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Biotechnology of Okpeye: A Nigerian Traditional Fermented Food Condiment

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Abstract

Most legumes and oil bean seeds used in condiment manufacture in Africa are inedible by nature. They contain antinutritional elements such indigestible oligosaccharides and phytate. Fermentation affects desired alterations by lowering anti-nutritional components and enhancing digestibility. *Okpeye* is a traditional West African seasoning prepared from *Prosopis africana* seed solid substrate fermentation. Many homes consider it as a family business because the preparation follows a passed-down habit from previous generations as an inexpensive source of plant protein. However, natural nature of the fermentation process raises concerns about the consistency, quality, and safety of the finished product. Because the seasoning is created on a small scale with less sophisticated equipment and manufacturing procedures, there are concerns about microbial safety. Thus, fermentation process and the range of microbial composition involved in *Prosopis africana* okpeye production were evaluated in this review. Potential spoilage agents, as well as biochemical and nutritional changes occurring during production of okpeye are gaining interest among researcher. This review highlights information that can help in developing starter cultures in a controlled fermentation process that ensures quality, longer shelf life, and microbiological safety.

Keywords

Okpeye, *Prosopis africana,* Fermentation, Legumes, Condiment

1. Introduction

Traditional fermented condiments are crucial for foods in West Africa due to their nutritional and sensory characteristics [1] [2]. Fermented okpeye condiment adds flavour to soups and has a pungent scent [3] [4]. When microorganisms, specifically yeasts, moulds, and bacteria, function enzymatically, they break down organic substances into acids or alcohol [5]. Fermentation has been demonstrated to improve food's organoleptic, nutritional, health-promoting, and preservation qualities [6]. Over the years, many fermented foods have been made through spontaneous fermentation and backslopping, with the most prominent natural microflora serving as a chosen starter culture [3]. Backslopping is a fermentation technique in which a small quantity of the previous fermentate is used as the raw material for the next fermentation step [7]. This technique reduces fermentation time, prevents failure, and standardizes the end product. Because fermented foods are traditionally manufactured in Africa, there are variances in the ingredients used, the way they are processed, how they are packaged, how they are handled, and how they are stored, which leads to significant differences in the final product [8].

The seeds of *Prosopis africana (Guill, Perr) Taub* are used to make the popular fermented soup condiment known as *okpehe* or *okpeye [9]. Prosopis africana* is a plant that grows throughout the African Savannah and Rainforest. Still, it is primarily used as a seasoning in the middle belt and some areas of South-East Nigeria [10]. *Okpeye* is dark brown with a characteristic of a pungent smell. *Okpeye* is rich in vitamins, protein, and minerals and may, as a food condiment, additionally contribute to improvements in the nutritional value of traditional dishes. As a result, *okpeye* contributes significantly to the nutrient intake of consumers [10]. The production and marketing of *okpeye* is also a primary revenue source for significant proportions of the rural population [10] [11].

Most research on African fermented foods has focused on the fundamental microbiology process: isolating and identifying suitable bacteria associated with the fermentation process. According to the findings of these experiments, *Bacillus* species are predominantly responsible for converting *P. africana* seeds during *okpeye* fermentation [12] [13] [14] [15] [16]. Other bacteria isolated during fermentation, including *Proteus, Staphylococcus, Escherichia coli, Alcaligenes, Micrococcus, Corynebacterium, Streptococcus, Pseudomonas,* and *Enterococcus*, appear to play no beneficial roles, and reproducible and acceptable products are produced without them [10] [11] [12] [13] [14]. Pathogen prevalence and growth in African fermented foods must be investigated further. Different microorganisms can participate in the natural fermentation process for *okpeye* production [17]. Thus, the survival or involvement of spoilage and pathogenic bacteria during manufacturing cannot be ruled out, mainly if fermentation occurs in African cooking technology under inadequate hygiene and sanitation settings. Product inconsistency caused by post-fermentation contamination and mixed-culture processing poses a significant threat to this product's microbial safety, quality, and other related food processes [17].

In terms of good manufacturing practice (GMP) and sanitation, okpeye manufacture in Nigeria is still a small-scale rural operation that only goes as far as the cleanliness of the largely rural illiterate producers [17]. Because of this, the microbiota that causes fermentation is frequently unpredictable, and diverse, and the tools are often rudimentary. In the same way, poor handler hygiene, potable water inadequacies, and other raw materials can lead to spoilage and contamination with pathogenic microorganisms. These elements affect the end product's quality and, subsequently, the consumers' health. Lack of uniformity in the manufacturing process is often the root cause of consistency issues and quality variation [17].

2. Production Process of *Okpeye*

The processing techniques for making *okpeye* and other condiments are based on personal expertise, custom, and indigenous knowledge. The primary or principal steps are similar despite minor variations arising due to location, local customs, and personal preferences. Environmental conditions, the type of raw materials used, and local customs that influence the choice of utensils and handling are usually the causes of specific differences. Fermentation usually occurs under conditions that local producers have discovered to be favourable for the reproducible production of the best product. These are the critical determinants in selecting appropriate fermentative organism growth and activity [1] [18].

Okpeye is a seasoning made from the cotyledons of *African Mesquite* (*Prosopis africana*). In process and usage, *okpeye* is similar to dawadawada. *Okpeye* is primarily used in Nigeria's south-eastern and middle belts to flavor soups and other traditional dishes. Details of the formal process may differ from one culture to the next. The most prevalent method used in parts of South Eastern Nigeria, according to Ugwuanyi [10], is to boil the seeds of *P. africana* for periods ranging from less than 6 hours to more than 12 hours, or until they are tender and the cotyledon is enlarged. By pressing the seeds between the thumb and finger, the cotyledons are retrieved. The retrieved cotyledons are washed several times, properly drained, and dried in a pot lined with *Alchonea cordifolia* leaves. The cotyledons are placed in the basket to a depth of 1 or 2 cm, then covered with additional leaves, reverse side down, and weighted with pebbles. Fermentation occurs over four days. At the end of the procedure, the cotyledons, which have turned dark brown and have a strong and pungent ammonia odour, are ground to a fine paste on a stone or mortar and shaped into various shapes and sizes. At this stage, the seasoning is ready to use. However, the balls are typically and preferably gently dried in the sun over many days, depending on the sun's intensity, until they harden and become black. The drying stage is a lengthy fermentation and maturation or curing stage in which the product becomes more aromatic and less pungent. Secondary fermentation, also known as maturation, is distinct in that it is self-protecting and devoid of microbial deterioration. After all, the pH is too high for spoilage organisms. The first four days of fermentation are the most delicate or risky, but regular sun drying away from dampness is essential for generating a mature product. **Figure 1** shows the conventional production process of *okpeye*. *Okpeye* in dried or mature condition can be preserved for several months with occasional re-drying in the sun or over a fire.

3. The Microbiology of the Traditional *Okpeye* Production Process

Several different microorganisms have been identified to ferment *Prosopis africana* seeds to *okpeye*, predominantly bacteria from the genus *Bacillus*, including *B. licheniformis, B. subtilis,* and *B. pimulus* [12] [16]. These organisms are usually introduced into the fermenting seeds fortuitously from processing utensils, air, water, and leaves used to wrap the seeds [19]. Often, microorganisms that drive traditional fermentation processes may be mixed populations of bacteria, yeasts, and mould from different genera. Sometimes more than one species from the same genus and more strains from the same species may be necessary. As a result, the microbiology of *okpeye* and other fermented foods is highly complex and poorly understood [20]. The kind of raw materials, the matrix condition (temperature, pH, and water activity), and other factors influence the microorganisms participating in the fermentation of different oil seeds. How each related microorganism affects the fermented product's organoleptic qualities is uncertain. While certain bacteria may interact simultaneously while others work in succession, the dominant flora may change throughout fermentation.

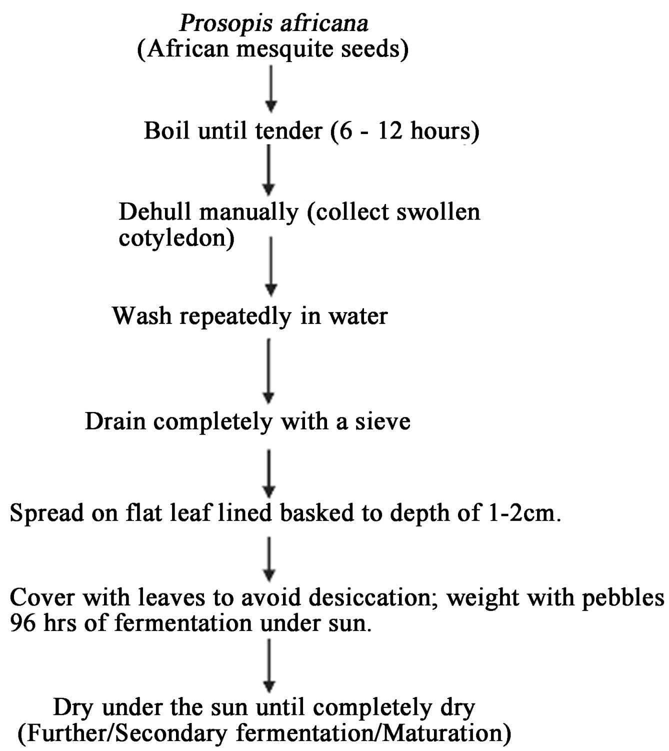

Figure 1. The conventional process of okpeye production (Source: Ugwuanyi, 2016).

These researchers discovered that indigenous fermented condiments are first aided by a broad microbial flora, giving way to *Bacillus* species. Although yeasts, moulds, and other bacteria genera are also observed, their functions are contentious. They may contribute to flavor formation and alter the product's nutrient composition through substrate alteration, but they cannot drive the fermentation independently [21]. Each organism's contribution to fermented condiments' nutritional and organoleptic properties is only partially understood.

4. Fermentation-Related Biochemical Changes

The main components of legumes and oil seeds responsible for most African seasoning production through fermentation are carbohydrates, fats, and proteins. As a result, the organisms in charge of fermentation must be able to utilize or hydrolyze these components [1].

4.1. Carbohydrates

Fructofuranosidase, galactanase, amylase, galactosidase, and glucosidase are among the carbohydrate degradation enzymes produced by *Bacillus* species [22] [23] [24] [25]. Microbial amylases break down starch into sugars that humans can easily digest. Galactanases, on the other hand, soften the structure of seeds and release sugars [1]. Non-digestible carbohydrates, such as raffinose, stachyosis, and arabinogalactan, are abundant in most legumes [26].

4.2. Proteins

One of the key factors affecting how the substrate's texture and flavor change in most fermented high-protein products is the degree of protein hydrolysis [27]. Enzymes that break down proteins provide soluble, low-molecular-weight peptides and amino acids that enhance flavor [28] [29] [30]. High-quality condiments smell strongly of ammonia and are dark in color. A steady rise in pH and a leveling up to 7.5 - 8.0 [12] [30] or higher to the level of up to 9.2 is caused by amino acids and ammonia produced as a result of protein decomposition. Alkaline pH values may be physiologically essential for fermenting microbes to adapt to and tolerate their environment [1] and to reduce undesirable processes caused by alkaline-intolerant species. Lysine and other essential amino acids diminish as fermentation progresses, although free amino acids grow [31]. This is one of the key arguments in favor of careful regulation of fermentation as compared to more conventional, uncontrolled procedures.

4.3. Lipid

Linolenic, oleic, and linoleic acids are the main products of leguminous lipolysis [21] [28]. This unspecific anti-tryptic action was associated with these free fatty acids, primarily linoleic, oleic, and linolenic acids [31]. Therefore, severe lipolysis might be detrimental to the nutritional value. Although up to 40% of the beans used in food fermentation are made of oil, substantial lipolysis rarely happens when dawadawa is produced. *Parkia biglobosa* was found to have low lipase activity [26]. The same author found low lipase levels in melon seed fermentation [26]. Similarly, Njoku and Okemadu [29] found that lipase played a minor role in ugba production in *Pentachletera macrophylla*. Due to adverse taste issues and rancidity production, low lipase activity in some fermented foods was deemed beneficial [26] [32].

However, even limited lipolysis can result in a significant flavor impact. It needs to be clarified whether this should be encouraged or discouraged and to what extent. Lipase has been reported to aid in developing distinct flavors and aromas [2] [27]. The absence of clarity related to the function of lipolysis in the fermentation of African seasoning agents requires further investigation. It would be interesting to look at how lipid amount and quality changes affect the organoleptic characteristics of fermented condiments.

However, after being treated with organic solvents, the aroma of fermented condiments created from locust beans, melon seeds, and soybean was liked [33]. The flavorful elements of fermented vegetable proteins used as condiments require additional study, creating the possibility for more study in this field [34]. Peptides, glutamic acid, and amines all undoubtedly contribute to flavor [1].

5. Nutritional Changes Associated with Fermentation of Okpeye

The nutritional value and flavor of the fermented product are markedly enhanced compared to the raw components during fermentation [35]. Minerals from legumes are a crucial part of the diet. Due to their role as structural elements in things like bones and as cofactors for specific enzymes, minerals are essential for the human body. Some critical components in the raw seed are shown by the mineral composition of fermenting *Prosopis africana*. In general, fermentation raises the concentration of minerals. As fermentation time rose, the mineral content of the condiment increases in calcium (24.1 - 68.3 mg/g), potassium (144.0 - 303.8 mg/g), phosphorus (131.3 - 540 mg/g), manganese (60.2 - 63.0 mg/g), and zinc (4.6 - 10.1 mg/g) [36]. Gberikon *et al.* [35] also reported nutritional values of the condiment viz, crude protein (24.70% - 30.79%), crude lipid (10.01% - 11.03%), carbohydrates (26.01% - 28.17%), fibre (5.01% - 7.07%). While fermenting African oil bean seeds, Enijiugha [15] discovered comparable increases in mineral concentration measured as the ash of up to 35%. The enhanced nutritional content of this condiment is likely due to the biochemical changes caused by organisms associated with its fermentation.

6. Flavour Development during Traditional Processing of Okpeye

The flavor is a crucial quality determinant for locally produced fermented condiments and significantly impacts consumer approval [10]. Many people utilize traditional fermented seasonings because of their distinctive flavour, aroma, and taste [2] [37]. Numerous volatile compounds produced by *Bacillus* spp. and other fermentation-associated bacteria through metabolic processes, including substrate molecule breakdown or biosynthetic processes, have been linked to these different flavors [34] [38] [39]. The taste, flavor, and aroma of *okpeye* are improved by drying as a secondary treatment or maturation process. As a result, the flavor becomes more aromatic and less pungent. Aroma is a very important quality parameter of any flavoring agent [40]. Esters which are major volatile compounds in African fermented seasonings are likely to be the product of reactions between microbial acidic and alcoholic metabolites and is associated with nice flavor [41] and have been reported to be responsible for quality sensory properties of various fermented foods [42]. Alcohols also contributed to the flavour of the condiment [43]. Raw okpeye seeds were dominated by hydrocarbons which do not play a significant role as flavour compounds as they possess a relatively weak aroma [34]. Azokpota *et al.* [38] identified essential fragrance components in afitin, iru, and sonru as tetramethylpyrazine 2, 5-dimethylhydrazine, 3-methyl butanol, chlorobenzene, 2-decanoate, 3, 5-dimethyl phenyl methanol and ethyl linoleate (three fermented soybean-based condiments).

7. Safety and Quality of Indigenous Fermented Seasoning Agents

7.1. Development of Starter Culture

Starter cultures, also known as starters, are single or mixed formulations of particular strains of microorganisms having the physiological ability to transform a substrate into a food product with a defined set of attributes when administered at prescribed quantities [44] [45]. The standard starter, modified for the substrate, enables better control of the final products' fermentation process and predictability [46]. Microbial starters' improvement, development, and standardization have been driving forces in transforming traditional food fermentations in developing countries from an art to a science. In many developing countries, the discovery of microbial starter cultures has also spurred innovation in developing machinery suitable for the sanitary preparation of traditional fermented foods under controlled conditions [3].

Developing a starting culture and improving bioreactor technology to control fermentation processes have been essential in industrialized nations for producing high-value products, including enzymes, microbial cultures, and functional food ingredients. These products are primarily made in wealthy economies as ingredients for food processing procedures, and less developed developing nations import them at a high cost [3]. The selection of microbial strains that can be utilized as starter cultures to produce different indigenous food flavoring compounds has been the subject of numerous research based on their dominance and enzymatic activity. It is known that microbial starters are necessary for indigenous fermented seasonings, especially okpeye. Mass-produced Japanese natto is made from a pure culture of *Bacillus subtilis var. natto* [47] [48]. Ouoba *et al.* [2] [28] demonstrated Soumbala, *Bacillus subtilis* B15, and B7 starters. Sakar and Tamang [49] employed *Bacillus subtilis* GK-2 and *Bacillus subtilis* KK-2: B10 strains as starter cultures for Kinema synthesis, and Visessanguan *et al.* [50] described Thuo-nao *Bacillus subtilis* TISTRO (BIOTEC7123) as a starter for Thuo-nao *Bacillus subtilis* TISTRO (BIOTEC7123). For the synthesis of soy dawadawada, Terlabie *et al.* [51] and Amoa-awua *et al.* [52] reported employing *Bacillus subtilis* FpdBP2 and *Bacillus subtilis* 24BP2. A few *Bacillus* strains have been examined and suggested as possible starter cultures for the fermentation of many native Nigerian food condiments. *Bacillus subtilis* mm-:B12 for ugba [53] and *Bacillus subtilis* for *okpeye* are examples of this [10]. Multiple laboratories study several fermentation organisms in depth to establish safe populations for commercial large-scale seasoning agent production.

7.2. Microbiological Safety of Indigenous Fermented Food

Bacillus spp. dominate protein-rich legume and seed fermentation. The long cooking time of alkaline fermented condiments such as okpeye produced from these protein-rich substrates is an important aspect of the production process, and this heating process may select spore formers that are more heat resistant [2] [18]. Furthermore, the degradation of proteins by *Bacillus* spp., most notably *B. subtilis, B. pumilus, and B. licheniformis*, results in the accumulation of peptides and ammonia. This causes an increase in pH, which promotes the growth of *Bacillus* spp [2].

Globally, food safety remains a major issue. According to the World Health Organization (WHO), 1 in every 10 people becomes ill, and more than 120,000 children under the age of 5 die each year as a result of consuming contaminated food. Africa bears a disproportionate share of the global burden of foodborne illness, with an estimated annual morbidity of 90 million people [54]. Foodborne pathogens and their toxins are primary aetiological agents of foodborne disease (FBD) and a growing public health concern. Fermented foods are generally thought to be safe. Fermenting organisms, particularly *Bacillus* spp., produce antimicrobial compounds such as organic acids, ethanol, bacteriocins, and hydrogen peroxide that inhibit the growth and survival of foodborne pathogens [55]. The presence of pathogenic and spoilage organism *E. coli* during *Prosopis africana* fermentation for okpeye production was reported by Fowoyo *et al.* [56]. Achi [1] made similar observations, noting the presence of *Enterobacter cloacae* and *Micrococcus* spp. in okpeye. This could also be an indication of faecal contamination as a result of product mishandling during production.

However, indigenous food production practises are frequently based on spontaneous fermentation, such as chance inoculation or the use of backslopping, in which utensils from a previous fermentation are reused [57]. Farmers, food producers, and handlers' lack of knowledge and application of Hazard Analysis and Critical Control Points (HACCP) and good manufacturing practises (GMP) can result in unsanitary production and processing. Because of these factors, the microbial profile of IFFs varies, and the presence of spoilage and pathogenic bacteria in these foods cannot be ruled out [16] [17].

8. Conclusions

Okpeye is a staple in the diets of the Igbos and other ethnic groups in Nigeria's eastern and south-eastern regions. It is made from *Prosopis africana* bean seeds that have undergone a natural solid-state fermentation process. *Bacillus* species are critical microbes in the process. Additionally, these microbes trigger the breakdown of other seed components by metabolizing the protein content of the seeds into ammonia and free amino acids.

Fermentation of *Prosopis africana* bean seeds increases the nutritional value of the product. The condiment's natural production procedure and the level of production quality raise questions about its safety and erode consumer confidence. Controlled fermentation of the product revealed that using starter cultures in the production process can overcome some of the observed drawbacks. As a result, there is a need to develop stable quality starters in forms that illiterate and semi-literate local processors can reproducibly utilize; and to bring such starters to play roles in the local industry.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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