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Microbiological and Chemical Changes During Fermentation of Crabs for *ogiri-nsiko* Production

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Abstract: Fresh water crab (*Sudananautes africanus africanus*) was processed and fermented to produce *ogiri-nsiko*, a type of condiment in Nigeria. During fermentation, bacterial populations, changes in pH, titratable acidity and proximate composition were measured over a 72 h period. The main microorganisms involved in the spontaneous fermentation of the crabs were, *Bacillus subtilis*, *B. pumilis*, *Staphylococcus saprophyticus*, *Micrococcus luteus* and *Pseudomonas* sp. Variations in the important microbial groups showed that *Bacillus* species were the most prevalent species and occurred until the end of the fermentation. However, significant contributions were made by *Staphylococcus* sp. which were present in low numbers until the end of the fermentation. Fermentation increased the pH of the substrate from 6.2 to 8.4. The titratable acidity increased in the first 24 h and then dropped as fermentation progressed. Proximate composition changes showed increase in protein, ash and crude fiber contents whereas crude fat decreased significantly in fermented samples. Fermented crab as a rich source of protein, offer potential substitute for meat and cultured dairy products.

Key words: Crab fermentation, condiment, *ogiri-nsiko*, microbial ecology, bacteria, *Sudananautes africanus africanus*

INTRODUCTION

Ogiri-nsiko is a fermented crab meal condiment prepared from the West African fresh water crab (*Sudananautes africanus africanus*) and are used mostly in soup preparation and as meat like seasoning for vegetable stews. It is popular in the southeastern areas of Nigeria just as *ogiri* made from melon and castor oil seeds, *dawadawa* from African locust beans, or *owoh* from cottonseeds (Achi, 2005). Fermentation provides an economic means of preserving fresh crabs by inhibiting the growth of pathogenic bacteria under conditions where refrigeration or other means of safe storage are not available (Motarjemi, 2002). Fermentation further demonstrates the exploitation of the process in the processing of crabs for value addition and for expanding the culinary base of crabs. This is because besides being used as an ingredient in a variety of local foods its use could be extended as a food ingredient incorporated into fabricated foods in order to increase its versatility and utility (Akapo *et al*., 1995).

The utilization of surplus freshwater and marine animals as condiments is not new. Thus, in South East Asia, the storage life of perishable fish can be extended by acid-fermentation with added carbohydrates in concentrated brine (Paludan-Muller *et al*., 1999; Steinkraus, 1996; Beaumont, 2002). However, in contrast, *ogiri-nsiko* is fermented without added salts or carbohydrates.

Traditionally, the production involves wrapping the crabs in fresh banana leaves and fermenting for 48-72 h. Smoking or sun drying then dries the fermentate. Despite the emerging significance of

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ogiri-nsiko in the diet of the people, little information is known about the microbiology and biochemistry of the fermentation process. No studies have been done on the spectrum of microorganisms associated with the fermentation and the development of flavour compounds during the process. The microorganisms associated with the fermentation may strongly affect the biochemical composition of the final product. The aim of this study was to determine the biochemical and microbiological changes occurring during natural fermentation of crab meal, which are essential for the selection of starter cultures for the improvement of the quality of processed product.

MATERIALS AND METHODS

Traditional *Ogiri-nsiko* Preparation

Samples of the common West African fresh water crab (*Sudananautes africanus africanus*) were purchased at the local market at Ariam Oboro, Abia State, Nigeria during the rainy season (May-October). The crabs normally hibernate in the dry season. *Ogiri-nsiko* was prepared conventionally in the laboratory of the Department of Microbiology, following the traditional techniques as practiced by the people of Oboro Abia State Nigeria. The whole crabs were washed to remove slime and soil. Two types of crab samples were used for the fermentation process. Juvenile crabs were fermented completely and with the carapace. Mature crabs that have carapace, which are course in texture, were usually crushed by hand along with other body parts before the fermentation. The cleaned crabs were then wrapped in 50-80 g amounts in layers of flamed banana leaves and left to ferment at 30±2°C for 3-4 days in a warm place to produce *ogiri-nsiko*. The product is sun-dried for 4-5 days. Fermenting samples were collected in triplicate every 24 h for analysis. Three separate fermentations were carried out.

Isolation and Enumeration of Groups of Microorganisms

Samples of the fermenting crabs were aseptically taken at 24 h intervals. Two gram of the sample were added to 30 mL of 0.1% peptone water diluents and agitated for 10 sec. The samples were plated in duplicate, using 3-6 dilutions of the 1/10 suspension on nutrient agar (NA, Oxoid) and tryptone soya agar (TSA, Oxoid) for determination of aerobic mesophilic bacteria. Fungi were determined on malt extract agar (MEA, Oxoid) containing 50 μg streptomycin per mL. The cultures were prepared within 24 h after the collection of the samples. Inoculated NA and TSA plates were incubated at 30°C aerobically for 48 h and MEA plates at 28°C for up to seven days. Colonies were counted in all petri dishes, which contained 30-300 colonies.

Systematic morphological and biochemical tests were conducted according to Cowan and Steel (1970) to classify the bacterial isolates into genera. Identification of bacterial isolates into species was done according to the tests and descriptions given in Collins *et al.* (1990) and Buchanan and Gibbons (1974). The tests employed include catalase, oxidase, coagulase, motility, nitrate reduction, starch hydrolysis, oxidation/fermentation of glucose, lactose, sucrose, mannitol, maltose, galactose, fructose and growth on MacConkey agar.

Chemical Analyses

The changes in pH of fermenting samples were monitored every 12 h for 72 h by using a standardized pH meter while temperature was monitored with a thermometer. The titratable acidity expressed as lactic acid, was determined by titration of fermented samples with 0.1 M NaOH to pH 8.1 (Speck, 1984). Proximate analyses for moisture, protein (N×6.25) and fat were carried out according to AOAC (1984) methods (moisture: 43.287; protein: 2.057 and fat 7.062). Triplicate determinations were made per sample.

RESULTS AND DISCUSSION

In this study, the microbial succession during the production of traditionally fermented crabs was investigated. Only bacteria were found to be associated with the fermentation. The fresh crabs invariably underwent natural fermentation following incubation and became *ogiri-nsiko* as readily identified by the characteristic development of ammoniacal odour. This became prominent after 3 days of fermentation. The fermented product was mucoid in texture due to slime production by the fermenting microorganisms. In all three separate fermentations, the counts of aerobic mesophilic bacterial population increased with increased fermentation time and reached a peak at 48 h (from 10^3-10^9 cfu g^{-1}) (Table 1). The isolated bacteria were identified as *Staphylococcus saprophyticus*, *Bacillus subtilis*, *B. pumilis*, *Bacillus licheniformis*, *Pseudomonas* sp. *and Micrococcus luteus. Bacillus* sp. markedly dominated the flora after 24 h whereas *Staphyloccus* sp. remained as part of the dominant flora without showing a noticeable increase during the fermentation (Table 1). *Bacillus* sp. has been reported to be responsible for the fermentation of African locust bean to produce *dawadawa* (Aderibigbe and Odunfa, 1990) *soumbala* (Ouoba *et al*., 2003), some Asian fermented foods (Sarkar *et al*., 1993;Wang and Fung, 1996) and other oil seeds (Barber *et al*., 1988; Achi, 2005). Their presence in these fermentations may be due to their ability to initiate fermentation of both nitrogenous and carbohydrate products (Omafuvbe, 1994). In addition, *Bacillus* sp. are known to produce a diversity of enzymes. Their metabolic activities can contribute to flavours and aroma generating reactions (Leejeerajumnean *et al*., 2001) and degradation of amino acids (Nomura and Sasaki, 1986; Janssens *et al*., 1992; Ouoba *et al*., 2003).

Staphylococcus were detected at the beginning of the fermentation and remained as part of the dominant flora without showing a noticeable increase until the end of the fermentation (Table 1). Present results are different from those of Ogbadu and Okagbue (1988) who reported the absence of *Staphylococcus* in the 48 h fermentation period for *dawadawa* and from those of Omafuvbe *et al*., 2000; Achi, 1992) who found that *Staphylococcus* was present only at the early stages of the fermentation. However, our results are similar to the findings of Antai and Ibrahim (1986) who reported the occurrence of *Staphylococcus* throughout the fermentation of African locust bean. *Staphylococcus* in fermented crab could have originated during handling and preparation of the materials before fermentation. Their survival may be a result of the chemical composition of the fermenting materials since they could not survive in other environments, which were acidic. Indeed, *Staphylococcus* sp. are known from other fermented foods such as a low-salt Thai fish product (Aryanta *et al*., 1991; Plauda-Muller *et al*., 1999), fermented sausages (Stahnke, 1994) and *iru* (Odunfa and Oyewole, 1986). Nevertheless, more investigations are needed to know whether *Staphylococcus* sp. are involved in the fermentation of crabs for *ogiri-nsiko* fermentation. Apparently, some *Staphylococcus* sp. involved in the fermentation of some foods, produce extracellular enzymes notably lipases and proteinases (Odunfa and Komolafe, 1989; Chukeatirote *et al*., 2006) to degrade macronutrients for food and energy. They may also produce heat stable toxins although fermented foods appear to be relatively safe for consumption (Odunfa and Komolafe, 1989; Achi, 2005). There

Table 1: Changes in microbial flora during crab fermentation for *ogiri-nsiko* production

		Microbial counts (log_{10} ef.u g^{-1})*				
Fermentation time (h)	TVC	*Bacillus subtilis*	*Bacillus pumilus*	*Staphylococcus saprophyticus*	*Micrococcus luteus*	*Pseudomonas* sp.
0	3.3	2.8	1.6	1.2	1.1	0.8
24	5.6	4.6	2.1	2.0	1.8	-
48	7.5	5.8	3.2	3.0	2.2	-
72	8.8	7.4	4.2	3.3	2.5	-

*Average of three replicates

Table 2: Changes in selected chemical attributes of fermenting crab meat

Fermentation period (h)	Moisture content (%)	pH	Temperature (°C)	Titratable acidity (% lactic acid)
0	30	6.24	27	0.3
12	28.01	6.28	29	0.2
24	26.0	7.00	30	0.2
36	24.5	7.24	30	0.1
48	24.0	8.00	30	0.1
60	23.0	8.20	29.5	0.05
72	22.5	8.40	29	0.05

Values are the average of triplicate determinations

Table 3: Proximate composition of fermented fresh water crab*

Fermentation time (h)	Protein	Fat	Crude fiber	Ash
0	33.60	3.80	11.82	41.68
12	33.95	3.62	12.94	45.00
24	34.65	3.54	13.98	50.44
36	36.23	3.34	14.14	53.24
48	36.68	3.14	15.26	57.02
60	37.45	3.14	16.28	60.16
72	38.85	2.94	16.42	67.92

*Average of three determinations

was a decrease in the number of *Pseudomonas* species to undetectable levels after 24 h. The development of a uniform micropopulation in fermented foods has been claimed in other studies (Omafuvbe *et al*., 1999; 2000). Among few studies based upon genotyping at subspecies level, Ouoba *et al*. (2004) investigated dominant isolates of *Bacillus* species from soumbala and Ogbadu *et al*. (1990) investigated dawadawa production. Both studies found that several strains of *Bacillus* were involved in the fermentation. Fermentation with starter cultures containing *Bacillus* sp. caused rapid production of the typical ammoniacal aroma, which is a critical parameter in the acceptability of fermented condiments.

The microbiological changes in fermenting milieu are concomitant with important physico-chemical changes that may vary according to the technologies and substrates used (Azopota *et al*., 2006). Table 2 shows increase in pH and temperature while titratable acidity declined with fermentation time. During the fermentation, an increase of the pH was observed for all samples. The average pH of the fermented crab samples was 8.4 with pH values ranging from 8.2 to 9.0. On the other hand, the percentage of titratable acidity decreased gradually as fermentation progressed, with a final value of 0.05. The increase of pH is attributed to the production of ammonia which is characteristic of proteinous food fermentations as a result of proteolytic activity of the fermenting microorganisms. The abundant production of ammonia, which is responsible for the unique aroma sometimes described as ammoniacal or pungent has been reported for other protein food fermentations such as *dawadawa, soumbala*, or *netetu* (Odunfa and Oyewole, 1998; Wang and Fung, 1996; Beaumont, 2002; Yong and Wood, 1977; Sarkar *et al*., 1993; Allegheny *et al*., 1996). The progressive decrease in titratable acidity with increase in pH during *ogiri-nsiko* production is similar to reports on similar fermentations (Barber *et al*., 1988). One explanation for this was the amount of protein in the fermenting medium which favours its capacity to buffer pH changes (Schaffner and Beuchat, 1991). On the other hand, the level of alkalinity may be attributed to the amount of total carbohydrate present in the fermenting medium.

All the determinations were on a dry weight basis. The ash, protein and fiber content all increased with the fermentation. The crude fat content was generally low and decreased with fermentation time (Table 3). The protein contents of crabs have previously been found to be of high quality similar to that of meat and fish (Muller and Tobin, 1980; Adeyeye, 2002). This means that crabs can be used as

substitute for fish and meat and can be recommended in the diet of communities where animal protein is expensive. According to Adeyeye (2002), the crabs can be ground to a paste, which is added to the meal and will be very appropriate as weaning food for children.

In conclusion, this study shows that the production of *ogiri-nsiko* from crabs is caused by microbial fermentation, with *Bacillus* species been the most dominant. Characterization and metabolism study of the dominant microorganisms involved in the fermentation could give more insight on the chemical changes occurring in *ogiri-nsiko* fermentation. This can contribute to a more in-depth and rapid understanding of the organisms, their roles, succession and prevalence in the fermented food.

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