic/spoilage organisms. This uncontrolled fermentation coupled with an unhygienic environment of preparation could result in the production of an ogiri with variable quality and unacceptable aroma, short shelf - life and one that can pose health hazards to the consumers.

Fermentation is a key unit operation in the production of ogiri. It is the process in which microorganisms degrade the food material or substrate, producing soluble nutrients, flavour or aroma components, a change in pH and texture of the material amongst other things in a process of digestion. Fermentation could be spontaneous or by direct inoculation of specified microorganisms into the food material. The conditions for growth of the microorganisms and the modification or degradation of substrate are established by the fermentor and these include basically the temperature within the fermentor and aeration where applicable. The process of fermentation in leguminous seeds is indicated by formation of mucilage, softening of the seeds and overtones of ammonia produced as a result of food degradation during fermentation (Onyenekwe *et al*., 2012). Stanbury and Whitaker, 1984 states that the main function of a fermentor is to create a controlled environment for the growth of microorganisms in order to obtain a desired product. Should the fermentor be equipped with a heating element and thermostat, then, it must support isothermal digestion of the melon.

The stainless steel, solid state fermentor fabricated by the Federal Institute of Industrial Research, Oshodi (FIIRO), Lagos in Nigeria is a non-heated, double walled, and lagged piece of incubating chamber that can be used for the fermentation of food materials of leguminous origin in the moist solid state. It is designed to enable a hygienic and controlled fermentation and to conserve the heat generated during fermentation. This will provide a fairly constant temperature for fermentation without loss of heat to the environment, thus providing a closed system. It can be utilized when kept within a building or outside of one, regardless of the prevailing atmospheric temperature. The incubating chamber is a piece of equipment that can be partitioned for varied utilization if the entire piece is too large for the volume of melon to be handled.

Various studies have been documented on ogiri viz: Microbiology and amino acid composition of ogiri (Odunfa 1981b), the biochemical changes taking place during the production of ogiri (Odunfa, 1983), Microbiology of ogiri production (Barber and Achinewu 1992) and Soluble nutrient production during the fermentation of melon varieties into ogiri using different leaf types (Onawola *et al,* 2011) to mention a few. However, there has been no information on the use of a non-heated incubating chamber for a possible hygienic and controlled fermentation of boiled melon seeds into ogiri and the accompanying soluble nutrient production, considering its uncontrolled and unhygienic nature as carried out by the traditional producers/village entrepreneurs. The objective of this study therefore is to investigate the possible use of a FIIRO-fabricated stainless steel, solid state fermentor for the purpose of a hygienic and controlled fermentation, and also to characterize the equipment.

2.0 Materials and Methods

2.1 Materials

A variety of unshelled melon seeds (*Cucumis melo*) was obtained from a local market in Mushin, Lagos state of Nigeria.

Leaves from the plant of *Musa spp*. were obtained from the garden of the Federal Institute of Industrial Research, Oshodi, Lagos in Nigeria.

Non-heated, stainless steel, double-walled and lagged solid state fermentor, fabricated by the Federal Institute of Industrial Research, Oshodi, Lagos, Nigeria.

2.2 Methods

2.2.1. Preparation and fermentation of melon seeds.

This was carried out according to the method of Onawola, *et al.,* 2011, but with slight modification. The boiled melon seeds in their wrappers were not further wrapped in cotton material and incubation was carried out at the prevailing ambient temperatures within the fermentor for a period spanning 0 to 168 hours (0-7 days).

The fermentor was disinfected with 70% ethanol just before use. Sixty percent (60%) of the fermentor was to be utilized and this was then loaded to about 70% capacity in a total of three trays. Loading was two-third, one-third and full capacity respectively for the individual trays. Fermentation was by a spontaneous process and sampling packages were done so as to allow 'a package – a day sampling'. Thermometers were fitted within the interior of the fermentor to determine the environmental / ambient temperature prevailing at time of fermentation and the average of the thermometer readings was taken as the temperature of the interior of the fermentor. This was taken to represent the temperature of incubation for the fermenting melon mash. At the time of sampling, temperatures of fermenting melon mash/samples were taken by the direct introduction of a thermometer into the mash, while preventing loss of heat.

2.3 Analysis

2.3.1. Proximate and mineral compositions. These were determined on raw and unfermented melon seeds after de-shelling according to the method of the Association of Official Analytical chemists (AOAC, 1995).

2.3.2. Preparation of soluble extracts. Samples of the fermenting melon seeds were collected at different processing periods of 0, 24, 48, 72, 96,120,144 and 168hours respectively and dried in a hot air oven at 60°C to constant weight. These were grinded and 5g of each was weighed into a 250ml conical flask and 50ml of 70%v/v alcohol solution was added to each. The mixture was shaken together for maximum extraction and allowed to settle. This was filtered and the resultant filtrate was washed with 20ml of petroleum ether in a separating funnel for fat extraction by vigorous shaking after which the layers were allowed to settle, the ether layer forming the upper layer was discarded. The alcohol layer was collected as extract for soluble/reducing sugar and amino acid determinations.

2.3.3 Quantitation of residual total soluble/reducing sugars present. This was estimated according to the method of Onawola, *et al*., 2012 after having done the following. To an equal volume of ethanolic extract for soluble sugar determination was added an equal volume of cold 10%w/v tetrachloroacetic acid solution and the resultant solution was cooled and centrifuged at 5000rpm for 10 min. The clear supernatant obtained was used for analysis. Analysis was carried out in triplicate and amount of soluble sugars present was calculated from a standard curve based on known concentrations of glucose.

$$\frac{\text{conc. corresponding to O.D. X dil.factor}}{\text{weight of sample taken}} = mg\ glucose/g$$

2.3.4 Quantitation of residual total free amino acids. This was determined on portions of ethanolic extract by the Ninhydrin reaction method of Rosen *et al*., 1957. The amount of free amino acids present was calculated from a standard curve based on known concentrations of leucine in triplicate determinations.

$$\frac{\text{conc. corresponding to O.D. X dil.factor}}{\text{weight of sample taken}} = mg\ leucine/g$$

2.3.5 pH. This was determined on a 10%w/v sample of ogiri according to the method of Onawola *et al*., 2012

2.3.6 Microbial count. This was determined according to the method of Harrigan and MacCance, 1976.

2.3.7 Moisture content. This was determined according to the method of Onawola, *et al*., 2012. Determination was carried out on 10g samples.

3.0 Results and Discussion

Table 1 shows the result of the Proximate and mineral analysis on raw unfermented melon seeds (*Cucumis melo*). It was observed that the melon variety is rich in fat and protein and high in crude fibre.

Table 2 shows the result of the temperatures of the fermentor interior and fermenting melon mash during fermentation. A steady increase was observed in the temperature of the fermentor interior over 48 hours, however, a decline which remained approximately constant at the expiration of 48 hours was observed until the end of the fermentation period with only a slight increase over 144 – 168 hours. In contrast to this, a sharp fall was observed in the actual temperature of the fermenting melon mash, beginning from the expiration of the zero hour, unto the end of the fermentation though with some fluctuations. The initial high temperature of the fermenting melon is due to its temperature at the time of packaging into leaves for fermentation, while the sharp drop observed could be accounted for by the loss of heat to the environment due to natural cooling of the boiled melon seeds prior to onset of fermentation. The rise in temperature of fermenting mash at the expiration of 24 hours unto the end of 72 hours could be accounted for by the heat generated during fermentation, heat being a normal by-product of fermentation. The heat released to the interior of the fermentor is responsible for the rise in incubation temperature which was kept approximately constant throughout the period of fermentation, and due to the constant temperature of incubation maintained by the lagging of the fermentor, the fermenting temperature was also kept approximately constant though lower than the incubation temperature. Thus, temperature of incubation and fermentation were kept constant throughout the period of fermentation and this sustained the fermentation, suggesting the fermentor to be adequate for solid state fermentations at the prevailing ambient temperature (whether kept within or without a building) and used accordingly.

Table 3 shows the results of the pH, residual soluble sugars and free amino acids in ogiri per period of fermentation. Steady pH increases were observed throughout the period of fermentation, leveling off to about 8.70. This is characteristic of leguminous seeds fermentation and is in accordance with the observations of Onawola *et al.,*(2012, 2011) in ogiri production, Ogunshe *et al.,* (2007) in *Aisa* production, Omafuvbe *et al.,* (2004) in *Iru* and Ogiri production, and Odunfa (1981) in ogiri production respectively. Sarkar *et al.,* (1997a), Barber and Achinewu (1992) reported that amino acids produced during fermentation due to protein metabolism are responsible for gradual pH increases, while Onawola *et al.,* (2012) reported that pH increases may also be due to the formation of ammonia from amino acids, a product of protein degradation. Hence, increases in pH would be due to a combination of both factors. Residual soluble sugar levels fluctuated during fermentation and this is in agreement with the findings of Onawola *et al.,* (2011), Ogunshe *et al.,* (2007), Omafuvbe *et al.,* (2004) and Odunfa, (1983) in melon seed and other leguminous seed fermentations respectively. The fluctuations

observed in residual soluble sugar levels may be attributed to high amylolytic activity coupled with high usage of sugar by fermenting organisms for metabolic activity. Onawola *et al.,* (2011) and Omafuvbe *et al.,* (2004) reported that the fluctuations in soluble sugar level with fermentation may be related to its utilization by fermenting organisms for their metabolic activities. Residual free amino acids were found to increase during fermentation unto the end of 120 hours without any fluctuations before its decline toward the end of fermentation. The steady increase is in agreement with the observations of Onawola *et al.,* (2012 and 2011), Omafuvbe *et al.,* (2004) and Odunfa, (1983). Also similar increases in the levels of free amino acids with fermentation have been reported in other seeds by Omafuvbe *et al.,* (2002). The increase observed in residual free amino acids is due to the ability of the fermenting organisms to degrade protein, leading to increases in pH, free amino acids and increased microbial count as some of the free amino acids would have been used up in cell generation. A daily increase in proteinase activity during fermentation has been reported by Onawola *et al.,* (2011) and Omafuvbe *et al.,* (2004) and this is a reflection on rapid increases in free amino acids generated. The decline in residual amino acid level obtained toward the end of fermentation could be due to increased usage and break down of the amino acids compared with its rate of generation. A high level of pH was also observed during this period, suggesting increased levels of ammonia, hence, leading to a reduced level of free amino acids. Also, some of the free amino acids might have been used up in cell generation and possibly for aroma, flavor and texture generation in a reaction with other components. Whitaker (1978) reported that in most fermented high - protein products, the extent of protein hydrolysis is one of the most important factors in the changes in texture and flavor.

Table 4 shows the results of moisture content and microbial count per period of fermentation. The moisture content levels were observed to fluctuate during fermentation but with increases toward the end of fermentation. In contrast the microbial count was found to increase steadily throughout the period of fermentation unto the end without fluctuating. Higher moisture retention toward the end of fermentation, may have served to provide a more favourable environment for microbial growth and hence, higher metabolic and enzymic activity. Onawola *et al.*, (2011), (Ogunshe *et al.,* 2007) and Omafuvbe *et al.,* (2004) reported daily increases in amylase, proteinase and lipase activities during fermentation. This is evidenced by a higher count as observed toward the end of fermentation. The fluctuations observed in moisture retention could be as a result of high metabolism of the organisms and possibly some evaporation at the temperature of incubation. Some water was observed to have condensed on the internal surface of the door of the fermentor in some of the days when the fermentor was opened for sample-taking. Increased levels of microbial count is a result of microbial cell multiplication and this leads to higher consumption of soluble nutrients, soluble sugars and free amino acids for cell multiplication and cell growth and for metabolic energy. This factor might also have contributed greatly to the reduced levels of free amino acids observed toward the end of fermentation.

4.0 Conclusion

Fermentation is a key unit operation in the processing of boiled melon seeds into ogiri. Stanbury and Whitaker, 1984 stated that the main function of a fermentor is to create a controlled environment for the growth of microorganisms in order to obtain a desired product. Should the fermentor be equipped with a heating element and thermostat, then, it must support isothermal digestion of the melon. However, the stainless steel, solid state fermentor fabricated by the Federal Institute of Industrial Research, Oshodi (FIIRO) in Lagos, Nigeria is a non-heated, double walled, and lagged piece of incubating chamber designed to enable a hygienic and controlled fermentation and to conserve the heat generated during fermentation. Heat generated during fermentation has been observed to be conserved leading to maintenance of a steady temperature of fermentation within the chamber. Also, other observations during fermentation were found to conform to those in previous experiments on boiled melon seed and leguminous seeds fermentation. Thus, the fermentor meets the desired requirements for fermentation of melon seeds in the solid state and may also be used for the solid state fermentation of other leguminous seeds. This fermentor may thus be utilized to achieve a hygienic fermentation and also production of an acceptable ogiri of non-obnoxious odour and flavor.

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Table 1. Proximate and mineral composition of raw melon seeds (*Cucumis melo*).

Parameter	**(%w/w)**
Ash	2.80
Moisture	4.79
Fat	45.65
Protein	30.45
Crude fiber	6.33
CHO (by diff)	16.31
Phosphorous (P_2O_5)	0.10
Calcium (Ca)	0.08
Magnesium (Mg)	0.42

Table 2. Temperatures of fermentor interior and fermenting melon mash during fermentation.

Fermentation period (hr)	Temperature (°C) fermentor interior	Temperature (°C) melon mash
0	33.0	60.0
24	35.0	32.5
48	39.0	36.5
72	36.0	33.5
96	35.0	32.0
120	36.0	33.5
144	36.0	33.0
168	36.5	33.0

Table 3. pH, residual soluble sugar and total free amino acid levels per period of fermentation.

Fermentation period (hr)	pH	Soluble/reducing sugars (mg glucose/g dry wt)	Amino acids (mg leucine/g dry wt)
0	6.88	5.25 ± 0.24	3.05 ± 0.04
24	7.08	5.50 ± 0.48	9.83 ± 0.85
48	7.86	5.04 ± 0.28	23.17 ± 0.24
72	8.16	6.58 ± 0.13	32.33 ± 0.47
96	8.64	6.50 ± 0.10	60.50 ± 2.83
120	8.66	6.79 ± 0.07	98.67 ± 0.94
144	8.66	8.53 ± 0.63	50.33 ± 0.47
168	8.70	6.72 ± 0.09	38.00 ± 0.71

Table 4. Moisture content and microbial count levels of melon mash during fermentation.

Fermentation period (hr)	moisture content (%w/w)	microbial count (cfu)
0	39.00	0
24	38.61	0.03×10^6
48	37.60	1.13×10^6
72	40.74	1.02×10^9
96	39.05	3.00×10^9
120	41.35	3.28×10^9
144	47.31	3.32×10^9
168	41.15	1.13×10^{11}