PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 11 (3): 243-246, 2012
ISSN 1680-5194

Isolation of Starch Degrading Spoilage Bacteria from 'Ogi' (Fermenting Maize Starch)

A.W. Ashiru[1], O.D. Teniola[2], N.N. Dibiana[1] and A. Apena[3]
[1]Department of Biological Science, [3]Department of Chemical Science,
Yaba College of Technology, P.M.B. 2011, Yaba, Lagos, Nigeria
[2]Federal Institute of Industrial Research Oshodi (FIIRO)

Abstract: Fermented maize starch known as 'Ogi' (Yoruba) or Akamu (Igbo) is a popular staple food and most popular traditional weaning food in West African countries. Its consumption by convalescents in these regions call for a safe product, free of pathogens and any potentially hazardous micro-organisms. The microorganisms associated with the spoilage of 'ogi' (fermented maize starch) porridge were isolated after seven day of fermentation. 'Ogi' off odour was first noticed at the 4th day of fermentation. Of all these bacteria and yeasts isolated, only bacteria could hydrolyze the starch in the ogi porridge and they were identified as *Bacillus magaterium* and *Bacillus subtilis*. The amylase activities of these organisms were studied under different temperature (20-80°C) and pH (2-8). The optimum temperature of both organisms was 40°C and optimum pH for *Bacillus megaterium* was four and that of *Bacillus subtilis* was two. *Bacillus megaterium* has higher amylase activity and thus was used to cause spoilage of sterile ogi porridge. The consistency of 'ogi' change (liquefy) on the fourth day instead of the normal seven-day duration. In other to prevent 'ogi' spoilage by *Bacillus megaterium*, a preservative, sodium benzoate was added to the sterile 'ogi' containing the inoculum, it was noticed that 'ogi' aroma and colour remained the same but there was a little change in the consistency after seven days.

Key words: Fermentation, 'ogi' spoilage, temperature, pH, amylase

INTRODUCTION

Cereals are the most widely cultivated and consumed crops on a global basis. In West Africa, cereals are the major sources of energy and protein in the diets of the people. For example in Nigeria, the maize is used in preparation of various dishes which include ogi (Banigo *et al*., 1974).

Fermented maize starch known as 'ogi' (Yoruba) or "Akamu" (Igbo) is a popular staple food of tropical West African countries. The reputation as the most popular traditional weaning food and its consumption by convalescents in these regions call for a safe product, free of pathogens and any potentially hazardous micro-organisms. Traditional fermentation processes of 'ogi' production are usually spontaneous and uncontrolled (Odunfa, 1985).

Many workers have worked on different aspects of 'ogi' production. Particular attention has been given to the various aspects such as process variations and mechanization and nutritional improvement (Akinrele and Bassir, 1967; Onyekwere *et al*., 1989). The microbiology of 'ogi' and related products during the processing stages up to the finished products has equally been studied (Odunfa and Adeyele, 1985; Adegoke and Bablola, 1988; Hountunigha, 1994). New attention is presently on the use of starter cultures, which is solving numerous problems associated with the product 'ogi' capable of prevention and treatment of many water borne diseases was developed by Olukoya *et al*. (1994) using bacteriogenic Lactic Acid Bacteria (LAB). Olasupo *et al*. (1997) increased the shelf-life of-'ogi' using a bacteriocin-producing *Lactobacillus* isolate. The nutritional value of the product has also been increased by applying lysin - and methionine-producing microorganism as starters (Teniola and Odunfa, 2001). Despite the delicate health position of some 'ogi' consumers, the role of spoilage micro-organisms has not been investigated, nor has their potential to produce harmful metabolites. Effective parameters useful in monitoring spoilage are also necessary in order to determine the appropriate time fermentation should be terminated to avoid spoilage and harmful metabolite production. The spoilage indices will be very useful industrially during large scale production of 'ogi', to avoid wastage and health hazards (Odunfa and Teniola, 2002). The paper reports our findings on:

- Identifying the microorganisms associated with starch degrading spoilage during 'ogi' fermentation.
- Estimation of amylase activity during spoilage.
- Controlling the spoilage of the 'ogi' by the isolated microorganisms.

Corresponding Author: A.W. Ashiru, Department of Biological Science, Yaba College of Technology, P.M.B. 2011, Yaba, Lagos, Nigeria

MATERIALS AND METHODS

'Ogi' fermentation: Raw 'ogi' was purchased from Oyingbo market and then cooked to produce a thin (porridge). The 'ogi' produce was left to ferment continuously for 6 days during which an offensive odour develops and the texture of consistency changes. Sample was then taken at the final day of 'ogi' fermentation.

Screening of microorganisms for starch degradation: Soluble starch was separately prepared and added to nutrient agar to give 1% soluble starch medium and was sterilized at 121°C for 15 min. 20 ml of the molten starch agar was poured into Petri-dishes and allowed to cool. The bacteria and yeasts were streaked once across the surface of the plates and incubated at 35°C for 2-3 days. After incubation, each plate was flooded with aqueous Gram's iodine and left for 30 sec. Clear zones around the growth colonies indicated starch hydrolysis by the cultures; unhydrolyzed starch formed a blue colour with iodine.

The supernatant for the enzymes was obtained by growing a fresh culture of bacterial isolates in one litre flask of nutrient broth at 30°C for 24 h in a shaker incubator. After incubation, the cultured broth was centrifuged to obtain the supernatant. Enzymatic extracts were prepared by ultra-filtration of culture supernatants using a Diaflo PM, lOmembrane (AMICON). Enzymatic activities were then assayed by adding 0.1 ml enzymatic extract to 0.8 ml of a solution containing 1.2% of soluble starch (probabo) in 0.1 ml phosphate buffer pH 6.0. After incubation for 10 min at 40°C, the reaction was stopped by addition of 0.1 ml of 5M NaOH. The increase in reducing power was determined using the method of Miller (1959), one enzyme unit is defined as-the amount of enzyme that releases one micromol of reducing power equivalent per minute under the conditions described.

Preparation of inoculum for spoilage test: Fresh cultures (24 h) of isolated microorganisms from the spoilage stage were used. A loopful of the organism was inoculated into 9 mls of sterile distilled water and thoroughly shaken to give an even distribution of the organism in the sterile distilled water.

Assessment of 'Ogi' spoilage: 7.5 g of 'ogi' from Oyingbo market was weighed separately into 3 tubes. 38 mls of hot water was used for each tube, to make the 'ogi' porridge. The tubes containing the 'ogi' porridge were sterilized in the autoclave for 15 min at 121°C. After sterilization, the tubes were allowed to cool. To the first tube, nothing was added. To the 2nd tube,' 1 ml of water containing 0.01 g of sodium benzoate was added and also 1 ml of the inoculum, to the 3rd tube was added 1 ml of the inoculum. The spoilage of this 'ogi' porridge was assessed based on physical properties like colour, aroma and change in consistency.

Table 1: Morphological and biochemical characteristics of bacteria isolates from spoilt 'ogi' porridge

	Isolates	
Characteristics	OB 6	OB 7
Morphology	Creamy surface with shiny surface, slightly rugose	Creamy dull surface with irregular shape
Gram stain	+	+
Spore stain	+	+
Catalse	+	+
Oxidaze	+	+
Starch hydrolysis	+	+
Shape	Rods	Rods
H_2S production	-	-
Indole production	-	-
Citrate	+	+
Methyl red	+	+
Vp test	-	-
Motility	+	+
Gelatin hydrolysis	+	+
Urease	-	-0
Nitrate	+	+
Sugar fermentation	OB 6	OB 7
Xylose	-	+
Cellobiose	+	W
Maltose	W	+
Salicin	+	+
Lactose	+	+
Manintol	+	+
Glucose	W	W
Arabinose	W	+
Sorbose	+	W
Sucrose	+	W
Galactose	+	-
Raffinose	+	+
Identification	*Bacillus magatrium*	*Bacillus subtilis*

Positive: +, Negative: -, Weakly reaction: W

RESULTS AND DISCUSSION

From the various biochemical tests carried out, the organisms coded OB 6 and OB7 were identified as *Bacillus megaterium* and *Bacillus subtilis* respectively (Table 1). Their amylase activities were studied under different temperatures and pH. From Table 3 showing the effect of temperature on the organisms, the optimum temperature of both organisms was at 40°C. The optimum pH for amylase activity of *Bacillus megaterium* is 4 (Table 3) and that of *Bacillus subtilis* is 2 Heat treatment of the amylase at 80°C for 10 min resulted in a great percentage loss in the starch degrading activities of the organisms.

From Table 2 *Bacillus megaterium* degrade starch better and was therefore used to cause spoilage by inoculating the organism into sterile 'ogi' porridge. After four days it was observed that the 'ogi' consistency changed i.e. liquefy instead of the normal seven day duration (Table 2).

Table 2 also shows that after adding sodium benzoate (preservative) and inoculating with *Bacillus megaterium*, the 'ogi' aroma and colour remained the same but there was a little change in the consistency after the 7th day.

Table 2: Spoilage of sterile Ogi by *Bacillus megaterium* and preservation using sodium benzoate

	Aroma							Colour							Consistency						
	Days																				
Sample	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
Sterile Ogi	5	5	5	4	4	4	3	5	5	5	5	4	4	4	5	5	5	5	4	4	4
Sterile Ogi inoculated with *B. megaterium*	5	4	4	4	3	2	2	4	4	4	4	4	3	3	5	4	3	2	1	1	1
Sterile Ogi containing sodium benzoate and *B. megaterium*	5	5	5	4	5	5	5	4	4	4	4	4	4	4	5	5	5	4	4	4	3

1 = Very poor, 2 = Poor, 3 = Fairly good, 4 = Good, 5 = Very good

Table 3: Effects of temp and pH on enzyme activity

Temp °C (10 min)	*Bacillus magaterium* amylase activity/ml	*Bacillus subtilis* amylase activity/ml
20	0.023	0.030
30	0.027	0.025
40	0.143	0.072
50	0.044	0.070
60	0.044	0.054
70	0.033	0.028
80	0.030	0.026
pH (10 min)	*Bacillus megaterium* amylase activity (U/ml)	*Bacillus subtilis* amylase activity U/ml
2	0.009	0.110
3	0.016	0.014
4	0.024	0.009
5	0.019	0.007
6	0.015	0.007
7	0.011	0.007
8	0.010	0.006

From the result, none of the yeasts could degrade starch in the 'ogi' while only two bacteria identified as *Bacillus megaterium* and *Bacillus subtilis* could hydrolyze the starch. The production of amylase however, had been described in certain species of yeasts e.g. *Saccharomyces cerevisiae* isolated from yam tuber was found to produce amylase (Olasupo *et al*., 1996). The presence of these starch non-degrading yeast and bacteria may be as a result of the utilization of simpler sugars that were produced after *Bacillus megaterium* and *Bacillus subtilis* had already degraded the starch in the 'ogi' porridge. The spoilage of the 'ogi' was however enhanced by these starch non-degrading yeasts and bacteria. This can be supported by the work done by Odunfa and Teniola (2002), that studied the changes during fermentation of 'ogi' up to the spoilage stage. Organisms as *Candida krusei, Candida valida* and *Geotrichium candidum* dominated the fermenting 'ogi' at the spoilage stage and contributed most significantly to 'ogi' off odour but none of these organisms could degrade starch.

From Table 3 *Bacillus megaterium* requires optimum pH of 4 while *Bacillus subtilis* requires optimum pH of 2 and both organisms have optimum temp. of 40°C. In contrary, works done on effect of temp. and pH on amylase activity of *Bacillus licheniformis* (Chandra *et al*., 1980) reported that the organism require an optimum temp. of 48°C and optimum pH of 6.5. This variation is as a result of the fact that amylase are known to exhibit similarities and differences in properties which include pH and temperature etc depending on the source (Ilori *et al*., 1997). In other to prevent the degradation of the 'ogi' starch, the optimum temperature and pH for amylase activities of *Bacillus megaterium* and *Bacillus subtilis* should be avoided.

From Table 3, at lower temperature (20°C) the amylase activities of both organisms were very low, thus, it could be drawn that if the 'ogi' porridge is kept at a very low temperature (e.g. refrigerator) the degradation of the starch in the 'ogi' would probably be reduced to the barest minimum. However, the amylase activities of these two organisms can be of importance. Mensah *et al*. (1997) reported that effective increase in the density of the energy is associated with reduction in viscosity and fermentation; this reduces the viscosity of some foods e.g. maize porridge. This reduction is probably due to the activities of amylase producing micro organisms that break starch down into simplex sugars, releasing bound water and thus reducing viscosity. The reduction in viscosity in food such as fermented maize porridge could satisfy the energy requirements of the healthy child (Gallat, 1989). In a study carried out in Ghana, it was shown that fermented maize porridge could sustain the growth of partially breast fed infants when introduced early enough (Amar, 1990).

Conclusion: Two bacteria namely *Bacillus megaterium* and *Bacillus subtilis* were isolated and identified as starch degrading spoilage organisms. The optimum temperature of both organisms was at 40°C and the optimum point for amylase activity of *B megaterium* was 4 and that of *B. subtilis*. The addition of a preservative sodium benzoate increases the shelf life of Ogi beyond 7th day.

'Ogi' is a popular staple food of tropical West African countries and the reputation as the most popular traditional weaning food and its consumption by convalescents in these regions call for a safe product, free of pathogens and any potentially hazardous micro organisms. Thus, in other to increase the shelf life or to obtain maximum satisfaction its consumption, it is recommended that:

- Good hygiene practice should be maintained at every stage of production in other to avoid contamination.

- 'Ogi' porridge should be consumed within 24 h of preparation so as to obtain maximum nutrient value and flavour.
- Or preservative such as sodium benzoate can be used during the production of the 'ogi'.

ACKNOWLEDGEMENT

Our appreciation go to Mr. Tope and other member of staff of Federal Institute of Industrial Research Oshodi (FIIRO) that contributed in one way or the other to the success of this work.

REFERENCES

Adegoke, G.O. and A.K. Bablola, 1988. Characteritisc of microorganisms of importance in the fermentation of 'fufu' and 'ogi' Nigerian foods. J. Applied Bacteriol., 8: 449-533.

Akinrele, T. and L. Bassir, 1967. The nutritive value of 'ogi' a Nigerian infant food. J. Trop. Med. Hygiene, 12: 279-280.

Amar, M.A., 1990. Maternal energy Status, Lcactation Capacity and Infant. Growth in Rural Ghana, A Study of the Interaction of Cultural and Biological Factors, Ph.D thesis, London School of Hygiene and Tropical Medicine, London, pp: 150.

Banigo, E.P., J.M. Deman and C.I. Duit-schaever, 1974. Utilization of high lysine corn for the manufacture of 'ogi' using a new improved processing system. J. Cereal Chem., 51: 559-512.

Chandra, A.K., S. Medda and A.I. Bhadra, 1980. Production of xtracellular thermostable α-amlyase by *Bacillus licheniformis*. J. Fermentation Technol., 58: 1-10.

Gallat, S., 1989. Preliminary study of the effect of lactic fermentation on the rheology and ph of sorghum porridge. J. Food Microbiol., 25: 71-75.

Hountunigha, D.J., 1994. Fermentation of Maize (*Zea mays*) Meal for Mawe Production in Benin; Physical, Chemical and Microbiology Aspects. Ph.D thesis. Agricultural University Wageningen, pp: 90.

Ilori, M.O., O.O. Amundi and O.O. Omidiji, 1997. Purification and properties of an α-amylase produced by a cassava fermenting strain *Micrococcus luteus*. J. Folia Microbiol., 42: 445-449.

Mensah, P., B.S. Drasar, T.J. Harrison and A.M. Tomkins, 1997. Fermented cereal gruels; Towards solution of the weanings dilemma. J. Applied Bacteriol., 70: 50-57.

Miller, S.L., 1959. Organic compound synthesis on the primitive earth. Science, 130 (3370): 245.

Odunfa, S.A., 1985. African fermented foods. J. Food Sci., 2: 155-191.

Odunfa, S.A. and S. Adeyele, 1985. Microbiological changes during the traditional production of 'ogi baba'. West African fermented sorghum gruel. J. Cereal Sci., 3: 173-180.

Odunfa, S.A. and O.D. Teniola, 2002. Microbial assessment and quality of 'ogi' during spoilage. World. J. Microbiol. Biotechnol., 8: 731-733.

Olasupo, N.A., D.K. Olnkoya and S.A. Odunfa, 1997. Assessment of bacteriocin producing lactobacillus strain in the control of cereal based African fermented food. J. Folia Microbiol., 42: 3134.

Olasupo, N.A., O.P. Teniola, B. Okosun, A. Omowaye, S.O. Olatope and M.E. Scoh, 1996. Studies on an amylolytic strain of *Saccharomyces cercvisiae* isolated from yam tuber. J. Basic Microbiol., 36: 283-288.

Olukoya, D.K., S.L. Ebigwesi, N.A. Olasupo and A.A. Ogunjimi, 1994. Production of dogik an improved 'ogi' (Nigeiran Fermented Weaning Food) with potential for use in diarrhoea control. J. Trop. Pediatrics, 40: 108-113.

Onyekwere, O.O., I.A. Akinrele and O.A. Koleoso, 1989. Industrializaiton of 'ogi', 2nd Edn., Steinkraus Publishers New York, pp: 360.

Teniola, O.D. and S.A. Odunfa, 2001. The effect of processing methods on the levels of lysine, methionine and the general acceptability of 'ogi' processed using starter cultures. Int. J. Food Microbiol., 24: 239-248.