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Probiotic microorganisms involved in cassava fermentation for *Gari* and *Attiéké*production

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ABSTRACT

Several fermented foods and beverages for human nutritionthat incorporate lactic acid bacteria and others beneficial microorganisms are produced throughout the world. Lactic acid bacteria (LAB) are widely distributed in nature and occur as natural microflora in many fermented foods (fermented milk, cereal fermented food, fermented fruit products, fermented roots products like cassava and others). This study gave characteristics, nutritional, Health and functional properties of probiotics microorganisms involved in cassava fermentation for*Gari*and *Attiéké* production**.** During cassava fermentation for *Gari*and *Attiéké* production many microorganisms with probiotic properties were involved and gave benefic properties. Lactic acid bacteria and yeast involved in food fermentation or production particular in cassava products may possess probiotic properties**.**Probiotics may have potential roles, as natural barriers to pathogens associated with intestinal disease with functional role.

Probiotic microorganisms role and importance in cassava fermentation for *Gari* and*Attiéké*production for healthy nutrition for consumers were developed in this work.

Indexing terms/Keywords

Probiotics, Cassava fermentedproducts, *Gari*, *Attiéké*

Academic Discipline and Sub-Disciplines

Biotechnology.

SUBJECT CLASSIFICATION

Food sciences

TYPE (METHOD/APPROACH)

Review

INTRODUCTION

Cassava (*Manihotesculenta*Crantz) is a food plant introduced in Africa from America by the Portuguese since 1558: it is brought from the New World to the Tropical Africa where it does now establish[1, 2].This food plant is also called manioc or tapioca-root, is a woody shrub of the *Euphorbiaceae* (spurge) family. It is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root. According to Lebot[3], cassava is the sixth most important food source in the tropics after rice, wheat, potato, barley, and maize. Cassava is the third most important source of calories in the tropicsafter rice and maize. Cassava products contain an important proportion of carbohydrates and minerals. Cassava leaves contain proteins,vitamins (A and C), and a lot of mineral salts [4]. Starchy root and tuber crops are second only in importance to cereals as global sources of carbohydrates[5] .

The main cassava food found in Africa are *attiéké, tapioca, gari, flour, starch, futu,* fermented flours, *akpissi*, *alebo*, *eberebe*, *ragout*, *kwadu*, ground fresh tuber, *kenkey*, *fede*, *agbelilakia*, *placali,yakayake*, *cossette, lafun, chikwangue*, etc. [6-8]. Cassava generates billions of income both for families and government and then contributes a lot to food security at several levels [9].

Fermentation process or technology is known and used by many people as one of the oldest forms of food conservation and preservation in different part of the world. The activity of microorganisms during or afterfermentation process may protect foods against spoilage and eliminate others antinutritional compounds also can increase the shelf-life of many food products. Preservation and protection offoods occurs through lactic acid, alcoholic, acetic acid andhigh salt fermentations [10].Lactic acid bacteria and yeast involved in food fermentation or production may possesses probiotic properties and contribute for food quality. Among them*Bifidobacterium* and *Lactobacillus* generaarerecognized having high potential in promoting good health. It is well known that Lactic acid bacteria are able to retard spoilage, preserve food as well as improve flavour and texture of foods. These microorganisms synthesize a variety of antimicrobial compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins which are important in fermented food characteristics and properties.

*Attiéké*is an essentially flavour starchy food produced from fermented cassava root.*Gari*is a grated, fermented and roasted cassava product. *Gari*is one of the popular cassava products consumed in Africa and others parts of the world. Cassava, when dried to a powdery (or pearly) extract, is called *tapioca*; its fermented, flaky version is named *Gari*.

This work deals with probiotics microorganisms involved in cassava fermentation for *Gari*and *Attiéké*production. Probiotic roles, characteristics, functional and nutritional properties were highlighted.

GENERAL INFORMATION ON THE PROBIOTIC MICROORGANISMS

1.Definitions

Fuller[11] defined a probiotic as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”.

Havenaar*et al.*[12]defined a probiotic as “mono- or mixed cultures of live microorganisms which, when applied to animal or man, beneficially affect the host by improving the property of the indigenous flora”, while in relation to food, probiotics were considered as “viable preparations in foods or dietary supplements to improve the health of humans and animals”[13]. Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host [14].

FAO/WHO defined probioticsas “live microorganisms which when administeredin adequate amounts confer a health benefit on the host”[15].

Beneficial association of lactic acid bacteria (LAB) with the human host was suggested in by Metchnikoff[16]and reported byFranz[17]

Kollath[18] and Holzapfel[19] were probably the first to introduce the term probiotic.Probiotics are defined as live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance.

2. Characteristicsand properties of probiotics

The probiotic microorganisms may have manyproperties and characteristics according to literature:

- Invitro bile salts resistance and bile salt hydrolase activity;
- Resistance to gastric acidity;
- Adherence to mucus and/or human epithelial cells. Probiotics cells should demonstrate the ability to adhere to gut epithelial tissue and to colonize the GIT;
- Antimicrobial activity against pathogenic bacteria. Producing some of several compounds such as organic acids, fatty acids, hydrogen peroxide, and diacetyl;
- Ability for Pathogen adhesion reduction;

Probiotics microorganisms are member of bacteria group that is Generally Recognized as Safe (GRAS). Probiotic LAB candidate must have or display include resistance to antibiotics, antimutagenicityproperties, rapid production of lactic acid, viability and retention of activity in delivery vehicles, ability to stimulate the host immune response, and the ability to influence metabolic activities such as vitamins production[20, 21].

According toMarteau[22], probiotics may theoretically be responsible for four types of side-effects: systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals, gene transfer.Probiotic products should be safe, effective, and should maintain their effectiveness and potency until they are consumed. For centuries folklore consideration has suggested that fermented dairy products containing probiotics microorganisms are healthful. Recent scientific investigation and work are supported some of these traditional views, suggesting the value of probiotics as part of a healthy diet. In addition, the emergence of some new public health risks suggest an important role for effective probiotics. Probiotics bacteria and others microorganisms have a potential in solving current and emerging lifestyle diseases [23].

3. PROBIOTIC MICROORGANISMS IN CASSAVA

The genus and species of the probiotic strainsinvolved in cassava fermented products as *Gari* and *Attièkè*are presented in table 1.

Many probioticswere used in poultry feed such as, *Lactobacillus* and *Bifidobacteria*[24], *Lactobacillus* strains [25-27], *Saccharomyces cerevisiae [28-30]*.

Many authors reported that Yeasts and lactic acid bacteria are implicated and play important roles in the fermentation of a wide variety of traditional food and beverage fermentations[31-35].

Fufu, a product of an acid fermented cassava root tuber serves as main course meals in most areas of Nigeria (Odunfa; 1985). Predominant Lactic Acid Bacteria involved in the traditional Fermentation of *Fufu*are: *L. plantarum, L. brevis, L. coprophillus, Lc. Mesenteroides,L. acidophilus, L. brevis, L. lactis*[36].

*L. mesenteroides*mostly occurs at early stages of most vegetable fermentations with *L. plantarum*predominating towards the end of the processes [31].

Lactobacillus sp. and *Pediococcus*sp. were isolated from cassava and sorghum fermentations [37].

Lactobacillus species were found dominated in *chicha*, cassava beerconsumed by theindigenous Shuar people of the Ecuadorian [38].

*Lactobacillus plantarum, Lactobacillus fermentum, Leuconostoc fallax, Leuconostocmesenteroides, Corynebacterium spp, Geotrichum candidum, Streptococcus faecium*are foundand isolated *in Gari[39-41]*..

In *Attiéké,* the species found and isolatedwere*Leuconostocmesenteroidessubsp.mesenteriodes; Lactobacillus salivarius, Lactobacillus delbrueckiisubsp.delbrueckii; lactobacillus fermentum; Lactobacillus confusus; Bacillus spp, Yeasts; moulds[42-45]*. The increase of proteins content in *attiéké* therefore depends on yeasts (unicellular protein organisms) in cassava dough [1, 46].

Actually new products are made from cassava like : chips [47, 48], fermented beer [38], bread [49], cake [50], *tapioca*[51, 52], biofuel [53-55], and medicine [56].

Table 1: Microorganisms responsible of cassava foods fermentation

Cassava fermented foods	Microorganisms	References
Tiquira	*Minilasitophila, Saccharomyces*sp	[57]
Lafun	*Saccharomyces cerevisae, Lactobacillus bulgaricus*	[58]
Cassavabeer	*Lactobacillus*sp	[38]
Attiéké /Gari	*Lactobacillus plantarium, Lactobacillus brevis, Lactobacillus casei Lactobacillus delbrukii*	[59]
Bêdêkouman	Lactic acid bacteria	[60]

Bêdêkouman:is a cassava fermented food. It is obtain after crumbling, fermentation and gridding of cassava roots.*Gari* and *Attiékè* used the same process of fermentation like *Bêdêkouman*.

Process is summarized in different steps: after crumbing, dough is inoculate with 8% (p/p) of cassava roots that were grated, washed, cuted and conserved in jute bag during 4 days at 30 ± 2°C. After 72 hours of fermentation, dough is sifted by pressing against stitch of steelsieve. Dough is packaging in a leaves, and cooked for first time in water during 20 to 25 minutes before to be pounded and packaging else. Package is cooked else during one hours and pound again, shaped like small bread and package again.

Tiquira:is a cassava fermented drink consumed in Brasil and originated from South America. It is processed into 3 steps which are: cassava root processing, saccharification of the gelatinized starch, alcoholic fermentation (Chuzel and Cereda, 1995).

4. FUNCTIONAL PROPERTIES OF PROBIOTIC MICROORGANISMS

Probiotics gave beneficial effects properties in poultry such as antibacterial and antifungal activities and the tolerance of fungi to gastrointestinal condition and antioxidant activity of the fungi [61]. According to Sugiharto*et al.*[62], fungi isolated from the Indonesian fermented dried cassava, particularly *A. charticola*, exhibited antibacterial, antifungal and antioxidant activity, gastrointestinal persistence and fermentative capacity that may be beneficial for poultry.

Probiotics have been shown to improve feed conversion efficiency [63, 64], improve weight gain and reduce mortality [64], reduce disease infection and stimulate the immune.

Bacteria are showed fermenting unabsorbed carbohydrate to short-chain fatty acids (SCFA) which have many health benefits related to heart disease and cancer prevention.

4.1 Improvement ofimmunity system

The human immune system acts to protect the host from infections against a large variety of noxious agents existing in the environment. The associate microorganisms in cassava fermented food are involved in the two functional divisions of the immune system: the innate and the acquired. Both components involve various bloodborne factors (complement, antibodies, and cytokines) and cells **[**65**]**. The ability of *Bifidobacterium* and *Lactobacillus* strains to influence the functioning of the immune system has been reported[66].

One of the arguments supporting the use of lactic acid bacteria fermentation to prevent diarrheal diseases is because they modify the composition of intestinal microorganisms and by this, act as deterrents for pathogenic enteric bacteria. Polyphenols compound are found in some cassava fermented food have several health attributes like anti-aging, anti-cancer, anti-immunodilation, protection against cataract, muscular degeneration and liver injury **[**67**-**69**]**. Several in vitro and in vivo studies have shown that polyphenols such as flavonoids have antioxidative and immunomodulatory actions. An high content of polyphenols might therefore be at responsible for the bioactive[70]. And there are several studies

indicating the stimulation of the host cell immunity, both innate and adaptive immunity, by *S. cerevisiae* var. *boulardii*in response to pathogen infections **[**71**]**.

4.2 Maintenance of epithelial barrier integrity

The columnar epithelial cells are responsible for maintaining the physical and functional barrier to harmful pathogen which included commensal organisms and their toxins. The preservation of the barrier function is dependent on the intactness of apical plasma membrane on the epithelial cells as well as the intercellular tight junctions. Various pathogenic organisms have developed strategies to either infect or traverse through the epithelial cells at mucosal surfaces, as part of the strategy to establish infection in the host. Successful probiotic bacteria are able to survive gastric conditions and colonize the intestine, at least temporarily, by adhering to the intestinal epithelium. Such probiotic microorganisms appear to be promising candidates for the treatment of clinical conditions with abnormal gut microflora and altered gut mucosal barrier functions. It has been shown that exposure of differentstrains of *S. cerevisiae* (human epithelial colorectal adenocarcinoma cell lines) increased the transepithelial electrical resistance [72] across polarized monolayers of cells. In another study, infection of T84 cells with enteropathogenic*E. coli* reduced the monolayer transepithelial resistance and distribution of tight-junction-associated protein *Zonula occludens*was altered, which caused disruption of epithelial barrier structure. And Induced rats showed a significant ($P < 0.05$) reduction in the membrane-bound ATPases and reduced expression of tight junction proteins in the membrane, coupled with their increased expression in the cytosol, indicating membrane damage. Transmission electron microscopic studies correlated with biochemical parameters. Pretreatment with combination of *L. rhamnosus* and *L. acidophilus* significantly prevented these changes **[**73**]**.

5 Importance and role

Certain Lactic Acid Bacteria (LAB) species are found not only as components of the human intestinal microflora but also they are present in ecosystem of fermented food. That is why fermented milks containing viable LAB are known to be beneficial to healh acting as prophylaxis against intestinal infections. Many studies have been conducted on their effect onthe incidence and duration of various types of diarrhea [74, 75]. LAB can be effective in preventing gastrointestinal disorders and in the recovery from diarrhoea of miscellaneous causes [22]. A decrease in the severty and duration of persistent diarrhoea has been reported with LAB [74].

The fiber content of cassava tended to be lower after fermentation.The fiber degrading capacity of fungi and their ability to increase protein may be beneficial for improving the quality of unconventional feed ingredients for chickens which commonly have high and low fiber and protein content, respectively.

LAB in improving the shelf life and nutritional quality of fermented foods and beverages,controlling diarrhea, as well as their antimicrobial properties have been established [76, 77].

The probiotics properties and antioxidant activity of *A. charticola*and *R. oryzae*isolated from the Indonesian fermented dried cassava were reported [62].

Some authors reported that association of Lactic Acid Bacteria and yeasts during fermentation may also contribute metabolites, which could impart taste and flavour to foods [78-80].

6.Health and nutritional properties

Archaic texts from Irac dating back to 3200 BC hold references to cheese, butter, and yoghurt indicating that fermented dairy foods have long been part of the daily human diet.

*Lactobacillu*s species are well adapted to fermentation of food both inside and outside the gastrointes[81, 82]. Lactobacilli have been positively associated with human health [83-87].In the case of fermented dairy products Metchnikoff [16] reported that they have long been associated with the ability to confer health benefits in those who regularly consume them. Also, their impact on the bacterial microbiota in the gut contributed to health and long life [16]. Probiotic-containing foods come in the form of fermented milk products, such as yogurt*, koumis*, and *kefir*, which have been consumed for 100s of years [88, 89]. Probiotic microorganisms can interact with the intestinal epithelial.

Guandalini*et al.*[90]reported that the administration of *Lactobacillus rhamnosus GG* to 287 children aged 1-36 months with acute diarrhoea significantly reduced the duration in infected children by rotavirus compared with those receiving placebo. Administration of *L*brhamnosus*GG also shortened the duration of the hospital stay. In Scandinavia regular intake of fermented foods to reduce the incidence of seriousdisease [91, 92].

The latter considered the longevity of Bulgarian peasants to be related to their high intake of fermented milk products, as he considered gut microbes detrimental rather than beneficial to human health [16].

Djoulde*et al.* [46] demonstrated that *Lactobacillus plantarum*and *Rhizopusoryzae*use as starter contribute to increase about 10 ± 2% of protein content.

Some species of *Lactobacillus* are considered to be beneficial to human health, given their ability to compete with pathogens, stimulate mucus production, and bind to the lining of the intestinal tract [93-95].LAB from fermented foods were recently found to play a positive role in mental health [23].

The use of LAB as probiotic for treating GIT disorders and as drug-delivery vehicles become increasing interest [66]. Their increase in antibiotic resistance, probiotics and their products, such as bacteriocins, promise to be good alternatives as

antimicrobials. Globally,many communities recognize the importance of probiotic microorganisms as evidenced by increasing demands for dairy products, especially yoghurt [23].

7. Production of metabolites

During cassava fermentation microorganismw have homolactic and heterolactic activities. The important organic acid synthetize by bacteria and others oragnisms during cassava fermentation are : butyric acid, Hexanoic acid, Octanoic acid, Decanoic acid, Nonanoic acid, Dehydro acetic acid, 2-Methyl propanoic, Oxalic, Citric, Tartaric, Mallic, Ascorbic, Lactic, Acetic, Fumaric, Propionic, Carboxylic acids, Hexanoic, Octanoic, Nonanoic[40, 96-104].

Somesvolatiles compounds are also produced during the fermentation of cassava like aldehydes, alcanes, ketones and others.

Plants, yeast and some bacterial species in fermented food contain the folate biosynthesis pathway and produce natural folates. But mammals lack the ability to synthesize folate and they are therefore dependent on sufficient intake from diet[105]. Several lactic acid bacteria and yeast have been identified to synthetize folates (vitamin B9) in different medium. Among them, some have been isolate in cassava fermented by products. It is the case of *Lactobacillus rhamnosus, Lactobacillus plantarum , Lactobacillus acidophilus, Lactobacillus reuteri, E. faecium, Lactobacillus fermentum, Lactobacillus brevis, Lactobacillus salivaris, S. cerevisiae* [69, 72, 73, 106].

CONCLUSION

Fermented food played a vital role in diet and human nutrition. Fermented foods are show as health foods, functional foods, therapeutic foods, nutraceutical foods or bio-foods. Probioticsmicroorganisms have a potential in solving current and emerging lifestyle diseases. Probiotics properties could be exploited either to enhance the nutritional value of existing foods, such as cassava, or in development of new food products based on traditional foods process.

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