International Journal of
AGRICULTURE AND BIOSCIENCES
www.ijagbio.com P-ISSN: 2305-6622 editor@ ijagbio.com

RESEARCH ARTICLE

Biochemical Changes of Melon Seeds (C*itrullus vulgaris)* Fermented by Pure Cultures of *Bacillus licheniformis*

Okpalla J*, CC Ubajekwe, KC Agu[1] and I Iheukwumere

Department of Microbiology, Anambra State University, Uli Anambra State; [1]Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka Anambra State

ARTICLE INFO

Received: November 13, 2012
Revised: November 27, 2012
Accepted: November 29, 2012

Key words:

***Corresponding Address:**
Okpalla J.
judyzuby@yahoo.com

ABSTRACT

The enzymatic activities, pH, temperature, reducing sugar and amino acid changes during the fermentation of melon seeds (*Citrullus vulgaris)* for "ogiri" production by *Bacillus licheniforms* have been established. The melon seeds were processed using standard procedures and at intervals of 12 h during which fermentation samples were collected for analysis. The protease activity reached a maximum value of 37.2 units/ml after 60 h, while the amylase activity reached its maximum value of 72.0 units/ml. The pH and temperature increased and reached their peaks at 8.1 and 37^{o}C at the end of fermentation respectively. The reducing sugar increased and reached its peak of 2.70 mg/ml at the end of fermentation, while the free amino acids increased and reached its peak at 3.80 mg/ml after 60 h. The result of the study revealed that various biochemical activities occur during fermentation of melon seeds for ogiri production using *B. licheniformis.*

Cite This Article as: Okpalla J, CC Ubajekwe, KC Agu and I Iheukwumere, 2012. Biochemical changes of melon seeds (C*itrullus vulgaris)* fermented by pure cultures of *Bacillus licheniformis*. Inter J Agri Biosci, 1(1): 42-45.
www.ijagbio.com

INTRODUCTION

The prevailing population pressure in Nigeria as in other less developed countries has resulted in an increasing demand for wild under-exploited nutritious plant products with aesthetic and organoleptic appeal in daily diet (Enujiugha, 2005). Seeds of legumes may account for up to 80% of dietary protein and may be the only source of protein for some groups (Achi, 1991). Their cooked forms are eaten as meals and are commonly used in fermented forms as condiments to enhance the flavor of foods (Odunfa, 1985; Aidoo, 1986; Onifiok, 1996).

Melon seeds (*Citrullus vulgaris* schrad) have been reported to contain 3.3% moisture, 15.5% crude fibre, 10.3% crude protein 8.2% carbohydrate, 52 % oil and 3.6% as (Omafuvbe *et al.*, 2004; Akobundu *et al.*, 1982). They are fermented to produce "ogiri" and used as condiments to season or flavour soups. Quite a number of *Bacillus* species have been isolated from various fermented melon seeds. Although other bacteria are also seen, only part of them can be considered to play a substantial role in fermentation processes. The biochemical changes which take place during food, fermentation have been overlooked. The understanding of the chemical changes during fermentation may help in the process control for the desired product.

In our immediate previous study (unpublished) we were able to isolate different types of bacteria from fermenting melon seeds (*Citrullus vulgaris*), with *Bacillus licheniformis* as the predominant one. The determination of the biochemical changes which occur during the fermentation of melon seeds with *B. licheniformis* as a starter culture is the objective of the study.

MATERIALS AND METHODS

Sources of Organism

Bacillus licheniformis was isolated from a previous work involving the fermentation of melon seeds for ogiri production. The culture was preserved in an agar slant at 4^{o}C until use.

Inoculum preparation for ogiri production

Two loofuls of 24 h culture of the *B. licheniformis* was used to inoculate 200 ml Erlenmeyer flask containing 50 ml of seed medium (peptone 10.0 g; yeast extract, 10.0 g; Nacl, 5.0 g and distilled water, 1L). The flasks were placed for 16-18 h on a rotary shaker at 120 rpm and

30^0C. After incubation, they served as seed culture used to inoculate the melon seed mash.

Preparation of ogiri using *Bacillus licheniformis*

300 gram weight portions of dehulled melon were cleaned and washed in tap water. They were put in wide mouthed container and then boiled for 6 h to soften the seeds. On cooling down to room temperature, each bottle was aseptically inoculated with 3 ml suspension of *B. lichenformis* using syringes and needles. Fermentation was allowed to proceed for 72 h at 30^oC. At interval of 12 h, changes in enzymatic (protease and amylase) activities, reducing sugars and free amino acids were determined.

Preparation of extract for determination of proteolytic and amyloytic activities

3 ml of cold distilled water was added to 5 g of fermenting mash in a mortar and crushed with clean pestle and then transferred to 100 ml Erlenmeyer flask. An additional 10 ml of Tris-HCL buffer was added and thereafter the flask was stoppered and shaken for 15min. The extract was then centrifuged at 2500 x g for 10min. The residue was re-suspended in 8 ml buffer and subjected to the same procedure. The supernatant was made up to 20 ml with the Tris-HCL buffer (Njoku and Okemadu, 1989). This serves as the crude enzyme supernatant.

Determination of Proteolytic activity

Protease activity was measured using the casein-digestion method of Hammed *et al.* (1996). To 1 ml of 1% (w/v) casein solution pH 8.5, 1ml of crude enzyme solution was added and incubated for 30 min at 40 ^{0}C. The reaction was stopped by adding 3 ml of 5% trichloroacetic acid by centrifugation at 10,000 g for 30 min and absorbance of amino acids and peptides released from casein by the proteases was read at 260 nm in a spectrophotometer. All protease activity assays were made in duplicate. Blanks were prepared in which 3 ml of trichloroacetic acid was added to the sample before incubation. One unit of protease activity was defined as the amount of enzyme that will release 10mg of tyrosine under the specified conditions (pH 8.5, 40^0C and 30 min).

Determination of amylolytic activity

To 1ml of 1% (w/v) soluble starch solution, in citrate phosphate buffer (pH 6.5), 1 ml of crude enzyme solution was added and incubated at 40^0C for 30 min. The reaction was stopped by adding 2 ml of dinitrosalycyclic acid reagent and boiled for 10 min and cooled and diluted with 20 ml of distilled water. The absorbance of glucose released from starch by α amylase was read at 540 mm on a spectrophotometer. All amylases activity assays were made in duplicate. Blanks were prepared in which 2 ml of DNS was added to the sample before incubation. One unit of amylase activity was defined as the amount of enzyme which liberates 1mg of glucose under the assay conditions.

Preparation of extract for the determination of total carbohydrate, total (soluble) sugar and free amino acids.

5 g of the ogiri sample was weighed into 100 ml conical flask and 50 ml of ethanol- water mixture (50:50 v/v) was added. The suspension was shaken and then washed with 5ml of petroleum ether to extract the oil, after which it was centrifuged at 5,000 rpm for 10 min, and the clear supernatant collected and used for analysis.

Determination of reducing sugar by DNSA method

1ml of the supernatant and 1ml of dinitrosalicyclic acid (DNSA) was heated in a water bath at 100^0C for 10 min. The volume of the mixture was adjusted to 12 ml with distilled water and reducing sugar determined as glucose by the colorimetric method as described by Miller (1959).

Determination of free amino acids

The ninhydrin method of Rosen (1957) was adopted. Aliquot (0.5 ml) of 1% (w/v) lead acetate was added to the extract to deprotenize it. The mixture was then centrifuge and 4 ml of 1% (w/v) ninhydrin solution was added to 1ml of the protein-free filtrate to give 5 ml reaction mixture. The mixture was then placed in a boiling water bath for 10 min, for the purple or blue colour to emerge. The absorbance was then measured using spectrophotometer at 420 nm.

A standard curve of tyrosine (0.2- 1.0 mg/ml) was used to determine the amount of amino acids present.

Determination of pH

One gram of the fermenting mash was introduced into 9 ml of distilled water and shaken, the pH was then measured using a digital pH meter.

Determination of temperature

This was determined by placing a clean thermometer in the fermenting mash and taking the reading.

RESULTS

The changes in protease and amylase activities during the fermentation of melon seeds for ogiri production are as shown in table1. The protease activity was not detected at the start of fermentation, but later increased from 7.4 unit/ml (12 h) to 37.2 units/ml (60 h) and thereafter decreased till the end of fermentation (72 h). The Amylase activity was also not detected at the start of fermentation, but later increased from 35 units/ml (12 h) to 72 units/ml (48 h) and thereafter decreased till the end of fermentation.

Table 2 shows changes in pH and temperature during fermentation. The pH increased from 6.9 at the start of fermentation to 8.1 at the end of fermentation. The temperature also increased from 30^0C at the start of fermentation to 37^0C at the end.

Table 1: Changes in enzymatic activities during the fermentation of melon seeds for ogiri production by *B.licheniformis*

Period of fermentation (h)	protease activity (unit/ml)	amylase activity (unit/ml)
0	ND	ND
12	7.40	35.0
24	21.5	51.2
36	29.3	67.4
48	34.0	72.0
60	37.2	64.6
72	23.0	54.1

Key: ND represents not detectable

Table 2: Changes in pH and temperature during the fermentation of melon seeds for ogiri production by *B.licheniformis*

Period of fermentation (h)	pH	Temperature (^{0}C)
0	6.9	30
12	6.9	30
24	7.1	32
36	7.1	33
48	7.3	35
60	7.8	36
72	8.1	37

Table 3: Changes in reducing sugar/ and free amino acids during the fermentation of melon seeds for ogiri by production *B.licheniformis*

Period of fermentation (h)	reducing sugar (mg/ml)	free amino acid (mg/ml)
0	1.30	2.10
12	1.42	2.84
24	1.82	3.30
36	1.91	3.70
48	2.23	3.80
60	2.55	3.80
72	2.70	3.70

Changes in reducing sugars and free amino acid during fermentation are as shown in Table 3. The reducing sugar increased from 1.3 mg/ml at the start of fermentation and reaches its peak of 2.70 mg/ml at the end of fermentation. The free amino acids also kept increasing from 2.1 mg/ml at the start of the fermentation to 3.8 mg/ml at 60 h before decreasing till the end of fermentation.

DISCUSSION

The ogiri samples were observed to be replete with a lot of bacteria during the study. This shows the dynamics of fermentation in any food matrix as a complex microbiological process involving interactions between different microorganisms (Daeschel, 1987). Various workers have identified different microorganisms in fermented melon seeds. These include *Bacillus* species, *E. coli*, *Proteus* species; *Pediococcus* species and *Alcaligenes* species (Sanni *et al.*, 2000; Barber and Achinewhu, 1992; Barimalaa *et al.*, l989). However, *Bacillus subtilis* and *B. licheniformis* have been identified as the main bacteria present (Barimalaa *et al.*, 1989).

The relative increase in free amino acid corresponded with increase in activity of protease. This is however not unusual since similar pattern have been reported (Odunfa, 1983; Omafuvbe *et al*, 2004). The activity of protease increases the digestibility of the product by breaking down the complex proteins to simpler peptides and amino acids that can be utilized or digested in the body (Ogueke and Nwagwu, 2007). Protease activity has been reported to be abundant in the fermentation of similar protein rich foods (Sarkar *et al.*, 1993; Omafuvbe *et al.*, 2002). The increase in amylase activity resulted in a corresponding increase in reducing sugars. Other workers have also reported similar trends observed in this study. Odunfa (1983) and Omafuvbe *et al.* (2004) reported an increase in α amylase activity which corresponded with an increase in total reducing sugar during the fermentation. Increase in amylase activity and an increase in total reducing sugar during the fermentation of soy sauce moromi has been demonstrated (Yong and Wood, 1977). *Bacillus* species have been reported as producer of amylases which are involved in the degradation of carbohydrates into sugars (Kiers *et al.*, 2000; Aderibigbe and Odunfa, 1990; Sarkar *et al.*, 1997).

It was observed that the pH increased throughout the fermentation periods. The increase in pH during fermentation of melon seeds has be reported (Omafuvbe *et al.*, 2004; Ogueke and Nwagwu, 2007; David and Aderibigbe, 2010).

The observed increase in pH could be attributed to active proteolytic activities and release of ammonia through deamination of amino acids (Achinewhu, 1987). The increase in pH into the alkaline range may be physiologically important for tolerance and adaptation of fermenting microorganisms in the environment. A pH rise was also observed during the fermentation of melon seeds to produce ogiri (Odunfa, 1981).

There was a gradual increase in temperature during the fermentation. Ogueke, *et al*, (2007) observed a temperature increase during the fermentation of melon seeds by *Bacillus* and *Proteus* species. Again, Odunfa (1981) reported an increase in temperature during the natural fermentation of melon seeds for ogiri production. The heat generated was due to metabolic activities of the microorganisms.

Conclusion

The present work indicates that biochemical activities occur during fermentation of melon seeds for ogiri production which results in increase in reducing sugars and free amino acids. The intake of ogiri is expected to increase the intake of these essential dietary components.

REFERENCES

Achinewhu SC, 1987. Carbohydrate and fatty acid composition of fermented melon seeds (*Citrullus vulgaris*). J Food Sci, Tech, 24: 16-19.

Adribigbe EY and SA Odunfa, 1990. Growth and extra cellular enzyme production by strains of *Bacillus* species isolated from fermentation African locust bean, iru. J Appl Bacteria, 69: 662-671

Adioo KE, 1986. Lesser-known fermented plant foods. Tropical Science 26:249-58

Akobundu EN, JP Cherry and JG Simmon, 1982. Chemical, functional and nutritional properties of egus, (*Colocynthis citrullus L.)* seed protein production. J Food Sc, 47: 829-35

Barber LA and SC Achinewhu, 1992. Microbiology of "ogiri" production form melon seed (*Citrullus vulgaris*). *Nigerian Food Journal* 10:123-35

Barimalaa IS, SC Achinewhu, I Yibatimal and EN Amadi, 1989. Studies on the solid state fermentation of Bambara Groundunt (*Vigna subterranean* L.) Journal of Science of Food and Agriculture 66:443

Daeschel MA, RE Anderson and HP Fleming, 1987. Microbial ecology of fermentation plant materials. *FEMS microbial Rev.* 46:357-367

Enujiugha VN, 2005. Quality dynamics in the processing of underutilized legumes and oil seeds. In: Crops,

Growth, quality and biotechnology (R. Dris, ed.), WFL publishers, Helsinks, Finland, Pp: 732-746.

Hameed A, MA Natt, and CS Evans, 1996. Production of alkaline protease by a new *Bacillus subtilis* isolate for use as a bating enzyme in leather treatment. *World Journal of Microbiology and Biotechnology*. 12:289-281.

Kiers JL, AEA VariLaekan, FM Romboat and MJR Nout, 2000. In vitro digestibility of *Bacillus* fermented soyabean. *Int Food Microbiol*, 60: 163-169.

Miller GL, 1959. Use of dinitro-salicyclic acid reagent for determination of reducing sugars *Anal Chem*, 31: 426-428

Odunfa SA, 1981. A note on microorganisms associated with the fermentation of African locust bean (*Parkia filicoidea*) during iru production. *J Plant Foods*, 3: 245-250.

Odunfa SA, 1983. Biochemical Changes during production of ogiri, a fermented melon (*Citrillus vulgaris* Schrad) product. Qualities planetarium. *Plant Food for Human Nutr*, 32: 11-18.

Odunfa SA, 1985. African Fermented Foods, In: wood, BJB (ed) Microbiology of Fermented Foods, Vol 11, Amsterdam, Elsevier Applied Science Publishers. p155-191.

Ogueke CC and A Nwagwu, 2007. Comparative study of melon seeds (*Citrullus vulgaris*) fermented with mixed culture and pure culture of bacteria isolated from ogiri egusi, *Life Science Journal*. 4(4): 62-67.

Omafuvbe BO, SH Abiose and OO Shounkan, 2002. Fermentation of soybean (*Gycine max*) for soy-daddawa production by starter cultures of *Bacillus. Food Microbial*, 19: 561-566.

Omafuvbe BO, OS Falade, BA Osuntogun and SRA Adewusi, 2004. Chemical and biochemical changes in African locust bean (*Parkia biglobosa) Pakistan Journal of Nutrition* 3(3): 140-145.

Rosen H, 1957. A modified nihydrin calorimetric analysis for amino acids. *Archives of Biochemistry and Bio physics*, 67: 10-15.

Sanni AI, GS Ayenor, E Sakyi-Dawson and S Sefa-Dedeh, 2000. Aerobic spore forming bacteria and chemical composition of some Nigerian fermented soup condiments. *Plants Foods for Human Nutrition* 55(2): 111-8.

Sarkar PK, PE Cook and JD Owens, 1993. *Bacillus licheniformis* of soybeans. *World Journal of Microbiology and Biotechnology*, 9: 295-9.

Sarkar PK, L Jones, GS Craven, SM Somerset and C Palmer, 1997. Oligosacharide profiles of soybeans during kinema production. Lett. Appl. Microbiol, 24: 337-339.

Yong FM and BJB Wood, 1977. Biochemical changes in experimental soy sauce Koji. J Food Technol 12: 163-175.