QUALITY ASSESSMENT OF ENRICHED AND LOCAL INSTANT FURA

BY

JOSEPH GOJI B.Sc. (Mlcroblolgy); ABU.

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Department of Microbiology,
Faculty of Sciences,
Ahmadu Bello Universitry,
Zaria

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DECLARATION

I, hereby solemnly declare that the work reported in this thesis was carried out by me in the Department of Microbiology, Faculty of Sciences, Ahmadu Bello University, Zaria, under the supervision of Dr. (Mrs.) V. J. Umoh and Dr. (Mrs.) L. J. Ogbadu.

No part of this work has been submitted for any degree or qualification before. The works of other authors were referred to during this work and were duly acknowledged.

25/4/1995

Joseph Goji

Ahmadu Bello University, Zaria

October, 1994

CERTIFICATION

This thesis titled **Quality Assessment of Enriched and Local Instant Fura** by Joseph Goji meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

Dr. (Mrs.) V. J. Umoh Chairman, Supervisory Committee	19-04-95 Date
Dr. (Mrs.) L. J. Ogbadu Member, Supervisory Committee	24/4/95 Date
Prof. N. Uriah External Examiner	09-02-95. Date
Dr. (Mrs.) L. E. Odama Head of Department	26/4/95 Date
Dean, Postgraduate School	29/1/96 Date

DEDICATION

This work is dedicated to my late father Mr. Goji Wada and Col. and Mrs. Vanduhe

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ABSTRACT

Enriched and local instant fura and non instant local fura samples were analyzed for total aerobic plate count, Bacillus cereus , yeasts and mould count and proximate composition. In addition the three instant fura sample were assessed or storage stability and sensory qualities. The B. cereus isolates obtained were tested for nterotoxigenicity. Bacterial counts obtained ranged from 2.0 x 10^2 to 7.8 x 10^3 cells/g germinated instant fura, 3.0 x 10^3 to 6.5 x 10^4 cells/g for soybean instant fura and x 10^3 to 9.7 x 10^4 cells/g for instant local fura and 2.6 x 10^3 to 9.3 x 10^4 cells/g for ion instant fura. B. cereus count ranged from 1.0 x 10^2 to 2.0 x 10^3 cells/g for germinated instant fura, 8.0 x 10^2 to 7.4 x 10^3 cells/g for soybean instant fura, 1.5 x 10^3 to 1.9 x 10^4 cells/g for instant local fura and 1.4 x 10^3 to 2.0 x 10^4 cells/g for the non instant local fura. Yeasts and mould counts also ranged from 2.0 x 10^2 to 2.3 x 10^4 cells/g for germinated fura, 4.0 x 10^3 to 2.8 x 10^4 cells/g for soybean fura, 3.0 x 10^3 to 3.0 x 10^4 cells/g for instant local fura while non instant local fura had counts of 3.5 x 10^3 to 4.0 x 10^4 cells/g. In all the counts there were no significant difference at 5% level of siginifincance. Soybean fura contained significantly highei protein content (9.42%) than the instant local fura (1.60%), germinated fura (1.69%) and non instant local fura (7.20%). The instant local fura had significantly higher aero Die plate counts (6.5 x 10^4 to 1.0 x 10^5 cells/g), yeasts and mould counts (2.2 x 10^3 to 2.5 x 10^3 cells/g) than the other two fura samples. Germinated fura had higher titratable acidity (1.35 to 1.36) than the other two samples. There was no significant cifference in the moisture content of the three fura sample at 5% level of significance. Ten (10) of the B. cereus isolates tested for enterotoxin production were toxigenic by the ileal loop test. Five (5) of the isolates (50%) were from local fura, 4(40%) from soybean fura and 1(10%) from germinated fura. In terms of preference, the local fura was more acceptable (61.67%) than the soybean fura (25.92%) and germinated fura (12.35%). There was no significant difference (P 0.05) between the local fura and the enriched samples in odour, texture, smoothness, acidity, sweetness, and over all

acceptability. However there was a significant difference ($p < 0.05$) in colour of germinated fura from those of soybean and local fura while the colours of soybean and local fura were not significantly different.

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CHAPTER ONE

INTRODUCTION

'Fura' is a cereal based food consumed by people of all works of life especially in the northern part of Nigeria. Depending on the consistency, 'Fura' is used as weaning food for infants, as appetizer,refreshing drink and food for adults and children. It is usually taken after lunch or dinner alongside with fresh or sour cow milk (nono) to give rise to a product referred to as fura -da-nono. The consumption of fura along with nono involves mixing fura in nono through the process of shearing and kneading action (Bolaji,1987).

Though fura is principally prepared from millet flour, other grains such as rice, maize and sorghum or their combinations could be used. Generally, the physico-chemical properties of millet,maize and sorghum are very similar, and this factor make it technically feasible for any of the cereals to replace one another (Oguntunde and Akinrele,1976).

The processing method for fura production involves cleaning and dry milling of the cereal grains into grits. Ingredients such as pepper and ginger are added to the grits and repounded to a fine flour. Water is slowly added as the pounding progresses to obtain a paste. The paste is moulded into balls manually and dropped into a boiling water to boil for about an hour until they float. They are then removed, repounded, cut into small sizes and rolled into balls. Flour is usually sprinkled on the balls to prevent them from sticking together. Fura is finally converted into fura-da-nono by the addition of sugar and sour cow's milk(Oguntunde, 1989). Fura is second to tuwo in acceptability and frequency of consumption. Tuwo is a well known traditional staple food in the diet of the people of Northern Nigeria.

Protein-, energy- malnutrition(PEM) stands out as the most serious of the nutritional problems in Nigeria as well as other developing countries. Protein malnutrition in Nigeria

maybe attributed to limited sources of protein of high biological values especially those of animal origin. It is an important nutritional deficiency condition that often occurs during the critical phase of weaning in infants, crippling their physical and mental growth leading to high death rates(Coursin 1965, Reddy, 1990). Pre-school children, pregnant and lactating mothers are the most vulnerable groups.

Cereals are low in protein contents and the traditional products such as fura are even lower due to the methods of processing(Umoh and Fields, 1981). Nono commonly used to enrich 'Fura' was also found to be significantly lower in protein content compared to raw milk (Umoh etal , 1989). The low protein content and the total solids was attributed to the common practice of adding water to increase volume and meet demand.

Therefore, there is need for a detailed study of the various methods of improving the nutritive value of traditionally available foods like 'Fura' and the acceptability pattern of such enriched products. Some of the various traditional techniques which have been used to improve the nutritive value of traditional foods are germination, fermentation, puffing, roasting and fortification or enrichment with protein rich legumes (Muddabi and Raja Gopal, 1979; Desikachar,1980; Kazanas and Fields, 1981 and Taur et al, 1984).

The arts of germination and fermentation which have been developed for African foods have achieved the following benefits: Longer keeping quality, variety in flavour, making inedible foods edible, enhanced nutritional values and decreased toxicity (Odunfa, 1987). Certain essential amino acids like lysine and tryptophan, vitamin C and the B-group vitamins are reported to increase during germination and fermentation (Fordham et al 1975, Dalby and Tsai, 1976). In addition, germination and fermentation have been reported to reduce the trypsin inhibitors (Bau and Delby, 1976) and other anti-growth factors (Chem et al, 1977) as well as phytic acid content (Ferrel, 1978).

Supplementation of cereal-diets with legumes is one way of improving their protein quality (Sefa and Plange, 1989). Soya beans, cowpeas, groundnut, melon and cotton

seeds have been used scientifically to enrich corn and ogi(Ojofeitimi et al,1984). Recently in Nigeria, a stimulated interest appears to have been developed in the use of soybeans for human food. One of such steps is the commercial production of soy-ogi a protein fortified Nigerian maize beverage which was developed, perfected and produced at the Federal Institute of Industrial Research, Oshodi (FIIRO). These foods therefore, provide an excellent means of improving nutritional quality through the incorporation of vegetable proteins.

The aim of this study is to prepare and enrich 'Fura' through combined germination and fermentation, and incorporation of soybeans; to determine and compare the microbiological and chemical qualities, storage stability and organoleptic qualities of the enriched products with the traditionally available fura and to assay for public health related organisms.

CHAPTER TWO

LITERATURE REVIEW

'Fura' which originated from the Northern part of Nigeria has been in existence for quite a long time (Mariga, 1980). It is a partial dehydrated food product made from cereal meals principally millet grains (furan gero) or rice (furan shinkafa). The nutritivr value of fura can be enriched by the addition of milk and legumes such as soyabeans or germination and fermentation.

2.1 MILLET

Millet (Pennisetum typoides) is a staple cereal in areas bordering the sahara desert where the climate is too dry for the sorghum harvest to be depended upon. It is the sixth most important cereal in the world's cereal production. It is widely cultivated in Asia, Africa and the United States (Salunkhe et al, 1984).

Millet is predominantly the cereal crop of northern Nigeria. It germinate with less moisture in the soil than maize, rice and sorghum. There are several varieties of millet and they are classified according to their maturity (early and late), smoothness or bristly,etc; but the commonest variety in Nigeria is the bulrush millet (Pennisetum typhoides,staph and Hubbard) (Irvine, 1953). Millet grains are conical in shape and are smaller in size than either sorghum or maize grains (Martz, 1969; Dogget, 1970).

2.1.1 NUTRITIVE VALUE AND USES OF MILLET.

Millet grain is rich in oil and protein but lower in soluble carbohydrates or total ash on dry matter basis (Irvine,1953;Oguntunde and Akinrele,19976). Millet grain appears to be higher in protein than most cereals. Typical figures for millet grains are:

Protein	9 - 10%
Fat	3 - 4%
Fibre	2%
carbohydrate	75 - 85%
Ash	1 - 2%
Food Energy	414 calories (Ihekoronye and Ngoddy, 1985).

Millet grain is a good source of thiamine and probably contains appreciable amounts of other B-vitamins. It contains good levels of calcium, phosphorus, magnesium and iron.

Pearl millet is a good source of the essential mineral iron,zinc,copper and manganese (Kumar and Kapoor,1984; Chanhan, Suneja and Bhat,1986). However, as reported for other plants foods (Maga,1982), human bioavailability of minerals from pearl millet may be poor because of the abundance of anti-nutrients such as phytic acid and polyphenols (Chauhan et al,1986; Dhankher and Chauhan, 1987a).

The pigment in pearl millet constitutes a serious problem which makes its products unacceptable and the bitter taste (Recichart and Yang, 1979). Due to the presence of certain pigments in the pericarp and the endosperm layers of seeds,coarseness and the absence of gluten characteristics in protein; the pearl millet has remained a food for the low socioeconomic groups. The wide use of pearl millet depends on removal of pigments to increase its acceptability.

Perhaps the most important use of millet flour in addition to making 'fura' in Nigeria is for "Kumun zaki" a well known traditional non-alcoholic beverage consumed mostly in the northern parts of Nigeria. Other traditional products derived from millet includes "Koko" a millet porridge consumed mostly in Sokoto, Kano, Kaduna, Adamawa, Borno, Bauchi and Yobe state. Others includes "Burabusko" a well known food commonly consumed in Borno state, "Dambo" or "Dakere" usually eaten with nono or fresh milk by the Fulanis and "Burukutu" a local beer made from malted millet.

2.2 SOYBEANS.

Glycine max(L) merril , known variously as soybean, soyabean, sojabean, chinese pea, or manchurian bean, is grown extensively throughout Eastern Asia, United States and the Sahel region of Africa. Although introduced into the United states during the 19th century,soybean received little commercial attention until the 1920's and 1930's.
In Nigeria, soybean is mostly produced in the Guinea zone, with Benue state and Abuja being the main producing areas (Ashaye,et al,1974). Most of the soybeans produced in Nigeria is exported,with little utilisation of the crop due partly to its unfamiliar flavour.

The seeds of most soybean varieties are nearly spherical. Average seed weight is 120

to 180mg of which the seed coat contributes about 10%. The large seed varieties, Kanrich, Disoy, Magna and Price , produce seed, weighing about 260mg. Short hairy pods containing from 2 - 3 seeds are attached to the plant. Shape and size of soybean vary from small,round pea-sized to longer, elongated beans, colours vary from yellow, brown or green to black. The bean consists primarily of a hull, hypocotyl and cotyledons.

2.2.1 NUTRITIVE VALUE AND USES OF SOYBEANS.

Soybeans (glycine max) ranks high among the vegetable protein sources in the world. The crop is particularly valued for its protein content. It is also an excellent source of edible oil and other major components.

The whole dry soybean grain contains about 40% protein and up to 20% fat. The seed has three major components namely the hull which constitute 8%, the cotyledons 90% and the embryo axis 2%. The over composition of soybean and its parts is as follows:

Fraction/Part	Proteins N x 6.25 %	Carbohydrate %	Fat %	Ash %
Hull (8%)	8.8	86	1	4.3
Cotelydons (90%)	43.0	29	23	5.0
Hypocotyl (2%)	41.0	43	11	4.4
Whole	40.0	34	21	4.9

Soybean contains about 35% total carbohydrates. The protein in soybean is of good quality and is measured in terms of the presence of essential amino acids. It is however deficient in methionine but adequate in lysine. Soybean oil is among the richest source of linolenic and arachidonic acid (essential fatty acids). The oil is also unsaturated and contains very little, if any, of cholesterol. It contains moderate amounts of minerals and vitamins.

Raw soybean contain mainly biologically active factors. Trypsin inhibitor and lipoxygenase are major factors responsible for the poor protein digestibility and beany-off-flavour respectively. The trypsin inhibitor (Collins and Sanders, 1976), lipoxygenase and urease(Iwanicki, 1971) activities have been reported to be increased during soybean maturation.

Soybean flours have been reported to have the following chemical and functional properties; protein (34.4-48.5%), Fat(14.8-26.2%) and carbohydrate content(16-30%). They also showed high water absorption capacity (153-340%), fat absorption capacity (127-173%), and very high emulsifying activity (6-11%) (Udensi and Onuora, 1992). The use of soyproteins for nutritional purposes is now becoming very important. Supplementation of cereals and meat extenders are examples of this application. Soyflours and fruits, used largely because of their low cost are added to a variety of foods.

Recently in Nigeria, a stimulated interest appears to have been developed in the use of soybeans for human food. One of such steps is the commercial production of soy-ogi a protein fortified Nigerian maize beverage which was developed, perfected, and produced at the Federal Institute of Industrial Research, Oshodi (FIIRO) Nigeria. Further still,International Institute of Tropical Agriculture (IITA, 1985) reported the utilisation of soymilk in the treatment of infant protein- malnutrition(Kwashiorkor) in kersey children's Home Ogbomosho, Oyo state, Nigeria.

In most Asiatic and American countries, soymilk has been the most largely consumed soy-product (Alden, 1975) and in Nigeria, it is currently being produced at the household level (Ogundipe and Oguntunde, 1990). In sahel region of Africa, Legumes are generally eaten fresh and prepared in many ways including boiling, roasting, frying and steaming . The traditional method of processing are soaking, decortication and conversion of legumes to a paste product before frying or steaming(Dovlo et al, 1976).

In Oriental countries, soymilk and fermented and non-fermented soybean products including tempeh, natto and tofu; have been important sources of good quality protein for ages. There is also a growing interests in the preparation of soybean in soups and stews (AERLS,1985 and IITA,1985). Attempts have also been made to improve the protein of

corn dough in Ghana, Kisra in sudan and many others (Akinrele,1970; Plahar and Leung, 1983; Akinyele and Fasaye,1988). Some of the products derived from soybeans includes soya drink, soya concentrate, soya ice cream, soya yogurt and others (Abiayi,1991).

2.3 TRADITIONAL TECHNIQUES OF ENRICHING FURA

2.3.1 GERMINATION:

Germination is a traditional practice in many developing countries,where it is used to improve the nutritional quality of cereals (Hamad and Fields, 1979). It has long been claimed that germination enhances the nutritional value of cereals and legumes (Finney, 1983; Boralkar and Reddy, 1985; Fernadez and Berry 1988; Price, 1988). It has also been reported to improve functional characteristics of some cereals(Lukow and Bushuk, 1984) and oil seeds(Canella et al ,1985).

It is a well known practice in India to germinate,dry and toast cereal and legume grains before eating. Pearl millet has been used locally in sprouted and dried form as a supplementary food for the child in the weaning period (Rajalakshim,1974). The use of sorghum malt as an additive has been introduced at the village level in Tanzania (Mosha,1987), and several studies have been carried out with millet (Brandtzaeg et al, 1981;mosha, 1983,1987),and rice (Gopaldas et al, 1986).

The process of malting consists of controlled germinating and subsequent drying of a seed. The conversion of grain into malt is essentially physiological in nature and is the result of the action of enzymes (α-amylase) (Manners,1974),

During the germination of cereals and legumes, a number of significant nutritional changes takes place. A considerable proportion of the insoluble protein is transformed into soluble components (Piendl and Wagner,1972). Dextrins and free sugars are liberated and there is an increased amount of damaged starch granules.

2.3.2 FERMENTATION:

Fermented foods such as bread, cheese, various soybean products,cassava, vegetables, and sausages, have made important contributions to human diets for thousands of years and continue to do so. Certainly, the most significant role of

fermentation in human nutrition has been to help make the nutrients naturally present in the starting food materials more palatable and more widely available than would be possible without fermentation. Thus, even if fermentations had no direct effect upon the nutrient content and quality of foods, these processes would be very important to the food supply. However, it is clear that fermentation processes can have significant direct effects on the nutritive qualities of foods.

Food fermentations are very complex processes because they normally involve the interaction of plant or animal tissues with a group of microorganisms. These means that changes depend upon the available nutrients and nutrient precursors in the starting materials,the metabolic abilities of the fermentative microorganisms, and possible interactions among all these elements. Many fermentations occur in solid or semi solid states so that particle sizes, diffusion rates of oxygen and nutrients distribution of fermentative organism may be important factors in both the organoleptic quality and nutrient content of the product.

The art of fermentation has been developed many centuries ago. Because of the emphasis placed upon plant materials as human foods, interest in traditional fermentation technology for food processing has greatly increased in recent years. Most of the fermentation processes were developed long before the existence of microorganism was recognised. The remarkable early man who had no concept of microorganisms not only developed the processes but also safely maintained the cultures through the centuries. These foods are priced for their palatability and wholesomeness and are important components of diets in many parts of the world especially South East Asia,the far East,the middle East,and parts of Africa (Wang and Hesseltine, 1981).

Fermented foods form a significant proportion in the diet of many Africans. Over thirty (30) different foods have been recorded (Odunfa,1987).They can be broadly classified into the following groups:

(a) Fermented starchy roots e.g. Gari,fufu,etc
(b) Fermented cereals e.g Ogi, uji, kamu.
(c) Alcoholic beverages e.g palm wine, pito etc.
(d) Fermented legumes and oilseeds e.g dadawa.

(e) Fermented animal proteins e.g fermented fish, fermented milk.

Corn, tef, cassava and soda crackers have been fermented by succession of lactic acid bacteria (Sugihara, 1978; Gashe,1985; Adegoke and Babalola,1988 and Oyewole and Odunfa, 1988). Millet is fermented by the hausas of Northern Nigeria to produce a starch - cake food called kamu (Oyeyiola, 1991).

Fermentation have been used to improve cereal protein quality, lysine and tryptophan (Umoh, 1979). The percent relative nutritive value (RNV) and limiting amino acids were increased significantly when cowpeas and chickpeas were fermented at 25°C for 4 days (Zamora and Fields, 1979).

Fermentation prior to cooking was found to improve the invitro and invivo digestibility of sorghum (maclean et al, 1981;Axtell et al 1982; Graham et al, 1986). It has also been found to increase pepsin digestibility of millet protein (Martz et al 1984),decreased the concentrations of phytic acid (Mahajan and chauhan, 1987) and polyphenols and improved the availability of minerals.

2.3.3 ENRICHMENT OF CEREAL DIETS WITH SOYBEANS:

Supplementation of cereal diets such as 'fura' with legumes such as soybeans and sunflower seeds is one way of improving their protein quality (Sefa-Dedah and Planges, 1989). Other legumes such as groundnut, cowpeas and melon and cotton seeds have been scientifically used to enrich corn and ogi (Ojofeitimi et al 1984).

In Nigeria, soybeans was used to improve nutrition at the kersey children's clinic in Ogbomosho. The Health clinic fed soymilk and other soybean products to children who were severely malnourished (e.g a 2 years old weighing 4kg). They received a diet of traditional foods fortified with soymeal prepared by mothers who attend weekly classes on the use of soybeans in making soups, soy milk and other foods. Malnourished children including those with kwashiorkor and marasmus gained weight after being fed with these diets (Weingartner et al,1987). There is a growing awareness in the production and utilisation of soybean in Nigeria within the last decade (Akinola, 1980; IITA,1985, 1988; Mijindadi, 1987) in preparation of daddawa, soymoi moi, soyakara and incorporation of soybean in soups and stews (AERLS 1985,IITA, 1989).

The increasing attraction of utilisation to soybeans has often been limited by several biological, physiological, manufacturing and social factors (Baker and Mustakas,1973;Rackis, 1974;Rackis et al, 1975; Carison, 1976; Nelson et al, 1976 and Yang, 1979). Notable among these factors are the rapid enzymic reaction in cracked soybeans which lead to off flavour development in soymilk(Nelson et al, 1979 and Weingartner, 1987). There is the natural long cooking time associated with soybean (Yang,1979 and Nelson et al, 1987) which generally hampers its utilisation especially at the home level. This problem also creates doubts in the consumers who may not be sure of what is "safe". Added to these are the short shelf-life of soyproducts as the milk (which last for just 24 hours) and the flatulence effects which are due to the presence of low molecular weight carbohydrate (Rackis, 1979). These problems are more pronounced in the rural areas where processing equipment and skill for soy-products handling are lacking.

2.4 NUTRITIVE VALUE AND MALNUTRITION.

Protein- energy-malnutrition in Nigeria maybe attributed to limited sources of protein of high biological values especially those of animal origin. For example, it is estimated that as at 1974 and 1975, animal products accounted for only 7.1g (12.5%) of the 56.6g per capita daily intake of protein in Nigeria(Olayide and Babatunde,1980). In recent years, uneven distribution of foods coupled with increase in population have worsened the situation.

Industrial manufactured cereal-based weaning foods such as Nutrend and Cerelac infant foods are very expensive even when lowcost alternatives are developed. In poor countries ,these products are normally available to urban children of the higher income families. Protein-energy-malnutrition can be prevented to a large extent by introducing lowcost weaning foods such as the soy-fura which can be prepared easily in home and community kitchens from locally available raw materials.

Therefore, there is need for a detailed study of various traditional methods of improving the nutritive value of locally available foods such as "fura" and the acceptability

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pattern of such enriched products.

2.5 MICROBIOLOGICAL QUALITY AND STABILITY OF FURA

"Fura" is a perishable cereal product due to its high moisture content. In addition, it contains starch, some sugar, available nitrogen compounds, minerals and accessory growth substances that favours the growth of microorganisms. In general, the basic condition necessary for the enzymic and microbial spoilage of fura is the presence of water.

Fura which tends to be low in moisture content absorbs moisture under storage up to water activity (^{a}w) of 10 to 15% (Bolaji; 1987). This increase in moisture content favours the growth of moulds, yeasts and bacteria which may subsequently lead to spoilage.

Opawole (1990) while working on the microbiological quality of fura, isolated bacteria, yeast and mould species. Notable among these species were Staphylococcus aureus, Bacillus spp, Aspergillus, Penicillium, Fusarium, Mucor and Rhizopus and Saccharomyces spp. Counts obtained ranged from 3.5×10^5 to 4.1×10^5 cfu/g. Of these species, moulds and yeasts were more associated with the spoilage of fura than bacteria. This is so because yeasts and moulds survive under lower moisture content than bacteria. Aspergillus flavours appears to be of public health interest due to its ability to release mycotoxins (aflotoxins) into food.

The shelf-life of fura has been improved upon by physical removal of water. This has been achieved through the production of dry fura powder known as instant fura (Mairiga, 1980; Ikharo,1988). This product has been shown to be less susceptible to microbial growth and spoilage and other chemical changes associated with the high moisture content (40-50%) of fura. It has also been shown to remain stable for 6 months without any significant changes in microbiological quality and moisture content (Ikharo, 1988). In addition to its stability, instant fura has the potential advantages to consumers including the quick and easy preparation method(Mairiga, 1980). It is easily dissolved in cold or warm water before the addition of nono to give rise to 'fura-da-nono'.

CHAPTER THREE

MATERIALS AND METHODS

3.1 ENRICHMENT OF FURA BY COMBINED GERMINATION AND FERMENTATION

3.1.1 Germination and Fermentation Procedure

Millet grains were purchased from the market, and mouldy and broken seeds were picked by hand. About 50g of the cleaned seeds were washed three times in tap water, soaked 16 - 18 hours in tap water at 25°C. After 18 hours of soaking at room temperature (25°C) all the samples were drained and uniformly spread on wet cloth. Grains were covered by another cloth and water was sprinkled on the top. Germination was carried out at room temperature for 2 - 3 days for some of the seeds to sprout. They were then washed in running tap water and mouldy seeds removed by hand. The whole cleaned germinated seeds were dried and milled into flour.

Two - hundred and fifty (250) grams of the germinated powder were poured into a container and 1 litre of tap water added. The contents were stirred, covered with aluminum foil and allowed to ferment at room temperature for 2 - 3 days with occasional stirring of the mixture. The mixture/paste was molded into balls, steamed at 100° C for an hour, pounded and remoulded into balls to give rise to germinated fura (Fig. 1).

3.2 ENRICHMENT WITH SOYBEANS

3.2.1 Soybean enrichment procedure:

Two hundred grams of soybeans were purchased from the market, cleaned, dehulled, dried, milled into powder and mixed with 1 litre of water to form paste. The millet - Soybean paste were mixed in different proportions or ratios of 90 : 10 (10%), 80 : 20 (20%), 70 : 30 (30%), 60 : 40 (40%), and 50 : 50 (50%) to obtain five samples. The millet - soybean pastes were then molded and steamed for 1 hour at 100° C They were pounded and remolded into balls to give rise to soybean fura (Fig. 2).

3.3 LOCALLY PROCESSED FURA AS CONTROL

Traditional fura were purchased from the market and used as control.

3.4 INSTANT FURA PREPARATION:

Both the germinated fura, soybean fura and the local fura samples were reduced to smaller sizes and dried in a dryer at 60° C for 3 - 4 days. The dried fura samples were then pounded using mortar and pestle into instant fura and stored in polythene bags for further analysis.

3.5 MICROBIOLOGICAL ANALYSIS

3.5.1 Total aerobic plate count:

Ten grams each of the fura samples were weighed into 90 ml of peptone water (1:10) dilution. Further serial dilutions of 10^{-2} and 10^{-3} were made. Aliquot (1 ml) of 10^{-2} and 10^{-3} dilutions of freshly prepared instant fura samples and non-instant local fura were pour - plated in duplicates on plate count agar (PCA) (oxoid) for total aerobic plate count, incubated at 32°C for 24 - 72 hours, and counted.

3.5.2 Yeasts and mould counts:

Ten grams each of the fura samples were used to prepare dilutions of 10^{-1}, 10^{-2} and 10^{-3} as above. Aliquot (1ml) of 10^{-2} and 10^{-3} dilutions of samples as above were pour plated in duplicates onto acidified potato dextrose agar (APDA) for yeasts and mould counts, incubated at 25 °C for 5 - 7 days, and counted. The yeasts and mould cultures were examined microscopically by gram stain and wet mounts for morphological characteristics i.e type of spores and hyphae.

3.5.3 Bacillus cereus counts:

Counts of B. cereus were determined by surface plating of 0.1 ml Aliquot of 10^{-1} and 10^{-2} dilutions of freshly prepared instant fura samples and non-instant local fura on Bacillus cereus medium (BC medium). Incubation was carried out at 32°C for 24 - 72 hours. The B.C medium was prepared by adding 1g of mannitol and 10ml of egg yolk

emulsion to 90ml of basal medium. The basal medium contains: $(NH_4)_2HPO_4$, 1g; KCl, 0.2g; $MgSO_4$ $7H_2O$, 0.2g; Yeast, 0.2g; bacteriological agar, 20g; 4ml of bromo cresol purple and distilled water, 1litre. The egg yolk was prepared by separating the eggs aseptically. The yolks was broken and mixed with an equal volume of physiological saline, filtered through a filter paper, distributed into sterile bottles and heated at 60°C for 30 min.

Typical gray white colonies with opaque zone and purple coloration of the surrounding medium were counted as B. cereus.Representative colonies of B. cereus species with egg yolk reaction were gram stained and the gram positive rods purified on nutrient agar plates, incubated at 32°C for 24 hours and stored on nutrient agar slants for further use.

3.5.4 Characterization of Bacillus cereus and Toxicity test.

3.5.4.1. Characterization:

All cultures on slants were restreaked on BCmedium. Colonies surrounded by an opaque zone (egg yolk reaction) with purple coloration of the surrounding medium were picked for further comfirmation as B. cereus. The confirmatory test included catalase, gelatin liquifaction, citrate utilisation, VP reaction, nitrate reduction, hemolysis of sheep red Blood cells (RBC) and motility test, anaerobic growth, Acid from Glucose and phenylalanine Deaminase (Appendix II). All the strains positive to the above tests were regarded as B. cereus.

3.5.4.2. Toxin Production.

Out of the 25 B. cereus isolates, 10(40%) were subjected to enterotoxin production. These includes 5 from local fura, 3 from soybean fura and 2 from germinated fura. Enterotoxin production was carried out by growing the test organism in brain heart infusion (BHI) broth (DIFCO) containing 0.1% (W/V) glucose supplement following the procedures of (Kramer et al, 1982).

The medium was filter sterilized using 0.45um pore membrane paper (millipore) and dispensed in 20ml volumes into 250ml sterile Erlenmeyer flasks using sterile 20ml

syringe. The medium was then inoculated after mixing 0.5ml brain heart infusion broth, and 0.1% glucose (BHIG) cultures grown at 32°C for 16 - 18 hours and incubated at 36°C for 6.5 hours on rotatory action shaker set at 200 rev/min. During incubation 1ml of 0.1N NaOH was added to the toxin production medium at the interval of 3.5 hours of the commencement of incubation. The addition of 0.1 NaOH into the medium helps to make it alkaline thereby counteracting the pH fall in the fermentation process towards acidic condition which does not favour toxin production. At the end of the 6.5 hours incubation, the pH was rechecked, and the cultures centrifuged, filter sterilized through 0.45um milipore membrane amd the filtrates stored in vials in deep freezer until needed.

3.5.4.2. Toxin test using Rabbit ileal loop.

Each of the two rabbits was injected with 0.5ml of pentamine hydrochloride for general anesthesia. The mid-ventral sides of the animals were opened up for ileum to be externalized and tied to form six test loops. Intraluminal injections of 2ml each of the extracts were made into each of the Six test loops. In each rabbit, one loop at the distal end of the ileum was injected with sterile brain heart infusion broth containing 0.1% glucose as control (Spira and Goepfert, 1972). The ileum was then returned to the abdominal cavity and the incision closed. Following recovery, the aminals were provided with water but no food and were allowed to live for 7 hours.

After the holding time of 7 hours, the rabbits were killed and opened up immediately for examination. The gross appearance of the loops were noted. In a case where the control loop or any of the blank loops contained fluid, all the tests in the rabbit were considered invalid. The length and fluid volume of each valid test was measured and the ratio of the fluid volume (ml) to loop length (cm) was determined and recorded. Ratios of fluid volume to loop length ranging from 0.2 to 0.4 was considered mild, 0.5 to 0.7 as moderate and 0.8 to 1.5 as severe.

3.6 PHYSICO CHEMICAL ANALYSIS

3.6.1 PH and Titratable Acidity:

The pH of the samples were determined with the 1:10 dilution of the fura samples using pH meter model 7. The titratable acidity was determined using 0.1 N NaOH and phenolphthalein indicator. Percentage titrable acidity (TA) was calculated as follows:

$$N_A = \frac{V_B N_B}{V_A} \times \frac{100}{1} \times \frac{1}{10}$$

N_A= % normality of the acid/ml

V_A= Volume of the acid or sample used (10ml)

N_B= Normality of the base = 0.1N

V_B= Titre value or volume of the base.

3.6.2 MOISTURE CONTENT AND TOTAL SOLIDS

The percentage moisture content was determined using hot air oven.Ten grams (10g) of the fura samples were each weighed in a porcelain bowl and oven dried at 120°C until there was no more loss or decrease in weight.

$$\%\ Moisture = \frac{\text{loss in weight}}{\text{weight before drying}} \times \frac{100}{1}$$

Total solids was determined by subtracting the value of percentage moisture from 100

Total solids = 100 - % moisture.

3.6.3 Determination of protein content:

This was determined as recommended by (AOAC, 1980) using standard micro-kjeldahl method and converted to crude protein using a conversion factor of 6.25. The use of this method is that organic protein in food is oxidized by concentrated H_2SO_4 in the presence of suitable catalyst and nitrogen converted quantitativelly to ammonia (NH_3). The ammonia liberated is titrated directly with standard HCl using a mixed indicator and the result calculated as percentage nitrogen. This involves three steps:

3.6.3.1 Digestion

Ten grams of the dry (instant) fura samples were weighed into a small kjeldahl digestion flask. A small amount of catalyst was added followed by 2ml of conc. H_2SO_4. This was then heated gently on the digestion stand until fumes of H_2SO_4 were freely evolved, then heated more strongly until the digest becomes apple green in colour. At this point, the digestion was continued for half an hour longer and stopped. The digestion was carefully done to avoid particles of the undigested carbon from sticking to the sides of the tube. Blank of the reagents alone was run at the same time.

3.6.3.2 Distillation.

The digested instant fura samples were poured into the distillation apparatus and the flask rinsed with 2ml distilled water. The round glass stopper was replaced and 10ml (40%) NaOH poured into the funnel and the stopper gradually lifted for NaOH to run into the digest for NH_3 liberation. The ammonia liberated was collected into 5ml (2%) boric acid in 50ml conical flask. The distillation was continued till 25ml of distillate was collected.

3.6.3.3 Titration.

The distillate was diluted with distilled water to 50ml mark in 100ml beaker. Two drops of indicator was added to the distillate and titrated with 0.02N HCl to give a faint pink colour.

Calculations:

$$\%N_2 \quad \frac{(A \quad B)\, N \times 14.007}{W} \times 100$$

Where A = quantity of HCl used to neutralize test samples

B = quantity of HCl used to neutralize blank.

N = normality of HCl (0.02)

14.007 = relative atomic mass of nitrogen in NH_3

W = weight of the sample in miligram (mg)

Thus % protein = % N_2 x 6.25

3.6.4 Determination of Ash

Ash was determined using 5grams of the instant fura samples weighed in clean crucibles and then gently heated over a bunsen burner in a fume cupboard. After heating the samples were put in a muffle furnace at 500°C for 15min till the samples became white (ash).

3.6.5 Determination of Fat

Fat was determined using ether extraction. Extraction was carried out for about 16 hours by warming the flask gently over a steam bath. The ether in the sample was evaporated to dryness living only the fat in the flask which were weighed.

3.7 STORAGE STABILITY

The instant fura products were packed for storage in polythene bags. The shelf life was monitored over a period of 4 weeks by weekly checking of

a) pH and titratable acidity (TTA)

b) Moisture content

c) Yeasts and mould counts

d) Total aerobic plate count.

3.8 SENSORY ANALYSIS

Consumer acceptability test was conducted using the ranking and five - point descriptive scale for sensory scoring.

3.8.1 Ranking:

The ranking test was conducted by 60 panelists (male and female, young and adult) that were familiar with fura. They were asked to choose the best out of the five soybean fura samples using a standard questionaire form (Appendix I). The best soybean fura, germinated and local instant fura were then subjected to preference test by 81 panelists using the standard questionaire form.

3.8.2 Hedionic test.

Five point descriptive test for sensory scoring was conducted using 7 trained penalists comprising of those that were familiar with fura. The scores were on such characteristics as colour, odour, texture, smoothness, acidity, sweetness and over all acceptability (Appendix IV). The test was repeated 4 times to obtain consistent point.

3.9 STATISTICAL ANALYSIS:

Scores from the hedionic rating tests were averaged to obtain mean scores which were subjected to analysis of variance. The multiple range test was then used to determine the significance of any differences between samples.

Figure 1: Flow sheet for the preparation of fura after germination and fermentation

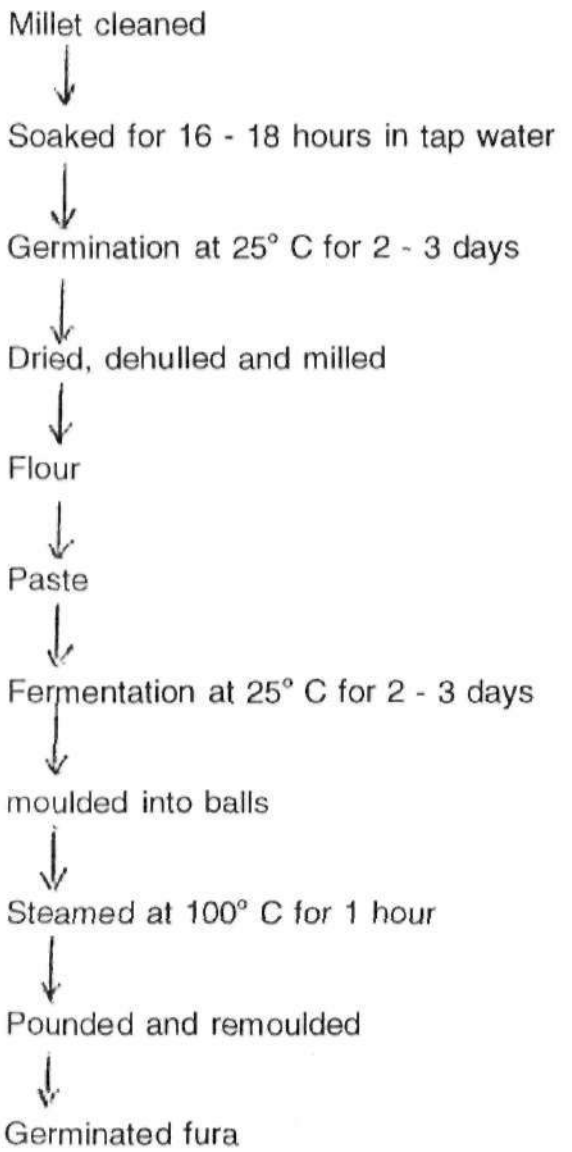
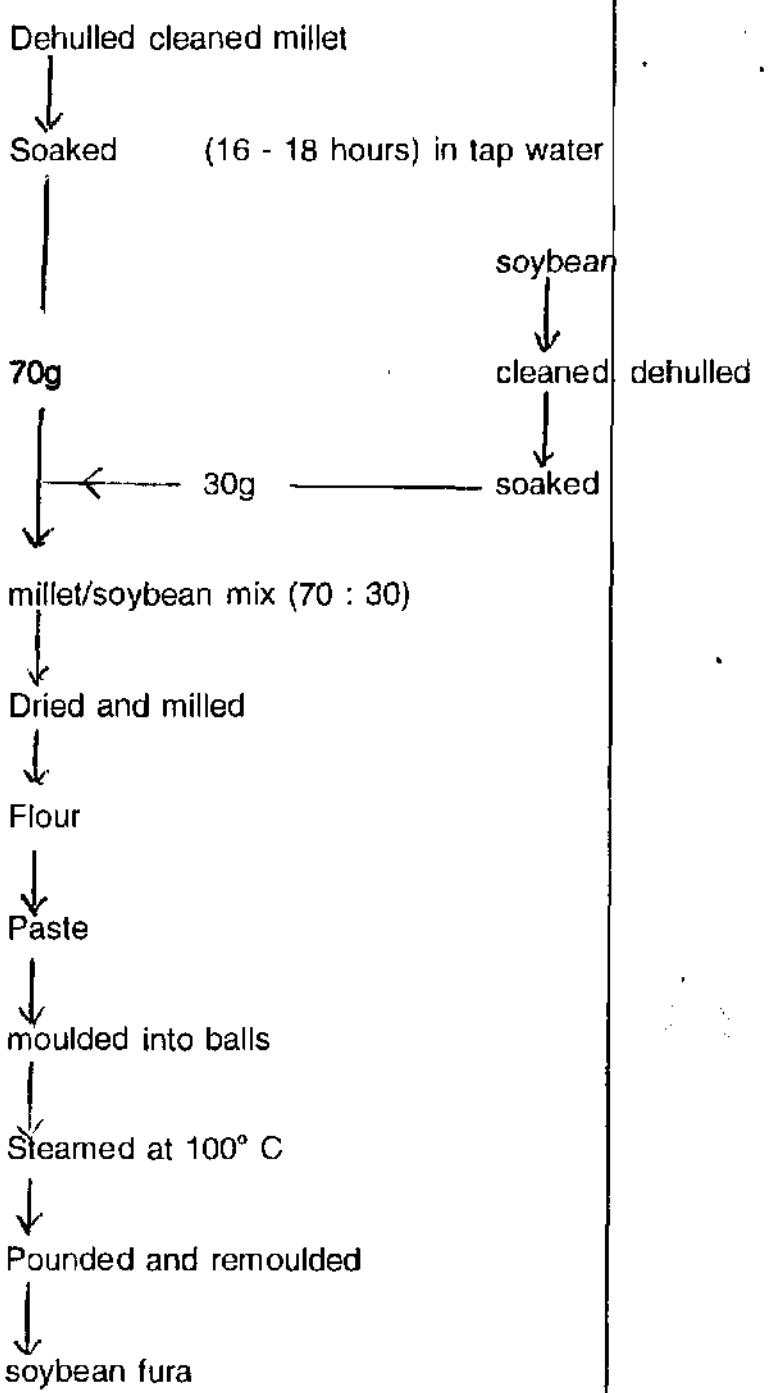


Figure 2: Flow sheet for fura preparation after enrichment with soybeans



CHAPTER FOUR

RESULTS

All the three instant fura samples (germinated, soybean and local fura) and non-instant fura were analysed quantitatively for total aerobic plate count, yeast and mould counts and Bacillus cereus counts. The fura samples were contaminated with these microrganisms at varying degrees. All the samples contain low bacterial count (2.0×10^3 to 3.0×10^4 cells/g), B. cereus counts(1.0×10^3 to 1.2×10^4 cells/g), and yeasts and mould counts (6.0×10^3 to 8.1×10^3 cells/g)(Table 1). Yeast dominated the yeasts and mould species.

There were no significant difference in the proximate composition of the samples except in protein and moisture content. Soybean fura had significantly higher (9.42%) protein content than the germinated and instant local fura while non-instant local fura had higher (45.20%) moisture content than the rest of the samples.(Table 2).

The result of storage stability of the instant fura samples over a period of 4 weeks showed no appreciable changes in the parameters tested for. However, the instant local fura maintained higher counts of bacteria (6.5×10^4 to 1.0×10^3 cells/g), yeast and mould counts (2.2×10^3 to 2.5×10^3 cells/g) than the soybean and germinated instant fura samples. There was no difference in the aerobic plate count, yeasts and mould counts of germinated and soybean fura. The moisture content of germinated fura and that of soybean fura was significantly different while that of soybean and local fura, local fura and germinated fura do not differ significantly. In terms of titratable acidity, the germinated fura maintained higher value throughout the period of storage while no two samples are identical in titratable acidity (Table 3).

Ten (10) out of the 25 B . cereus isolates were tested for enterotoxin production. 6 (60%) of the isolates had fluid/lenght ratio betweyen 0.2 to 0.4 (mild), 4 (40%) had 0.5 to 0.7 (moderate) and none was senere (Table 4). The characteristic fluid colour produced by the isolates ranged from bloody to straw. (Appendix III).

Results of ranking test performed by 81 tasters showed that the local fura was still

more preferred with 50 (61.67%) people, followed by soybean fura with 24 (25.92%) people and germinated fura with 10 (12.35%) people respectively (Table 6).

The results of five - point hedionic rating test scored by seven trained penalists to determine the extent of the differences in the sensory attributes of the 3 instant fura samples are summarized in Table 7. The differences in the sensory attributes were not statistically different for odour, textture, smoothness, acidity, sweetness and overall acceptability at 5% level of significance. However, there was a significant difference in colour of germinated fura from those of soybean and local instant fura while the colours of soybean and local fura were not statisticlly different.

TABLE 1: Mean and Range of aerobic plate count, B. cereus and yeast and mould count of the instant and non-instant fura samples.

Samples	Aerobic plate count (cfu/g) *		B. cereus (cfu/g) *		Yeast and mould counts (cfu/g) *	
	Range	Mean	Range	Mean	Range	Mean
Instant soybean fura	3.0×10^3 - 6.5×10^4	1.7×10^4	8.0×10^2 - 7.4×10^3	4.0×10^3	4.0×10^3 - 2.8×10^4	7.7×10^3
Instant Germinated fura	2.0×10^2 - 7.8×10^3	2.0×10^3	1.0×10^2 - 2.0×10^3	1.0×10^3	2.0×10^2 - 2.3×10^4	6.6×10^3
Local Instant fura	2.8×10^3 - 9.7×10^4	3.0×10^4	1.5×10^3 - 1.9×10^4	1.0×10^4	3.0×10^3 - 3.0×10^4	8.0×10^3
Local Fura (non instant)	2.6×10^3 - 9.3×10^4	2.8×10^4	1.4×10^3 - 2.0×10^4	1.2×10^4	3.5×10^3 - 4.0×10^4	8.1×10^3

* Counts were made in duplicates.

Table 2: Proximate composition of instant and non -instant fura samples

Samples	Protein (%)	Fat (%)	Ash (%)	Moisture (%)	Total Solids (%)	pH	Titratable acidity (% lactic acid)
Soybean instant fura	9.42a	2.04a	2.06a	8.30a	91.70a	4.3a	0.40a
Germinated instant fura	1.69b	2.52a	2.15a	9.90a	90.10a	3.9b	1.35b
Local instant fura	1.60b	2.47a	1.89a	9.30a	90.70a	4.5a	0.25a
Local fura (non instant	7.20a	2.10a	1.70a	45.20b	54.80b	5.8a	0.22a

Note: Means for each attribute follwed by the same letter are not significantly different at 5% level of significance

Table 3: Storage stability of the three instant fura samples for four weeks at room temperature (25° C)

Week	Sample	Aerobic Plate mean count/g	yeast/mould mean count/g	moisture content (%)	pH	Titratable acidity (% lactic acid)
1	Soybean fura	1.5×10^3	<10	9.05	4.34	0.40
	Germinated fura	<10	<10	9.88	3.91	1.35
	Local fura	1.0×10^5	2.5×10^3	10.10	4.50	0.25
2	Soybean fura	1.4×10^3	<10	8.95	4.33	0.40
	Germinated fura	<10	<10	10.20	3.90	1.36
	Local fura	1.0×10^5	2.3×10^3	9.08	4.53	0.25
3	Soybean fura	1.4×10^3	<10	8.62	4.23	0.41
	Germinated fura	<10	<10	9.78	3.84	1.36
	Local fura	8.0×10^4	2.2×10^3	9.00	4.44	0.26
4	Soybean fura	1.3×10^3	<10	8.59	4.21	0.41
	Germinated fura	<10	<10	9.81	3.82	1.36
	Local fura	6.5×10^4	2.2×10^3	9.00	4.40	0.26

* < 10 cfu/g = No growth in the plates

Table 4: Degree of toxigenicity of B. cereus isolates using fluid volume/loop length ratio.

Sample	Number of Enterotoxigenic B. cereus	Positive (%) with fluid length ratio		
		02. - 0.4	0.5 - 0.7	0.8 - 1.5
Soybean instant fura	4	3	1	-
Germinated instant fura	1	-	1	-
Instant local fura	5	3	2	-

Table 5: Results of ranking tests for five millet and soybean blends of fura samples

Ratio of millet and soybean fura	Reference rate (%) n = 60
90 :10	14 (23.33)
80 : 20	14 (23.33)
70 : 30	20 (33.33)
60 : 40	7 (11.66)
50 : 50	5 (8.33)

Table 6: Results of ranking tests for the three instant fura samples

Samples	Reference rate (%) n = 81
Local instant fura	50 (61.67)
Soybean instant fura	21 (25.92)
Germinated instant fura	10 (12.35)

Table 7: Results of five point descriptive tests for sensory attributes of the three instant fura samples.

	Sensory attributes and mean scores						
Samples	colour	odour	texture	smoothness	acidity	sweetness	overall acceptability
Soybean instant fura	3.29 a	3.29 a	3.14 a	1.86 a	2.14 a	3.71 a	3.14 a
Germinated instant fura	2.29 b	2.71 a	3.43 a	2.00 a	2.71 a	3.57 a	2.86 a
local instant fura	3.57 a	2.86 a	3.57 a	2.14 a	2.43 a	3.71 a	3.43 a

NOTE: means for each attribute followed by the same letter are not significantly different at 5% level of significance.

CHAPTER FIVE

DISCUSSION

Quantitative analysis of the instant and non instant fura samples shows that they were contaminated with aerobic mesophilic bacteria, Bacillus cereus, yeasts and moulds at varying degrees. The total aerobic plate count of about 10^4 cells/g obtained in these samples fall within the acceptable limit set by the international commission on microbiological specifications for food (ICMSF). The ICMSF suggested the acceptable limit for total bacteria in food as 10^4 cells/g as acceptable, 10^4-10^6 cells/g as marginal and above 10^6 cells/g as unacceptable limit (ICMSF,1974). Similarly the occurrence of 10^4 cells/g of Bacillus cereus is within the acceptable limit for B cereus since 1×10^4 cells/g is regarded as tolerable and $> 1 \times 10^5$ cells/g as above the acceptable limit (ICMSF,1974).

The common occurrence of B. cereus in these samples may not be unique since several dry food samples have been reported to contain some of this organism (Kim and Geopfert,1971; Blakey and Priest,1980). It has been established that very high numbers of B. cereus cells of about 1×10^7 cells/g of food, are required to cause outbreaks of disease although in young children 1×10^5 is enough to result in an outbreak (Mossel,1967).

The yeasts and mould counts were dominated by yeasts (Saccharomyces spp) with few species of mould (Mucor and Rhizopus) isolated from local fura. Aspergillus, penicillium and fusarium species were not isolated in any of the samples.

The predominance of yeasts as observed in this study, is a common occurrence in food and beverage fermentations. This has been attributed to a symbiotic relationship that usually occurs between lactic acid bacteria and yeasts in such circumstances. In this relationship, the lactic acid bacteria provides an acid environment for the yeast growth, while the yeasts provides the lactic acid bacteria with vitamins and other growth factors (Odunfa, and Adeyele,1985). The absence of Aspergillus, Penicillium,

and Fusarium species may be due to the low pH and high acidity observed in the samples. Similar results were obtained during the preparation of ogi from maize grains (Akinrele,1970), and ogi-baba from sorghum grains (Odunfa and Adeyele,1985).

Generally, lower microbial counts were observed in all the samples. This could be due to the method of 'fura' preparation which involves washing, milling, soaking and steaming or boiling. All these stages have a significant effect on the microbial populations of the fura samples.

The result of proximate analysis shows a decrease in protein and fat content in all the samples compared to the whole grains of millet and soybean. The whole undehulled millet and soybean grains is reported to contain 10% and 40% protein respectively. Similarly, the fat content of undehulled millet and soybean grain is about 4 and 21% respectively (Ihekoronye and Ngoddy,1985). This decrease in protein, 1.60% for instant local fura, 7.20% for non instant local fura and 9.42% for soybean instant fura may be attributed to the practice of dehulling and milling to remove the germ of the grains. This germ is believed to be rich in this nutrient. Similarly, there was a drop in the fat content of the local fura (2.47%) and the enriched fura samples (2.04-2.52%) as compared to the whole undehulled grains of millet and soybean.

Throughout the period of storage stability, the local fura purchased from the market dominated the improved samples with higher moisture, total aerobic bacteria, B cereus and yeasts and mould count. This may be responsible for the short shelf life of the non instant fura as compared to the dry fura powder (instant fura). Mairiga (1980) showed that the shelf life of fura can be improved upon by physical removal of water to make it less susceptible to microbial growth and some other chemical changes. On the other hand, there was no appreciable changes in the yeasts and mould counts, and the moisture content of the instant fura sample during 4 weeks of storage. The presence of yeasts and mould, and B cereus in higher numbers in local fura purchased from the market might be due to improper sanitary practices employed in the processing and preparation. In all the samples, the soybean instant fura have the highest protein contain.

This result agrees with the result of previous workers (Rahotra,1974 and Weingatner,et,al,1987) who reported an increase in protein content of meals fortified with soybeans. There was a slight increase in the protein content of germinated instant fura over the instant local fura. Similar results was obtained by Malleshi and Desikachar (1986) who reported an insignificant increase in protein content between germinated and ungerminated millet.

There was a progressive fall in the microbial counts, pH and titratable acidity of the instant fura samples during the four weeks of storage at room temperature. These are characteristics of fermenting cereal grains (Akinrele,1970; Au and Fields, 1981; Umoh and fields,1981). Yeasts and mould were eventually absent in the germinated instant fura while the soybean and local instant fura samples maintained low or no growth.

CONCLUSION AND RECOMMENDATION

In conclusion the result of this study demonstrated that the nutritive value of cereal based foods such as fura could be improved by local technologies such as germination/fermentation and addition of protein rich products like soybeans. It also revealed that the instant fura could be stored for a period of 4 weeks without appreciable changes in the properties studied. The incidence of B. cereus, total bacteria, yeasts and mould counts and enterotoxigenic strains of B. cereus was not so alarming which may be due to the methods of processing. In terms of likeness, the local fura was more preferred to the traditionally enriched ones but there was no significant different between the three products in terms of sensory qualities except for colour.

Further studies should be geared towards upgrading and standardizing the traditional methods of enriching and preserving fura to obtain a product of consistent characteristics and anticipated qualities comparable with commercially available weaning foods. Display of fura in the open air in the market should be discouraged. Instead, proper packaging of the product in transparent polythene bags should be encouraged to avoid contamination from buyers and atmosphere. The Hausa Fulanis who are mostly involved in the preparation of fura should be enlightened on the

importance of personal hygiene so as to avoid or minimize contamination by microorganisms.

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APPENDIX I

Questionnaire for Ranking Millet - Soybean Blends and Fura Samples.

Name..... Date.....

Product.....

Please rank these samples for preference. Rank the sample you like best as first and the sample you like least as last. Rinse your mouth after testing each sample.

First.....

Second.....

Third.....

Fourth.....

Fifth.....

Comments

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APPENDIX III

Enterotoxigenic Strains, amount of fluid accumulation and colour during ileal loop test

ISOLATES	VOLUME (cm^3)	VOLUME/LENGTH RATIO	CHARACTERISTIC FLUID COLOUR
LF1	4.0	0.40	Bloody
SB1	2.0	0.20	Straw
LF4	3.0	0.30	Straw
GF1	5.5	0.55	Straw
LF2	5.0	0.50	Bloody
SB2	5.1	0.51	Straw
LF5	2.7	0.27	Straw
SB4	3.8	0.38	Bloody
SB3	2.2	0.22	Bloody
LF3	6.0	0.60	Bloody

APPENDIX IV

Five - Point Descriptive Scale for Sensory Scoring

Name..... Date.....

Sample Code.....

Please taste each sample and circle the one that best describes the attribute of the fura. Answer the questios too. Rinse your mouth after tasting each sample.

1. **Colour**

    1. Yellowish brown
    2. Chocolate brown
    3. Camel brown
    4. Light brown
    5. Pure brown

2. **Texture/Thickness**

    1. Very watery
    2. Slightly watery
    3. Neither watery nor thick
    4. Slightly thick
    5. Very thick

3. **Smoothness**

    1. Very coarse
    2. Slightly coarse
    3. Neither coarse nor smooth
    4. Slightly smooth
    5. Very smooth

4. **Acidity**

    1. Not sour
    2. Very slightly sour
    3. Slightly sour
    4. Moderately sour
    5. Very sour

5 **Sweetness**

1. Bitter
2. Slghtly bitter
3. Niither bitter nor sweet
4. Slightly sweet
5. Very sweet

6. **Overall Acceptability**

1. Very poor
2. Poor
3. Fair
4. Good
5. Excellent

(a) Which sample(s) do you like best ?
(b) Which sample(s) do you like least ?
(c) General comments on the sample(s) you like best.

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