BACTERIAL SAFETY AND PRESERVATTON OF "KUNUN ZAKI": A NON-ALCOHOLIC PRODUCT

RY

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DECLARATION

This is to certify that I carried out the work reported in this thesis at the laboratories of the Department of Microbiology, Ahmadu Bello University, Zaria and also at the laboratories of the National Animal Product Research Institute (NAPRI), Shika, Zaria, under the supervision of Drs. V.J. Umoh (Mrs) and J.P. Kwaga.

This thesis is an original work carried out by myself and no part of it has been presented elsewhere for a degree or a diploma. The work of other investigators referred to is acknowledged in this presentation.

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CERTIFICATION

This thesis titled "Bacterial Safety and Preservation of "Kunun Zaki": a non-alcoholic beverage" by Oranusi Solomon 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.

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DEDICATION

This work is dedicated to my family.

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To God be the glory for making everyday of my life a day of joy.

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ABSTRACT

Bacteriological safety of "kunun zaki", a non-alcoholic beverage prepared with three different packaging methods was carried out. Aliquot, of diluted and concentrated samples were spread on Nutrient agar for total aerobir. plate count, Baird parker agar for staphylococcal count., Eos in methylene blue agar for coliform count, Dextrose tryptone agar for Baci1lus count, Salmonella - shigella agar for isolation of Salmonella and shiqel1 a and lactose broth for coliform count. Bulk packaged samples in bowls had the highest contamination with mean total aerobic plate count of 2.5 x 10* cfu/ml, mean Baci1lus count of 1.0 x 10^4 cfu/ml and mean staphylococcal count of 7.3 x 10^2 cfu/ml. Bottled samples were least contaminated with mean total aerobic plate count of 9.7 x 10^5 cfu/ml, mean Baci1lus count of 1.0 x 10^3 cfu/ml and mean staphylococcal count of 6.8 x 10^1 cfu/ml. However cellophane packaged samples had the highest mean coliform count of 3.6 x 10^3 cfu/ml while bottled and bulk packaged samples had mean coliform counts of l.8 x 10^J cfu/ml and 1.5x10^2 cfu/ml respectively. Only one Salmonella isolate was recorded and other pathogens of concern such as B cereus, E. col i and S. aureus were found to be present . Shigella spp was not isolated. Bulk packaging and cellophane packaging of "kunun zaki" should be discouraged due to its high rate of contamination.

Studies on sources of contamination and hazard analysis of "kunun zaki" preparation was carried out on three producers around Zaria. It revealed some sanitary deficiencies to include

inadequate sorting and washing of raw materials, possible faecal contamination from animals in or around food preparation area, use of unprotected well water and potential sources of spores from **raw** materials and sweetening agents and dust at point of display for sale. Inadequate boiling. Mixing and seiving of product with hare hand were also observed as possible mean of contamination.Education of producers on the importance of hygienic environment, is imperative.

Laboratory products were prepared containing preservatives such as acetic acid, benzoic acid, sodium benzoate, sodium citrate and citric acid at concentrations permitted by national and international regulations. The control products had no preservative. At 28+2*C. of storage, sensory analysis by a panel of six judges revealed that, sodium benzoate alone extended the shelf life for up to 24 weeks, sodium benzoate/sodium citrate and sodium benzoate/citrie acid/sodium citrate extended the shelf life for 16 weeks, acetic acid, benzoic acid and sodium citrate lost the effect after 4 weeks. Citric acid had no preservative effect on "kunun zaki". The control samples deteriorated after two weeks while the commercial samples had a shelf life of one day. The preservation of "kunun zaki" for 24 weeks at ambient temperature is a pointer that this work could be the basis for a pilot production of bottled "kunun zaki". Tt is therefore recommended that a starter culture he developed and controlled industrial production of "kunun zaki" be encouraged.

Ten R^ cereus isolates were tested for enterotoxin production using the ligated rabbit, ileal loop test.. Five (50%) were enterotoxigenic! with mild, moderate and severe enterotoxin reactions. The severe enterotoxigenic strain was from the bulk packaged samples. Ten S. aureus isolates were tested for haemolysis on human and sheep blood agar. Six (60%) were alpha haemolytic while 4 (40%) were heta haemolytio. This raises serious public health concern since 6-haemolytic strains are likely to be human biotypes and more enterotoxigenic than animal biotypes which are often beta haemolytic.

A study of the resistance patterns of the 37 isolates of B. cereus, R. coli and S aureus to 16 commonly used antimicrobial agents showed no significant, difference (P>0.05) in resistance of isolates from various packaging methods. Overall resistance of 15 strains of E. coli to antimicrobial agents tested were Cephalexin 86.6%, Cefuroxime 79.9% Streptomycin 53.3%. The resistance of 10 strains of S. aureus to tested antimicrobial agents were Penicillin and Streptomycin 70% each, Sulphafurazole 60%, while the resistance of 11 strains of B. cereus isolates tested were erythromycin 9.7.3%, penicillin and oloxacillin 18.2% each.

The predominant multiple resistance patterns for E. coli were streptomyoin-ampioi11 in 40%, streptomyoin-ampicillin-sulphafurazole 40% and amplici11in-sulphafurazole 33.3%. The common patterns for S.aureus were sulphafurazole-streptomycin 60%, penicillin-sulphafurazole 50%, penioi 11in-ampici11 in 30% and ampioillin-cloxacillin 20%, while the patterns for B. aureus were penicillin-

ampioillin 18.2% and penici11in-amplicil1in-cloxaoillin 18.2%. These high percentage resistance to antibiotics may be a reflection of misuse of these antimicrobial agents in medical and veterinary practices. The indiscriminate use of antibiotics should be discouraged to reduce the incidence of drug resistance isolates.

Apart from lead (Pb) content, there were no significant differences in proximate composition of commercial and laboratory produced "kunun zaki" but there were nutritional losses in sorghum and millet due to processing into "kunu". There may therefore, be the need for enriching "kunun zaki" with nutrients from other sources during processing or before consumption.

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CHAPTER 1

INTRODUCTION

The current economic hardship in Nigeria and the devaluation of the Naira has led to an increased cost of imported raw materials generally used for the production of various beverages, including the non-alcoholic ones (Ogbonna and Obi, 1991; Oranusi et al, 1994). This has led to the increase in cost of soft drinks, thereby encouraging the use of local and unconventional raw materials for the production of local beverages with improved production practice and enhanced quality and shelf-life (Ogbonna and Obi, 1991).

Kunun zaki is one of the many indigenous non-alcoholic beverages produced in Nigeria. It is widely consumed by the people in the savannah region of Nigeria. "Kunu zaki" is consumed any time of the day by both adults and children. A large number of people consume "kunun zaki" as refreshing drink, appetizer, food and to quench thirst. It is also used as substitutes for or to complement soft drinks and wines at social gatherings. The production of "kunun zaki" from unmalted grains and the short period of fermentation needed to produce the desired taste makes it adequate for consumption by people of all works of life.

"Kunun zaki" is a non-alcoholic beverage prepared from either guinea corn, Sorghum bicolor, millet, Penisetum typhoides, maize, Zea mays, rice, Oriza sativa or wheat, Triticum aestivum. Traditionally, "kunun zaki" production involves steeping of whole grains for 6-24 hours, wet milling with spices and sweet potato,

pitching of about 3 part of the mixture gelled in hot water with about 1 part fresh (ungelled) part of the mixture and allowing for overnight fermentation. The supernatant is ready for consumption after sieving.

The short shelf-life of one day for "kunun zaki" due to unhygienic conditions of local production and pitching with mixed culture from the fresh wet milled mixture, and the resultant inconsistent taste and flavour from different producers are limitations to massive production of this product.

The microbiological profile of "kunun zaki" and other similar non-alcoholic drinks of grain and non-grain origin have been reported to include bacteria such as Bacillus spp; Lactobacillus spp; Pediococcus spp; Aerobacter spp; Staphylococcus spp; Corynebacterium spp; Micrococcus spp. Yeasts and moulds isolated were Candida spp; Saccharomyces spp; Aspergillus spp; Penicillium spp; Rhizopus spp; Fusarium spp and Cephalosporium spp. (Akinrele, 1970; Okafor and Aniche, 1980; Odunfa and Adeyele, 1985; Oyeyiola, 1991; Ososanya and Whong, 1991; Oranusi et al, 1994). The possibility of contamination by S. aureus, Salmonella, Shigella, B. cereus, pathogenic strains of E. coli and other pathogenic or opportunistic pathogenic microorganisms associated with poor sanitary condition and unhygienic and dusty environment cannot be overlooked.

Consumption of "kunun Zaki", should be encouraged among Nigerians because it is ~~being~~ a product prepared from grains rich in group B vitamins, carbohydrate and reasonable amount of protein

(Donald, 1981; Jarrold, 1991) and it contains spices rich in essential oils. Secondly it is more natural than most industrially produced non-alcoholic soft drinks. The materials required for preparation are available locally, making it cheaper than other non-alcoholic drinks in the market.

There is scanty information available on the chemical composition of "kunun zaki" (Edward et al, 1988), though the chemical composition of most Nigerian sorghum, millet and maize products are well documented (Oke, 1967; Akinrele, 1970; Mertz, 1970; Banigo and Muller, 1972; Adeniji and Potter, 1978; Umoh and Field, 1981). To our knowledge, no information is available on the total microbiological safety and nutritional quality of kunu zaki and only a scanty information is available on attempts to produce "kunu zaki" commercially (Sopade and Kassum, 1992).

The effect of spices, PH and temperature on survival and multiplication of S. aureus and E. coli in "kunun zaki" have been documented (Onuorah et al, 1987).

For reasons of utilizing "kunun zaki" nation wide like other industrially produced non-alcoholic beverages, this study aimed at determining:

i. the bacterial safety of "kunun zaki"
ii. to study possible sources of contamination of the traditional product using hazard critical control analyses (HAAC)
iii. the physiochemical characteristics of both local and laboratory product
iv. Enumeration, Identification and the antibiotic resistance and

toxicity of possible pathogenic bacterial isolates from "kunun zaki"

v. to develop a formulation and study the effects of the following chemical preservatives, citric acid, acetic acid, benzoic acid, sodium benzoate, sodium citrate and their combinations on shelf-life of "kunun zaki".

CHAPTER 2

REVIEW OF LITERATURE

2.1 Introduction

The quest for foods that are more natural and more refreshing coupled with the poor quality of water supplied to the public has resulted in massive consumption of locally produced non-alcoholic beverages including "kunun zaki" (Adesiyun et al 1983, Alabi and Adesiyun, 1986). However, people sometimes complain of stomach ache, diarrhoea, vomiting, headache and high temperatue after consuming some of these products (personal communication).

2.2 Socio-economic Importance of Sorghum, Millet and Maize

Sorghum, millet and maize have been important staples in the semi-arid tropics for many centuries. They have been cultivated in so many countries mainly by small-holder cultivators. It is thus not surprising that the common and vernacular names by which they are known are many and varied. In some records no distinction is made between sorghum and millet. Production statistics quoted even by international authorities, often group the two cereals together (Hulse et al, 1980).

Sorghum, millet and maize are of much greater importance in human diet, particularly those of the poorest people of the semi-arid tropics than the attention they have received would suggest. They constitute a major source of dietary energy and protein for millions of people in Asia and Africa. According to the statistics of the Food and Agriculture Organisation (FAO) of the United

Nations (FAO), the average annual global production of sorghum amounts to over 68.7 million tonnes. Over 52 million hectares are planted yearly with numerous varieties and hybrids of sorghum. The annual millet production was estimated to be over 34.3 million tonnes from an area of 53.8 million hectares. Thus sorghum and millet contribute annually over 100 million tonnes to the global food supply (Hulse et al, 1980). Maize ranks third among cereals in terms of hectares under cultivation after sorghum and millet. In Nigeria, the total production of these cereal grains amount to several million metric tonnes grown over millions of hectares of land (Olatunji and Edward, 1980). Sorghum is the main cereal crop in Nigeria, occupying 46% of the area applied to cereal cultivation and 44% of the total cereal production. Millet covers over 40% of the annual land sown with cereals, while maize covers an area of 13-14% of the land sown with cereals annually (Olatunji and Edward, 1980; Fussel and Werder, 1988; Joseph, 1992).

2.3 Processing of Sorghum, Millet and Maize for Human Consumption

Cereal processing in Nigeria as in many other African countries is still largely by the traditional method of pounding in a mortar followed by winnowing. It is usually performed by women and is the major occupation for many of them (Olatunji and Edward, 1980). Perten (1976) showed that by this traditional processing less than 2kg of flour can be produced per hour per woman at an extraction rate of about 60% of the grain. Water is usually added to facilitate milling but this leads to an increase in the moisture

content of the flour to about 25-40%. The keeping quality of such flour is reduced and the indigestible fibre is high and therefore hinders the digestion of other constituents of the diet (Olatunji and Edward, 1980). However, Muller (1970) reported the availability of small scale processing using motor operated mills and located in corners of local markets.

Flour from these cereals are boiled in water over fire to produce "tuwo" which is normally eaten with soup. Whole grain of sorghum, maize and millet are fermented for different food products. Umoh and Field (1981) fermented corn for the production of "Agidi", a Nigerian starchy meal fed as a post-weaning food for infants and is eaten by adults with vegetable soup or with bean-ball (akara) as breakfast food. Banigo and Muller (1972) produced "ogi" from fermented maize. "Ogi" is a traditional staple food eaten by many Nigerians as breakfast meal. It is the first food generally introduced to babies at weaning. Oyeyiola (1991) produced "kamu", "gasara" or "kulli", from fermented millet wet milled with small quantities of pepper (Capsicum frutescens), ginger (Zingiber officinale) and cloves (Eugenia calophylloides) added as spices. "Burabisko" and "fura" are food products from powdered unfermented millet prepared after removal of the coat (pericarps) (Oyeyiola, 1991) Banigo and Muller (1972), Au and Field (1981), Odunfa and Adeyele (1985), fermented sorghum for the production of "ogi" and "ogibaba". "Kunun zaki", "kunun tsamiya" and "kunun kanwa" are all products of millet, maize and sorghum consumed mainly in the northern part of Nigeria.

Fermented cereal foods are produced for daily consumption in most African countries. Similarities have been found in traditional food preparations over ecological zones. For example Mawè is a fermented maize dough prepared and consumed in Benin Republic. It is used in the preparation of cooked dishes including gels (ogi-like products), steam-cooked bread (ablo), or porridge (koko, aklui, akhuyonu) (Hounmonigan et al , 1993). "Galette-masa-wayna" is an unleavened fermented bread consumed in Niger Republic. It is a thick pancake, made from rice, millet, corn or sorghum flour or a combination of these flour (Jeanette et al, 1987). "Galette-Gwomi" is a fermented, fried bread eaten plain or with a soup in Mali. "Tana" is a porridge maize eaten with a soup or plain in Cameroon.

Unfermented whole millet grain contain about 84% carbohydrate and 9% protein (Oyenuga, 1968). Cereals are deficient in Lysine, tryptophane and methionine. Therefore, they are deficient in protein quality (some essential amino acids) (Inglett, 1970; Mertz, 1970; Tsen et al, 1976; Adeniji and Porter, 1978; Umoh and Field, 1981). The processing methods used for these cereals reduce the nutrient value of the various food product derived from them (Oke, 1967; Akinrele, 1970; Banigo and Muller, 1972; Olatunji and Edward, 1980). However, adequate fermentation procedure has helped to increase the nutritive value of cereal products over the unfermented ones (Hamed and Fields, 1979; Umoh and Fields, 1981).

2.4 Microbiological Profile of Kunun Zaki and Similar other Products

Studies on microbiology of kunun zaki" include that of Onuorah, (1984) and Onuorah et al, (1987), who reported a high prevalence of Coliform and Staphylococci and enterotoxin production in 20(8.1%) of the 246 staphylococcal isolates from kunun zaki and utensils used in its preparation. Onuorah (1987), reported a $2.4x10^{11}g^{-1}$ Staphylococcal count for mixture of spices used in kunun zaki preparation. It was observed that ginger Zingiber officinale was most contaminated and red pepper Capsicum annum was the least with total aerobic plate counts of $9.5x10^{10}g^{-1}$ and $7.3x10^{6}g^{-1}$ respectively. Ososanya and Whong (1991) isolated Staphylococcus spp; B. cereus, Salmonella spp, and Coliform, except E. coli, Penicillium spp; Aspergillus and Fusarium spp from kunun zaki. Yeast was also reported to be of high prevalence.

A report by Akinrele (1970) showed that "ogi" a traditional African starch cake was contaminated with moulds such as Cephalosporium spp; Fusarium spp; Aspergillus spp, and Penicillium spp. Of the aerobic bacteria, only Corynebacterium spp and Aerobacter spp were isolated. In a similar study by Oyeyiola (1991) on fermentation of millet to produce kamu, a Nigerian starch-cake boiled into either a thin porridge called "koko", or into dumplings such as "eko" or "agidi" which are eaten with vegetable soup or with bean-balls (akara). Organisms involved in kamu production include moulds such as Aspergillus versicolor, Penicillium nigricans and Rhizopus stolonifer. Pediococcus pentosaceus and Lactobacillus plantarum were the bacteria isolated.

Yeasts included Saccharomyces cerevisiae and Candida krusei. Odunfa and Adeyele (1985) reported on the microbiological changes that occur during the traditional production of "ogi-baba", a West-African fermented sorghum gruel. They found the contaminating microbial flora to include Aspergillus spp; Penicillium spp; Rhizopus spp; Candida spp; Pediococcus spp and Aerobacter spp while Saccharomyces spp and Lactobacillus spp were implicated in fermentation.

In the production of "mawè, a fermented maize dough of Benin Republic, Hounnouigan et al (1993), showed that the dominating microbes are lactic acid bacteria and yeasts, with Enterobacteriaceae counts of less than 400 and 3600 cfu/g in commercial and home produced "mawè", respectively. Fermented cereal foods have been found to be similarly prepared traditionally in most African countries. It could equally be expected that the microbiological profile of these foods will be similar to those stated above.

Commercially produced soft drinks could be similar to kunun zaki in that they are non-alcoholic beverages highly prone to microbial contamination. Odunfa (1987) carried out analyses of carbonated soft drinks produced in Nigeria. He reported the microbial contaminants to include Bacillus spp; Lactobacillus spp; Pedoicossus spp; Saccharomyces spp and Aspergillus spp. Oranusi et al (1994) also carried out a study on bottled non-alcoholic drinks produced in Nigeria, and found the microflora to include bacteria such as Bacillus sphaericus, B. megaterium, B. subtilis,

Lactobacillus fructivorans, L. brevis, L. delbrueckii, L. kafir, S. epidermidis and Micrococcus cycae and fungi including Saccharomyces cerevisiae and Aspergillus niger. Frazier and Westhoff (1978) and Odunfa (1987) attributed contaminants of commercial soft drinks to environment/or the raw materials and factory personnel, and their survival in the products was attributed to the Osmo and thermotolerant nature of the Bacillus spp and acid, CO_2 and salt tolerance of the Lactobacillus (Odunfa, 1987; Frazier and Westhoff, 1978).

The microbiology of certain local alcoholic beverages of cereal origin are well documented. Ekundayo, (1969); Faparusi et al, 1973, Ogbonna et al, 1983, reported the presence of Candida mycoderma, C. tropicalis, Hansenula anomala, Kleokera apiculata and Saccharomyces pastorianus and Pediococcus cerevisiae in "Burukutu" an alcoholic beverage prepared from guinea corn Sorghum bicolor or S. vulgare and widely consumed amongst the population group within the Savannah region of Nigeria.

Akinyemi (1990), in a similar report on Burukutu, identified the bacteria flora to include many spp of Bacillus : B. cereus, B. subtilis, B. mycoides, Micrococcus leteus and Micrococcus roseus, Enterobacter agglumerous and Serratia spp. A single spp of the genus Acetobacter was said to be responsible for the vinegary taste of Burukutu, while the other bacteria were likely to cause spoilage. The predominant moulds observed were Aspergillus spp, Mycelia sterilia, Rhizopus spp and Fussarium spp. The presence of Aspergillus was said to be associated with the presence of

aflatoxin in the beer. Nine yeast belonging to four genera were obtained, which included three Saccharomyces spp, two Candida spp, a Pichia spp and Rhodotorula spp.

The microbiology of "Bussa", a Kenya opaque maize beer as reported by Nout (1980) showed that the microorganisms present during storage of uncooked grits were mainly yeasts such as Candida kruzei and Saccharomyces cerevisiae. The bacteria include Lactobacillus spp, L. helviticus, L. salivarius, L. brevis, L. viridensces, L. plantarium and Pediococcus spp, P. damnosum and P. paralus. The organisms identified in the malt were Corynebacterium spp, Bacillus spp, Listeria spp, Eubacterium spp, Lactobacillus casei (Var pediocossus damnosus) Enterobacter cloecea, Klebsiella pneumonia, Propionibacterium spp, Rhizopus spp, Mucor spp, Candida kruzei. The alcoholic fermentation of Bussa was dominated by Saccharomyces cerevisae, while the lactic fermentation was initiated by hetero- and homo- fermentatative Lactobacilli such as L. brevis, L. bucheri, L. plantarum, L. salivarius (Var saicinius), L. viridences and L. casei (Var salicinius).

Vander Walt (1956) reported that the souring process of kaffir beer is done mainly by typical lactic acid flora such as Lactobacillus delbrickii, L. plantarum, L. brevis, L. fermenti, Pediococcus damnosus, Leuconostoc mesenteroides and Leuconostoc dextranicum and the alcoholic fermentation phase involved the activities of such yeasts as, Saccharomyces cerevisiae, Candida cruzei and Kloekera apiculata. The spoilage of the beer was attributed to excessive acetic acid formation which was found to be

caused by the development of the common malt and beer Acetobacter spp.

2.5 Chemical Characteristics of Kunun Zaki and other Similar Products

There is evidence in the literature indicating that biochemical analyses of kunun zaki and similar other products have been conducted. The biochemical analyses carried out on "kunun zaki" and "kunun tsamiya" by Edward et al (1988) showed that both types of kunu had lower levels of nutrient content when compared with those of the raw materials (the sorghum and millet grains) reported by other workers (Oyenuga, 1968; Olatunji et al, 1980; Joseph et al, 1980; Thekoroye and Ngoddy, 1985). Twenty-four hour aged samples of kunun zaki were reported to contain 9.5% dry matter, 90.5% moisture, 3.0% crude protein, 1.5% ether extract, 37.3% nitrogen free extract, 1.46% ash, 0.05% Ca, 24.0 ppm Na and 23.0 ppm K. The figures for kunun tsamiya were given as 13.3% dry matter, 86.7% moisture, 9.4% crude protein, 1.2% ether extract, 8.1% nitrogen free extract, 1.62% ash, 0.06% Ca, 14.4ppm Na and 23.0ppm K. Sopade and Kassum (1992) reported the proximate composition of kunun zaki as 0.3% protein, 1.0% fat, 1.5% ash and 12.2% carbohydrate. The comparative studies by Umoh and Fields (1981) on fermented and non-fermented agidi, revealed that fermented agidi was more acidic than non-fermented agidi and contains 8.68% protein, 3.37mg/gN tryptophan, 30.17mg/Gn lysine. Akinrele (1970) in his fermentation studies on maize for the preparation of ogi, showed that percentage total N, amino N, non

protein N, thiamin, Riboflavin, Niacin, folic acid and pantothenic acid were higher in fermented than in non-fermented ogi.

The biochemical analyses carried out on burukutu by Ogbonna et al (1983), showed that the beverage had such values as 52% sugar, 0.46% ash, 0.52% protein, 7.0µg/100ml vitamin c and 3% alcohol. In a similar work in Southern Nigeria, Faparusi (1970) reported the presence of many sugars such as fructose 0.2%, maltose 0.05%, glucose 0.6%, galactose 0.36%, galaturonic acid 0.48%, sucrose 0.2%, isomaltose 0.38%, starchyrose 0.43% and maltotetrose 0.16% in Burukutu.

Hounhouigan et al (1993) assessed the chemical composition of "mawè" and reported that home-made mawè contained 46.8% moisture, 9.2% crude protein, 2.3% fat, 0.7% fibre, 1.1% ash and 86.7% soluble carbohydrate. These figures were slightly higher than the commercially produced mawè. They attributed the differences to the fact that more hull and germ is retained during home production.

Aucamp et al (1961) assessed the nutritional value of some kaffir corn beer from malted and unmalted corn and indicated that kaffir is rich in protein, fat, ash, fibre, thiamine, riboflavin and nicotinic acid and is a rich source of vitamins C and B.

Nout (1980) in his studies on Bussa showed that Bussa contains 0.5-1.0% lactic acid and 2.4% volume of ethanol. He noted that the beer becomes unacceptable after further fermentation and souring to about 2% lactic acid.

2.6 Traditional Kunun Zaki Production

Onuorah (1984), Sopade and Kassum (1992) reported on the methods of processing of kunun zaki based on the information collected from their locality. The basic method of preparation involved the use of Sorghum bicolor (Guinea corn) or Penisetum typhoides (millet). During the course of this study, investigation and interview of producers in Zaria area revealed that maize Zea may, rice Oriza sativa or wheat Triticum aestivum could be used. The grains are steeped for 12-24 hours, wet-milled with spices, about ¼ quantity of the wet-milled mixture is removed and mixed with wet-milled sweet potato. The larger portion is gelled completely with hot water, allowed to cool and pitched with the aliquot mixed with sweet potato. Souring process is usually allowed overnight before sieving, following which the supernatant is then sugared to taste. Cocoa yam can be used in place of sweet potato and sieving can be done before division into unequalled portion.

The spices: ginger Zingiber officinale, cloves Eugenia calophylloides, black pepper Piper nigrum and Xylopia aethiopica impart the desired flavour to kunun zaki while the sweet potato or cocoa yam serve to lighten up the gelled portion, making it easy to filter and also serve as a sweetener. Fig. 1 gives a flow chart of traditional kunun zaki production in Zaria.

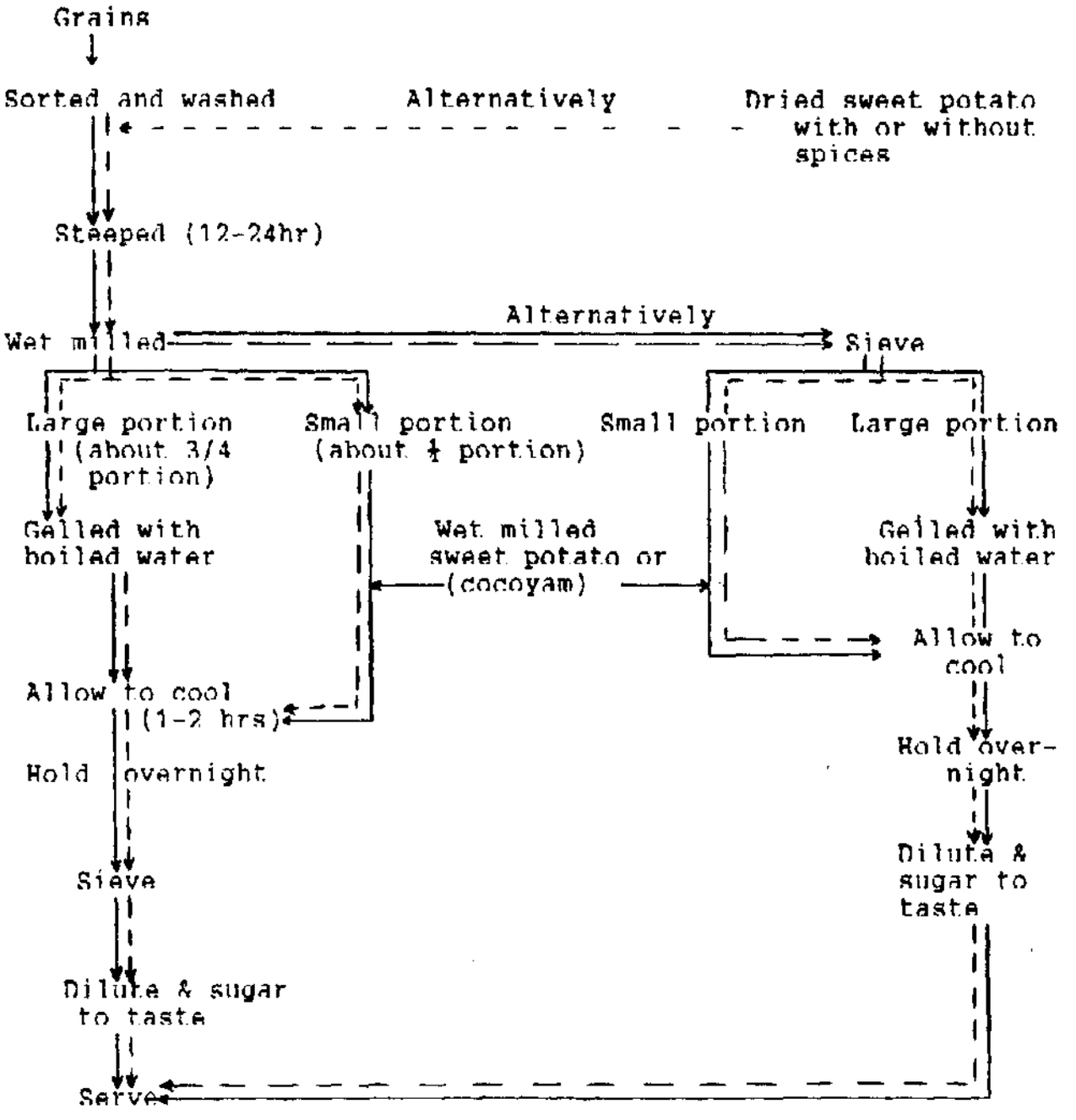


Fig. 1: Flow Chart of Kunun Zaki Processing in Zaria

2.7 Chemicals in Food Preservation

The purpose of food preservation is to prevent or at least delay enzymatic and/or microbial changes in food so that the food is made available at some future time. That time may range from a few hours to several months. Preservation involves the basic principles of preventing enzymes and microbial activities in the food so as to retain as far as possible, the original characteristic of the food and impair its nutritive value as little as possible (Brian and Allan, 1980). The principles are employed in several basic techniques of food preservation, including use of high temperature, use of low temperatures, removal of water, the addition of inhibitory chemical substances and irradiation.

The Federal Food, Drug and Cosmetic Act (1975) designates any chemical which, when added to a food tends to prevent or retard its deterioration as a chemical preservative. Every chemical added to a food during the process of its fabrication is not necessarily considered to be a preservative. For example, in canning, if the air in the head space is displayed with nitrogen, the gas is not considered a chemical preservative added to the food (Desrosier and Desrosier, 1977). The general regulation also exclude natural preservatives or condiments from the classification of chemical preservatives. These include common table salt, sugars, vinegars, spices and their oils, and those substances incorporated into food by direct exposure of the product to wood smoke. The presence of these materials in foods need not be disclosed as preservatives although natural preservatives may have the same general action as

chemical preservatives. In as much as the natural preservatives do not appear to meet the same prejudice as artificial preservatives encounter, the natural ingredients ar excluded from this consideration (IFT, 1975).

2.8 Effects of Sodium (Na), Potassium (K) and Lead (Pb) in Human Health

Of the 92 natural chemical elements, more than 50 are found in human body tissue and fluids. Na and K belong to the first group which are the essential macro elements required in amounts of 100mg/day or more. Calcium, phosphorus, chlorine, magnesium and sulphur being the other essential macro elements.

Lead belongs to the 4th group occurring as trace contaminants in man with no known function. Mercury, Barium, Strontium, Boron, Aluminium, Lithium, Beryllium, Rubidium, Gold and Silver being other members of this group. The 2nd and 3rd groups constitute the essential micro or trace elements required in amounts of no more than a few mg/day and sometimes only a few Mg e.g. Iron, Copper, Iodine, fluorine among others and those necessary for animals but not proved for man include tin, nickel, vanadium and most recently, Arsenic and possibly Cadmium (Donald, 1981).

The major sources of these elements to the body include air, food, water, beverages and other drinks and Pb treatment of cancer patients (Doyle et al, 1993). The need for close and constant monitoring of the level of lead in human diet is essential considering the health implication of this heavy metal and the ease with which this metal can contaminate human food and water through

the use of petrol and petroleum products (lubricants) in grinding machine. These petrol and its products more often than not contain Pb in the form of lead tetraethyl used as antiknock agents.

The functional role of Sodium-potassium (Na-K) pump in the maintenance of water equilibrium, ionic gradients and particularly in the regulation of non-electrolyte transport, the maintenance of acid-based equilibrium and osmotic pressure and the role of K in metabolism, excitability of nerve tissue, transmission of impulses, contractility of muscle coupled with the adverse health implication of deficiencies and excess of these element calls for the need for regular check on these elements in human diet (Goldsmith, 1953).

Na and K requirements are similar, Na is more in the extracellular fluid while K is largely found in the intracellular fluid. The possible link between Na intake and hypertension are at present inconclusive, though Jerrold (1991) suggested that it may contribute to high blood pressure in sensitive individuals when consumed in excess amounts. Giround (1973) noted that an excess of Sodium (Na) is dangerous for the mother and foetus and facilitates toxemia, in which the alteration of the spiral arteries of the myometrium and of the placental arteries reduce the placental circulation inducing growth retardation. On the other hand, hyperpotassemia causes mental confusion, numbness and tingling of the extremities, weakness, pallor, cold skin, disturbances in cardiac rhythm and peripheral collapse (Goldsmith, 1953).

Na and K deficiencies caused impaired fluid balance, impaired cellular function and death. Dietary sources for Na are table

salt, processed foods, meat, fish, poultry, milk and eggs. The dietary sources for potassium include fresh fruits and vegetables, oranges, bananas, peanut, butter, dried peas and beans, potatoes, yoghurt and meat (Jerrod, 1991).

Lead is a cumulative rather than an acute poison. It is absorbed from the gastrointestinal tract and from the respiratory tract and stored in tissues especially bone (Louis, 1970). Lead is a poison of enzymes, probably denaturing their protein moiety. It interferes with porphyrin and heme synthesis, and thus with haemoglobin formation resulting in anaemia that is usually hypochromic but may be normochromic (John, 1970). Children absorb P_b more than adults (Barltrop and Strehlow, 1977). It is especially dangerous to the infant brain and a number of reports have documented an association between low level of P^b and various adverse neurophysiological effects and reproductive toxicology as regard various health and developmental parameters of the foetus and new born (Donald, 1981; Ernhart, 1992a&b).

2.9 Bacterial Food Poisoning

Food infection and food intoxication associated with bacteria have been attributed to organisms such as Staphylococcus, B. cereus, Salmonella, E. coli, Shigella, Clostridium, Yersinia, Vibrio, Aeromonas, Campylobacter and Listeria. Others include Legionella, Brucella, Streptococcus, Klebsiella, Cyanobacteria, Borrelia and Citrobacter among others (Doyle et al, 1993).

Over the year, Salmonella, E. coli, S. aureus and B. cereus

have been in the fore-front of organisms associated with food infection and toxicity probably due to the ubiquitous nature of these organisms and their close association to man and animal.

2.9.1 *Salmonella* Food Borne Infection: A number of Salmonella serotypes have been implicated in human and animal disease the most prominent in human being *S. typhi* which is the causative agent of enteric fever. A less severe form of enteric fever is also caused by *S. paratyphi* B & C. The symptoms of typhoid and paratyphoid fever include anorexia, headache, malaise, non-reproductive cough, abdominal pain, fever, constipation is more common than diarrhoea, rash and splenomegally (Litchfield, 1980; Cheesbrough, 1991; Gregory *et al*, 1991). Other *Salmonella* serotypes implicated in food borne outbreaks include *S. typhymurium*, *S. enteritidis*, *S. blockley*, *S. muenden* and *S. saint paul* among others. These are associated with Salmonella enteritis and extra intestinal infections.

Salmonella toxins include endotoxin, A-cytotoxin and enterotoxin. Virulence is mediated by chromosomal and plasmid encoded factors. In contrast to food borne intoxication resulting from the ingestion of food containing toxins, in *Salmonella* "food poisoning", the intact *Salmonella* cells are the causative agents and must be consumed with the food or water to cause illness.

2.9.2 *Escherichia coli* Food Infection: The pathogenic strains of *E. coli* produces a number of enterotoxin including heat stable (ST) and heat labile (LT) toxins, verocytotoxins (shiga-like toxins),

haemolysins, cytotoxic necrotizing factors and cytolethal distending toxin (Doyle et al, 1993).

The Enterotoxigenic E. coli (ETEC) strains cause severe and often fatal pediatric diarrhoea and often major cause of travellers diarrhoea and residents of developing countries. The LT producing strain causes choleraic diarrhoea, sudden explosive watery stool without blood, often accompanied by abdominal pain, vomiting, temperature may be sub-normal and dehydration being the cause of death. The ST strain produces watery diarrhoea without blood or mucus, sometimes accompanied by nausea, general malaise, fever, abdominal cramp and vomiting (Cheesbrough, 1991; Takeda et al, 1993).

Enterohaemorrhagic E. coli (EHEC) strains, otherwise known as shiga-like toxin producing strains of E. coli (SLTEC) cause hemorrhagic colitis due to colonic mucosa oedema, erosion and hemorrhage. Disease is manifested by sudden severe abdominal pain, watery diarrhoea followed by bloody diarrhoea, nausea and vomiting. Haemolytic uremic syndrome (HUS) begins with diarrhoea, thrombocytopenia, acute renal failure, long term kidney damage and death (Karmali, 1989; Lother et al, 1995).

Enteropathogenic E. coli (EPEC) strains cause a specific type of attaching and effacing lesion. The organism adhere intimately and remove the microvilli (effacement) on mucosal absorptive cells. EPEC may be causing diarrhoea by loss of absorptive power of mucosal cells. It causes travellers diarrhoea (Cheesbrough, 1991).

Enteroinvasive E. coli (EIEC) strain invade colonic mucosa as

in Shigella. The invasion of epithelial cells of colon causes dysentry-like illness (Cheesbrough, 1991).

Enteroadherent aggregative E. coli (EAggEC) strains have been implicated as etiologic agents of gastroenteritis in infants in developing countries, most predominantly among prolonged cases of infection lasting up to 14 days (Azizul et al, 1994). Many children infected with Eagg Ec experience bloody diarrhoea. Eaggec are non-invasive and do not cause any obvious histological lesions (Azizul et al, 1994).

2.9.3 Staphylococcus Food Toxicity: In most food poisoning incidents due to S. aureus, some form of temperature abuse has occurred during preparation or storage of the food, to enable the pathogens to proliferate to high numbers and produce enterotoxins (Robert, 1988; Tranter, 1991). Staphylococci produces a number of enterotoxins, exfoliative toxins and a variety of proteins including coagulase (predominantly by S. aureus), protein A, alpha, beta and delta toxin and Leucocidin, all of which could contribute to the virulence of the organism (Adesiyun, 1984b; Adesiyun et al, 1991; Michelle et al, 1994). Six of the enterotoxins have been identified, purified and characterized (SEA, SEB, SEC, SEC_1, SED and SEE) (Bergdoll, 1980).

Recently, enterotoxin H & G has been identified in S. aureus (Su and Wong, 1995). Enterotoxin A is the type most commonly implicated in food poisoning outbreaks with SED next in order of frequency (Bergdoll, 1980). The amount of enterotoxin necessary to

cause illness in human is not known, but evidence from food poisoning outbreaks indicate that as little as 0.1mg or less might be sufficient for some individuals (Bergdoll, 1980). Symptoms of staphylococcal food poisoning include abdominal pain, nausea, vomiting, diarrhoea, fever, chills and prostration. These symptoms are concluded to be due to direct action of the enterotoxin in the abdominal viscera (Bergdoll, 1980).

2.9.4 Bacillus Cereus Food Poisoning: B. cereus has been implicated in isolated cases and outbreaks of food poisoning (Lubys et al, 1993; Ahmad et al, 1995). B. cereus produces a variety of exoproducts that contribute to its pathogenicity in varying degree and have been characterized based on production of enterotoxin, permeability and lethal factor, haemolysin, cytotoxin and Lecithinase (Doyle et al, 1993). It causes two distinct food poisoning syndromes: The emetic syndrome appears 1-5 hrs following the ingestion of the contaminated food and is due to a thermo stable toxin (Shinagawa, 1990). The diarrhoea syndrome is characterized by abdominal pain with diarrhoea and appears after 8-16 hrs (Shinagawa, 1990).

In most food poisoning incidents, some form of temperature abuse has occurred during preparation or storage of food to enable the pathogens to proliferate to high number and produce enterotoxin (Roberts, 1988).

2.11 Antibiotic Susceptibility of Food Borne Bacteria

Misuse and sub-therapeutic levels of application of antimicrobial agents in food production and disease control in livestock and man has led to increase in the incidence of resistant bacteria and drug resistance transfer (Kwaga and Adesiyun, 1984; Umoh et al, 1990). Although disease control and economic benefit have been derived from the use of antimicrobial agents in man and animals, the prevalence of antibiotic resistance among microbial isolates from food is a cause for concern. Adekeye, (1981); Adesiyun, (1984a); Kwaga and Adesiyun, (1984); Umoh et al, (1990); reported the resistance of staphylococci to commonly used antibiotics and the widespread interchange of animal and human biotype of Staphylococci between man, animal and food. Multiple antimicrobial resistance of B. cereus have been documented (Gill et al, 1986; Hin-Chumg et al, 1988). The antibiotic resistance of E. coli were recorded by Ojo and Moomow (1972) while Pocurull et al, (1971) and Hatha and Lakshman, (1995) reported multiple drug resistance of Salmonella. Walton (1971), suggested the economic and cost benefits of the use of antibacterial agents in food production and disease control must always be matched against the public health implications of the development of multiple antibiotic resistant strains of pathogenic bacteria.

2.21 Enterotoxin Test

The Ligated Rabbit Ileal-Loop Test: Various enteropathogenic bacteria of man have been studied using the ligated loop of rabbit

small intestine (Moon et al, 1970; Kuboto and Liu, 1971). Spira and Goepfert (1972) studied B. cereus toxin using rabbit ileal loop test. The ileum of the rabbit were externalized after acclimatizing the rabbit to the laboratory and fasted for 40 hours. The ileum was ligated into six loops each measuring approximately 10cm long, and separated by 5cm blank. Loop response measured by fluid accumulation using the ileum of older big rabbits was sporadic and rarely reproducible, but loop response in young rabbits was reproducible (Spira and Goepfert, 1972). Enterotoxin alone caused fluid accumulation in the loop as neither the number of cells nor the development of spores in the test loop played significant role in eliciting fluid.

2.31 Hazard Analyses Critical Control Point (HACCP)

The ability to predict microbial growth as a function of the food environment enormously reduces the extent of experimentation and monitoring required to ensure safety. To check the numerous factors contributing to unsafe food, monitoring must start during the design of a product. To ensure an adequate assessment of safety, it is necessary to fully understand the processes, know the type and sources of ingredients and all other components that make up the product. This must be done to predict the types of controls that will be necessary, so that whatever goes out of the plant door is safe.

Silvia et al, (1986) ICMSF, (1988) Sperber, (1991) Schothorst and Jongeneel, (1992) noted that the HACCP approach consist of the

following elements: identification of hazard (a chance for, or the risk of an unacceptable biological, chemical, physical and/or economic property in a food product which may cause consumer distress or illness) and assessment of their severity and risk (hazard analyses).

* Determination of critical control points (ccps) required to control identified hazards.
* Specification of criteria that indicate whether an operation is under control at a particular ccp.
* Establishment and implementation of monitoring systems.
* Execution of corrective actions when criteria are not met.
* Verification of the system.
* Establishment of documentation concerning the above points and their application.

The hazard analyses portion of HACCP is the identification of sensitive ingredients, sensitive areas in the processing of the food or ingredient, control of handling process, etc. from which we can identify the critical points that must be monitored to ensure safety of the product.

The critical control points are those areas in the chain of food production from raw materials to finished products where the loss of control can result in an unacceptable food safety risk. The system hase control over the process, the raw materials, the environment and the people, thus the finished product needed no testing other than for monitoring purposes. The system can be used to control any area or point in the food production system that

could contribute to hazardous situation, whether it be contaminants, pathogenic microorganisms, raw materials, a process, consumer use directions, the distribution system or storage conditions. With adequate recording of each control/monitor the final product is sure to be safe.

HACCP differ from other plants in that the various quality control procedures at various points of the process were all put together as a system, thus the system cannot go out of control without the information being picked up through the monitoring systems (Sperber, 1991; Tisler, 1991; McMeekin et al, 1992).

HACCP can be applied in homes, food processing and service establishments. Unlike the traditional method, HACCP approach is based on an understanding of the factors that contribute to outbreaks of food borne diseases and on applied research on the ecology, multiplication and inactivation of food borne pathogens. The HACCP provides a greater assurance of food safety than any other approach and monitoring critical points is less costly and more effective than analysis of samples and inspection of processing plants.

CHAPTER 3

MATERIALS AND METHOD

3.1 SAMPLING METHOD

Sample Collection

Two hundred and forty samples comprising of 130 cellophane packaged, 90 bottles packaged and 20 bulk packaged kunun zaki were purchased from different producers/vendors around Zaria namely ABU Main Campus Samaru, Sabon-Gari, Danmagaji/Wusasa and Zaria City. The samples were transported to the laboratory within 2 hours for analysis in well labelled sterile universal bottles, except for the cellophane packaged samples which were transported in the cellophane pack in which they were sold.

Three of the producers, one each from ABU Campus, Samaru, Sabon-Gari and Danmagaji/Wusasa were identified and used as case study for Hazard Analysis Critical Control Point (HACCP).

Enumeration of Microorganisms

The cellophane packs were cleaned externally with 70% ethanol to disinfect it. Cellophane was punctured using a sterile needle and 100ml aliquot evacuated into sterile centrifuge tubes and sampled along with the bottled and bulk packaged samples using two distinct sampling methods.

Method I: Concentration by centrifugation:

Duplicate aliquot of samples (100ml each) were placed in sterile centrifuge tubes, centrifuged at 5,000 rpm for 5 min and

supernatant decanted, leaving about 2.0ml in each tube. After vigorous agitation, 0.2ml aliquot of these residues were used to inoculate Nutrient agar (NA) for total aerobic plate count, Baird parker agar (BPA) for Staphylococcal count, Eosine methylene blue (EMB) agar for coliform count, Dextrose tryptone agar (DTA) for Bacillus count, Salmonella, Shigella agar (SSA) for isolation of Salmonella and Shigella by spreading on agar plates and into each of Lactose broth for coliform test and peptone water for pre-enrichment. Incubation of plates and tubes was done at 37°c except for a tube of Lactose broth which was incubated at 44°c for 24 hours.

Method II: Serial dilution:

Each sample was serially diluted in sterile normal saline and 0.2ml aliquot of 10^{-1} dilution were spread on or into NA, BPA, EMB, DTA, SSA, Lactose broth and Peptone water as in method I and incubated accordingly.

Total aerobic plate count:

The spread-plate method was used. About 0.2ml aliquot of concentrated/diluted samples were placed on nutrient agar plate and spread with a sterile glass spreader. Following plating, the surface of medium was allowed to dry after which the plates were inverted and incubated at 37°c for 24 hours. Enumeration of microorganisms was done after 24 hours.

Coliform Count and Confirmation:

The counts for coliforms were performed as for total aerobic plate count except that EMB agar was used in place of Nutrient agar. Confirmation of coliform were carried out as follows: The Lactose broth inoculated with samples was incubated at 37 and 44°c for 24 hrs and examined for evidence of gas production (indicated by Durhams tube in the broth), and reincubated for another 24 hrs in the absence of gas production. The presence of gas constitute a positive presumptive test and the broth streaked out on EMB agar and incubated at 37°c for 24 hrs. Typical colonies on EMB plates appearing bluish black with greenish metallic sheen characteristics of E. coli or brownish colonies often convex and mucoid characteristics of Enterobacter aerogenes were counted. Typical colonies producing gas in broth and grown on EMB plates were Gram stained and stored on nutrient agar slants at 4°c. Gas production in Lactose broth evidenced by Durhams tube and Gram negative non-sporing bacilli revealed by microscopical examination confirms the presence of coliform organisms.

Bacillus and Staphylococcal Count:

Counts were performed as for total aerobic plate count using Dextrose tryptone agar and Baird Parker agar respectively. Large, flat irregular wrinkled or smooth, "ground-glass" colonies, 4-6mm in diameter were counted as Bacillus. Black, shiny, convex colonies, 1-1.5mm in diameter on BPA were counted as Staphylococcus.

Isolation of Microorganisms

The peptone water pre-enriched samples were sub-cultured onto Selenite F broth for selective enrichment, incubated at 37°c for 24 hours and subsequently streaked out on SSA for Salmonella and Shigella isolation. BPA and DTA were inoculated with the pre-enriched sample for Staphylococcus and Bacillus isolation respectively. Typical colonies were Gram stained and stored on nutrient agar slant at 4°c. Pure cultures were obtained from mixed colonies following repeated subculture. Prior to biochemical characterization, the isolates were checked for viability and purity

3.2 HAZARD ANALYSES CRITICAL CONTROL POINT (HACCP)

Hazard analyses and critical control points were of three products (cellophane, packaged, bottled and bulk packaged) from three different producers around Zaria. Producers were not given special instructions, but were observed as they carry out the kunun zaki preparation as usual.

The hazard analyses consisted of observing the raw materials used, environment, kunun zaki preparation and packaging practices to identify sources and modes of actual or potential contamination. Samples were taken at sequential stages of preparation and subsequently tested for total aerobic plate count and for certain pathogens.

The critical control point consisted of measuring temperatures immediately after gelling, during holding and when ready for

serving (consumption). Time and temperature exposure/variation were also recorded. The thermometer was washed, cleaned with 70% alcohol and allowed to dry before inserting into the product and samples were collected in sterile universal containers and taken to laboratory for analysis as appropriate.

Observations made during the analysis were indicated in the schematic diagram of kunun zaki during preparation and holding. Potential sources of contamination from raw materials, equipment, utensils and persons preparing the kunun zaki as well as likelihood of microbial survival or destruction and likelihood of microbial multiplication were noted on the diagram. Critical control points that need to be monitored were indicated at appropriate steps of the operations.

Description of production sites and producer:

Producer A lived in a two-bedroom flat located close to A.B.U. Main Campus, North Gate. Water for kunun zaki preparation was obtained in metal and plastic buckets from tap and stored in drums and big plastic containers which were all well covered. The producer keeps poultry, a cobbler workshop is cited few yards from the house. Producer has a family of 5 which at the time of study appeared all healthy. Two of the children occasionally helped in the processing under the supervision of their mother (producer). The finished product is measured with a plastic cup into white cellophane packs, tied and sold to members of the campus community. Left-over kunun zaki were refrigerated and sold the next day.

Producer B lives in a two-room apartment in a large compound with eight other families. The house is located amidst other houses and close to a motor park and a busy market (Sabon-Gari market). Water is obtained from a tap in buckets and stored in a drum and clay pots, some were covered and some were left open. Chicken, goats and dogs are usually seen moving freely around the compound. Finished product is measured into bottles and vended in Sabon gari market, Zaria and motor park by the children. No left-over were observed on the two days the producer was visited for the purpose of analysis.

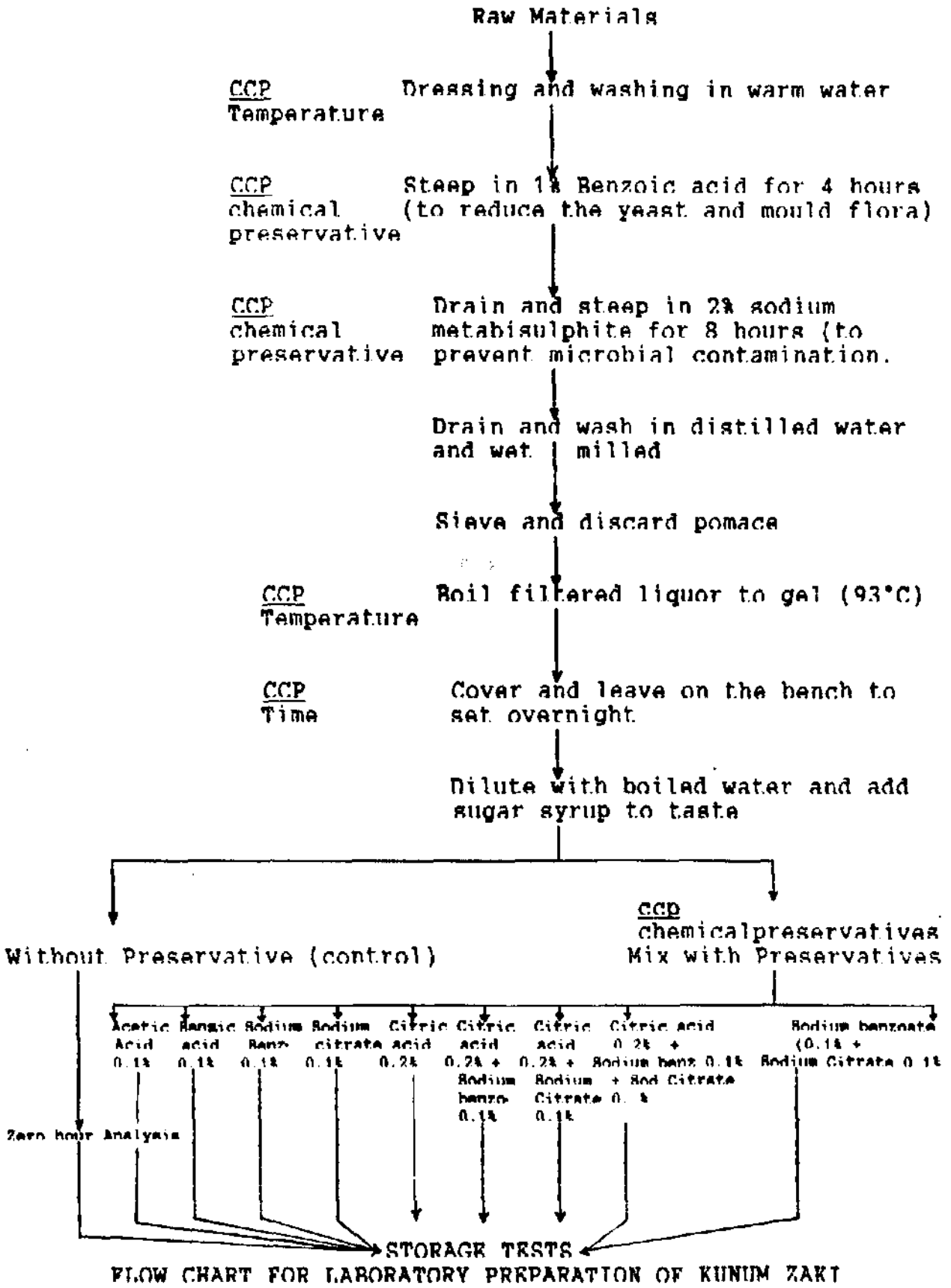
Producer C lives in a well-fenced compound with the normal Islamic setting. There is a common kitchen for the two wives and 8 children of the family. Water for kunun zaki preparation was obtained directly from a well at the centre of the compound. Goats, sheep and chickens were seen roaming around the compound. Finished products were sent in bulk to a canteen (Cafeteria) located in the market at a suburb of Zaria (Danmagaji market and the remainder were vended in bulk by the children. Leftover kunun zaki were consumed by the members of the family.

3.3 LABORATORY PREPARATION OF KUNUN ZAKI

Materials - Millet (Pennisetum typhoides) and Guinea corn (Sorghum bicolor) were used. These grains and ginger (Zingiber officinale), sweet potato (Ipomoea batatas), red pepper (Capsicum annum), cloves (Eugenia calophylloides), black pepper (Pipper nigrum) and a fruit of Xylopia aethiopica were obtained from the

market, Samaru, Zaria.

Preparation: The kunun zaki was prepared as illustrated in the flow chart in Fig. 2. The grains and spices were picked over by hand to remove all unwanted particles. About 3.2kg of sorghum were debranned in a decorticator. This was then mixed with 3kg of millet, 1kg sweet potato, 5g cloves, 5g black pepper, 1g red pepper and 5g fruit of Xylopia aethiopica and 120g ginger. The ingredients were washed in warm water and steeped for 4 hours at room temperature in twice its volume containing 1% Benzoic acid to reduce the yeast and mould flora. The 1% Benzoic acid was drained off and the grains, sweet potato and spices steeped in 5% sodium metabisulphite for 8 hr to prevent microbial contamination (Akinrele, 1970). The grains, sweet potato and spices were drained, washed with distilled water and wet milled, sieved through a fine wire mesh screen (pore size 0.5mm by 0.8mm) to remove the pomace, which was discarded. The filtrate was gelled by boiling for 20 minutes, covered and left on the laboratory bench to set overnight. Boiled water was used for dilution to a moisture content of about 95%.



FLOW CHART FOR LABORATORY PREPARATION OF KUNUM ZAKI AND STUDY OF SHELF LIFE

Fig.2

KEY:

CCP = Critical Control Point.

3.4 STORAGE TESTS

Batches of the kunun zaki were prepared as untreated without preservative (control) and treated with preservative - acetic acid (0.1% w/v), Benzoic acid (0.1%), sodium benzoate (0.1%), sodium citrate (0.1%), citric acid (0.2%) and sodium benzoate/citric acid (0.1% and 0.2%), citric acid/sodium citrate (0.2% and 0.1%), citric acid/sodium benzoate/sodium citrate (0.2%, 0.1% and 1.0%) and sodium benzoate/sodium citrate (0.1% and 0.1%) respectivaly. Each batch of 12 bottles were corked without pressure (CO_2) in 35cl bottles. All were stored at room temperature (28±2°c). Periodically, at daily intervals for 1 wk, weekly intervals for 1 month, monthly intervals for 2 months and at intervals of 2 months for 6 months, bottles were removed and the PH, titrable acidity, Pb, Na,K, total organic matter, total solid, ash, moisture, carbohydrate, lipid, nitrogen, crude protein and sensory qualities were measured over a period of 24 weeks.

3.5 SENSORY ANALYSIS

The commercial and laboratory produced samples were evaluated for colour, odour, texture, sweetness, sourness and flavour on a 4-point scale (four for V.good and one for V.poor) and for acceptability by a panel of 6 judges (all men aged 28-31) selected from within A.B.U., Zaria. Their selection was based on availability, interest and familiarity with kunun zaki.

Acceptability rating during storage was monitored on daily basis for 1 week, weekly intervals for one month, monthly intervals

for 2 months and at intervals of 2 months for 6 months. Panelists were each served with the kunun zaki samples and a master sheet (Appendix 1) and were required to taste the sample and to rate their overall impression on a 4-point descriptive scale from V.good, good, poor to V.poor, which for analysis, were scored 4-1.

Assessors were provided with water (28+2°c) with which to rinse their mouths after tasting each sample to avoid methodological bias.

3.6 BIOCHEMICAL AND PHYSIOLOGICAL CHARACTERIZATION AND CONFIRMATION OF THE ISOLATES

The biochemical and physiological identification of isolates were carried out as described by Buchanan and Gibbons, (1974); Collins and Patricia, (1976); Cheesbrough, (1991). Briefly, the tests were carried out as follows:

A. Catalase Test:

Presumptive Staphylococcus and Bacillus isolates on nutrient agar slant were tested for catalase production. Isolates were suspended in a drop of 3% H_2O_2 solution on clean glass slides. The evolution of gas bubbles indicated catalase production.

B. Coagulase Test:

The tube coagulase test was adopted in testing for production of coagulase from presumptive Staphylococcus isolates using freshly prepared human and sheep plasma. 1/10 dilution of plasma were made using physiological saline and inoculated

with test organism, incubated at 37°c for 6 hr. The tubes were checked every 30 minutes for 6 hours for evidence of the production of clot, gelling or formation of a loose web of fibrin all of which were recorded as coagulase positive.

C. Haemolysis of Human and Sheep red blood cells:

Presumptive Staphylococcus and Bacillus isolates were inoculated on blood agar base (oxoid) containing 10% human and sheep blood respectively. The plates were incubated at 37°c for 24 hrs and zone of haemolysis around colonies were observed and recorded as either alpha (α) where there is incomplete haemolysis with greenish or hazy outline round the organism, beta (β) where there is complete haemolysis with clear zone of haemolysis surrounding the organism or gamma (γ) where there is no haemolysis.

D. Phosphatase Test:

Presumptive staphylococcus isolates were inoculated onto phenolthalein phosphate agar and incubated for 24 hrs. at 37°c after which it was exposed to ammonia vapour. Colonies that turned pink were recorded as phosphatase positive.

E. Indole Production:

All the Gram negative (presumptive E. coli, Salmonella and Shigella) isolates were inoculated into Bacto tryptone broth and incubated for 48 hours at 37°c and five drops of xylene

was added to the broth culture and were shaken gently. Five drops of Rhrlich's reagent was added and the tubes observed for a rose-pink colour of indole production.

F. Methyl Red Test:

Presumptive Gram positive and Gram negative rods were tested for acid production. About 0.5ml of MRVP broth (dextrose phosphate medium) (Difco) were inoculated with presumptive isolates and incubated over-night at 37°c. A drop of methyl red solution was added and a bright red colour was recorded as methyl red positive.

G. Voges-Proskauer (V.P) Test:

2ml of MRVP broth (Difco) were inoculated with presumptive isolates and incubated for 2, 5 and 7 days at 37°c and tested for acetyl methyl carbinol (acetoin) production. A trace amount of creatinine and 1ml of 40% KOH were added, shaken and observed for slow development of pink-red colour after 1 hour.

H. Citrate Utilization:

Slants of Simmon's Citrate agar (Oxoid) were inoculated with test organisms, incubated at 37°c and observed daily for 4 days. Colour change of medium from green to blue was recorded as positive.

I. Urease and Motility Test:

Tube of motility indole urea (MIU) medium (Bacto) were stab - inoculated with test organisms and incubated at 37°c for 24 hours. Colour change from yellow to pink in the medium was recorded as positive test for Urease. After 24 hours incubation, turbidity of the entire medium were recorded as positive for motility while growth only along and the line of inoculation were noted as negative for motility.

J. H_2S Production:

Test organisms were stabbed into and streaked on the surface of Triple Sugar Iron (TSI) agar slants (oxoid). The tubes were closed loosely and incubated for 24 hours at 37°C. A blackening of the medium was recorded positive for H_2S production. Pink-red slop and yellow butt were noted as positive for glucose fermentation yellow slop and butt indicated fermentation of all the sugars (glucose, lactose and sucrose) while cracking of the media were noted as positive for gas Production from glucose fermentation.

L. Nitrate Reduction:

Tubes containing nitrate broth (oxoid) was inoculated with test organisms, incubated at 37°c for 24 hours, following which broth was tested for the reduction of nitrate to nitrite by adding sulphanilic acid reagent and alpha - naphthylamine. A pink-red colour development was recorded as positive for nitratase activity, when there was no colour development, small amount of zinc dust was added and pink colour development indicated negative nitratase activity and no colour indicate complete reduction of nitrates.

M. Starch Hydrolysis:

Starch agar plates (Difco) were spot inoculated with test organisms and incubated for 24 hours. The plates were flooded with dilute iodine solution. Formation of clear zone around colonies compared to the background colour change to blue-black was taken as hydrolysis.

N. Lecithinase Test:

Egg yolk agar was inoculated with test organisms and plates incubated for 48 hours at 37°c. Zones of opacity around colonies were recorded as positive for lecithinase activity and absence of opaque zone has negative results.

O. Reaction to Litmus Milk:

Test organisms was inoculated into tube of litmus milk and

incubated for 3 days (72 hours) at 37°c. Formation of stormy clots and colour change of indicator from mauve to pale yellow was recorded as positive.

P. Carbohydrate Utilization:

Presumptive isolates were inoculated in phenolphthalein peptone water containing 0.5% glucose, 1% mannitol, 1% lactose, 1% maltose and 0.5% xylose each and incubated at 37°c for 1-5 days. Acid production was indicated by colour change from pink-red to colourless while gas production were indicated by air bubbles in Durham's tubes. Positive and negative results were recorded accordingly.

3.7 B. cereus TOXIN PRODUCTION AND EXTRACTION

The methods of Kramer et al (1982) and Trunbull and Kramer (1983) were used for enterotoxin production in Brain heart infusion broth (oxoid) with 0.1% (w/v) glucose supplement (BHIG). The medium was inoculated with 0.5ml B HIG culture grown for 18 hours at 37°c and incubated for 6½ hours at 37°c in a shaker at 200rpm. At intervals of 3½ and 4½ hours of commencement of incubation, 1ml of 0.1N NaOH was added to each toxin production medium. This was to alkalinize it to counteract the fall in PH in the fermentation. This is because acidic conditions do not favour toxin production. At the end of the 6½ hr incubation the cultures were centrifuged at 500 rpm. The supernatant was carefully decanted and stored frozen in sterile bottles at 20°c until required.

3.8 RABBIT ILEAL-LOOP TEST FOR THE SCREENING OF ENTEROTOXIGENIC B. cereus

The test was conducted following the procedure of Duncan et al (1968) and Spira and Goepfert (1972). Four rabbits weighing between 1.5-2.5kg were acclimatized in the laboratory for 1 week before the test. The rabbits were examined at the Small Animal Clinic of Veterinary Teaching Hospital, A.B.U., Zaria and were treated for coccidiosis. The rabbits were fasted for 48 hours prior to the operation (externalizing the ileum).

The surgery and loop test were carried out at the Department of Veterinary Surgery and Medicine, A.B.U., Zaria. The ventral side of the rabbits was shaven clean, about 1ml Ketamine hydrochloride (100 mg/ml) was injected intramuscularly into the rabbits as general anaesthesia and Lidocaine as local anaesthesia. The mid-ventral side of the rabbits were incised, the ileum externalised, ligated to form test loops, each approximately 10cm long and separated from each other by a 5cm blank loops.

Two millilitre of each of the enterotoxin extract were injected intraluminally into each of the loops using 25 gauge ½ inch needle. One loop at the distal end of the ileum was injected with sterile BHIG broth to serve as the control. The ligated ileum was then returned to the abdominal cavity and the incision stitched. Following recovery from anaesthesia, the animals were provided with water, but no food and were allowed to live for 6 hours, after which they were euthanized and opened up immediately for examination. The length and fluid volume of each loop were measured. The index of fluid accumulation was derived from the

ratio of loop-fluid volume (ml) to loop length (cm). If either the control loop or any of the blank loops contained fluid, all the tests in the rabbit were considered invalid. Ratio of fluid volume to loop length ranging from 0.2 to 0.4 were considered to be mild, 0.5 to 0.7 as moderate and 0.8 to 1.5 as severe enterotoxin activity (Spira and Goepfert, 1972; Yusuf et al, 1992).

3.9 SUSCEPTIBILITY OF ISOLATES TO ANTIMICROBIAL AGENTS

The disc diffusion method of Bauer et al (1966) was used for antibiotic susceptibility testing with slight modification using Muller Hinton agar (oxoid), Brodisks and oxoid antibiotic multodisc containing the following antimicrobial agents and concentrations: Brodisks: Ampicillin (25µg); Cephalexin (25µg); Cefuroxime (3µg)' Co-Trimoxazole (50µg); Floxacin (10µg); Gentamicin (10µg), Nitrofurantoin (200µg) and Tetracycline (50µg). Oxoid multodisks: Ampicillin (2µg); Chloramphenicol (10µg); Cloxacillin (5µg), Erythromycin (10µg); Penicillin G (1.5iµ); Sulphafurazole (100µg), Streptomycin (10µg) and Tetracycline (10µg)

Brain heart infusion broths (oxoid) were inoculated with test organisms, incubated for 16 hours at 37°c and used for the test. One percent barium sulphate standard solution was used to standardize the turbidity of the culture medium by visual comparison aided by the black line on the tube.

Where the suspension of the organism was lighter than standard, the tube was centrifuged to concentrate the cells, and where turbidity exceeded that of the standard, sterile saline was

added until the two suspensions were of the same concentration as judged by the visibility of the black lines. A sterile swab was immersed into the broth culture and used to streak the surface of a Muller-Hinton agar (oxoid), and allowed to dry before placing antibiotic discs using sterile forceps. The plates were inverted and incubated at 37°c for 18 hours. The standard positive and negative control organisms used for this test were oxford S. aureus and sensitive E. coli (K.12 185Nx), Lactose positive and nalidixic acid resistance.

A test organism was considered susceptible to an agent when the difference in the zone of inhibition in the test were not more than 3mm as compared to the zone of inhibition of the control strains (Stokes, 1975; Raughnath et al, 1981).

3.10 CHEMICAL ANALYSIS

(i) PH and Titrable Acidity:

The pH of different samples were measured by using Philips digital PH mater model Pw9409.

Titrable acidity were determined on 10ml samples against 0.1N NaOH using phenolphthalein as indicator. A percentage titrable acidity was calculated as follows:

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

where

N_A = % Normality of the acid/ml (sample used)

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

N_B = Normality of the base (NaOH 0.1N)

V_B = Volume of the base or titre value

(ii) Nitrogen and Crude Proteins

The official micro-Kjeldahl method (AOAC 1980) was used to determine the nitrogen content. Crude protein were calculated by multiplying the % N by a factor 6.25.

The principle of this method is the conversion of the nitrogen of the nitrogenous substance into ammonia by boiling (oxidized) with concentrated H_2SO4 which is fixed by the excess of acid as ammonium sulphate. Suitable catalyst being essential to increase the speed of the reaction. The ammonia liberated is treated directly with standard Hcl using a mixed indicator and the result calculated as %N. Steps involved include:

Digestion:

Procedure: 10ml samples were measured into a small Kjedahl digestion flask. A small amount of $CUSO_4$ and powdered KSO_4 mixture were added as catalyst, and 2ml of concentrated H_2SO_4 added. Flask was placed in inclined position and heated gently on the digestion stand until frothing ceases. Then heated more strongly to boil gently until solution clears and the digest becomes apple green in colour, the digestion was continued for about 30 minutes longer and stopped. Particles of undigested carbon were avoided from sticking

to the side of the tube. Blank of the reagents alone were run at the same time as blank determinations of reagent.

The digested samples were poured into the distillation apparatus which has been steamed thoroughly to render it free of nitrogenous substances. The flask was rinsed with 2ml distilled water. The round glass stopper were replaced and 10ml (40%) NaOH poured into the digest for NH_3 liberation. The NH_3 liberated were collected into 5ml (2%) boric acid in 50ml conical flask. The flask was rotated to mix contents thoroughly until approximately 25ml of distillate has been obtained. A blank determination exactly as above but with water in place of sample was carried out. Distilled water was used to dilute the distillate to 50ml mark in 100ml beaker. Two drops of methyl red/Bromocresol green mixture were added as indicator and were then titrated with 0.2N hydrochloric acid to a faint pink colour.

Calculation:

$$\% \text{ Nitrogen} = \frac{(V_2 - V_1) \times N_A \times 14.007}{W} \times \frac{100}{1}$$

where

V_2 = Volume in ml of standard Hcl used in the titration

V_1 = Volume in ml of standard Hcl used in the blank titration

N_A = Normality of Hcl (0.02)

W = Weight of the sample in Mg

14.007 relative atomic mass of Nitrogen in NH_3

Thus % protein = % Nitrogen x 6.25.

(iii) Determination of Lipid:

The Lipid content of the different samples was determined using Soxhlet ether extraction procedure of AOAC (1980).

10ml of each sample was measured into a Soxhlet extractor to which 25ml dimethyl-ether was added. The extractor was rotated to mix content thoroughly and were allowed to extract for 1 hour. The ether/lipid mixture were extracted and ether evaporated to dryness leaving only the lipid whose weight was taken.

$$\% \text{ lipid} = \frac{\text{weight of lipid}}{\text{weight of sample}} \times \frac{100}{1}$$

(iv) Determination of moisture content and total solid:

These were determined using the hot air oven procedure of AOAC (1980).

10ml of samples were weighted in a crucible of known weight and oven dried at 120°c to constant weight.

Moisture content = weight of sample - weight of total solid

$$\% \text{ moisture} = \frac{\text{loss in weight (moisture content)}}{\text{weight before drying}} \times \frac{100}{1}$$

Total solid were determined by subtracting the value of moisture from 100.

% total solid = 100 - % moisture or

$$\frac{\text{weight of total solid}}{\text{weight of sample}} \times \frac{100}{1}$$

(v) Determination of Ash content:

The determination of ash was by the use of muffle furnace AOAC (1980).

10ml sample was measured into a crucible of known weight, placed in a muffle furnace at 550°c for about an hour until sample become ash.

$$\% \text{ Ash} = \frac{\text{weight of ash}}{\text{weight of sample}} \times \frac{100}{1}$$

(vi) Determination of Carbohydrate:

This was assayed by difference assuming the weight of the mineral elements to be negligible and without taking other products such as organic acids and other products of microbial degradation into consideration.

Carbohydrate = Total solid - (Ash+Lipid+Crude protein) or 100 - (Ash+Lipid+CP+moisture).

% Carbohydrate = % Total solid - (% Ash+%Lipid+%CP) or 100 - (% Ash+%Lipid+%CP+%moisture).

(vii) Determination of organic matter:

This was determined by difference

Organic matter = Total solid - % Ash or CP + lipid + carbohydrate

% Organic matter = % Total solid - % Ash or % CP + % lipid + % carbohydrate.

(viii) Determination of Mineral elements Na, K, Pb:

These were quantified with the use of flame photometer and atomic absorption spectrophotometer (AAS).

The principle of this method is that Na, K and Pb in the sample is aspirated and the air (oxidant gas) compressor spreads it as aerosol (droplets). In the presence of the flame, the elements are converted to free atoms and they absorb energy at a wavelength

specific to the elements and become thus excited. On cooling they fall back to the ground state liberating energy related to the concentration of the free atoms in the flame (and hence in the solution). The heat energy on falling to a photo-sensitive detector, is converted to current. This is now detected by the dial of the instrument as optical density (OD) of the analyte element (white side and Milner, 1984 $^{(4th)}$). This OD is then compared with given standards to ascertain the level of the mineral (element) as contained in the sample.

Procedure: 10ml of the samples were measured into a flask and digested with 60% perchloric acid mixed with concentrated HNO_3 and concentrated H_2SO_4 (All of analytical grade) Contents were mixed very well and heated gently until white fumes appear and then heated strongly for 30 seconds. The mixture is then cooled and, distilled water, added and further boiled for 30 seconds. After which it was cooled and then filtered. Na, K and Pb content of the digest were then determined.

STATISTICAL ANALYSIS

Analysis of variance and correlation coefficient were used in analyzing the results (Spiegel, 1987).

CHAPTRR 4

RESULTS

Out of 240 samples of "kunun zaki" collected, 130 were cellophane packaged with a total aerobic count range of $3.4x10^4$-$4.2x10^6$ cfu/ml, 90 were bottled with count ranging from $4.5x10^4$-$3.2x10^6$ cfu/ml and 20 were bulk packaged with count ranging from $1.5x10^4$-$6.2x10^6$ cfu/ml (Table 1). Though method 1 (concentration) gave higher microbial counts, analysis of variance showed no significant difference in rates of microbial isolation between the two methods ($P > 0.05$). While the bulk packaged samples had a significantly higher mean total Bacillus count ($1.04x10^4$ cfu/ml) compared to cellophane packaged and bottled samples, the cellophane packaged samples and bottled samples had a significantly higher mean coliform counts of $3.60x10^3$ cfu/ml and $1.8x10^3$ cfu/ml compared to bulk packaged samples which was $1.5x10^2$cf/ml. Table 1 also revealed that bulk packaged and cellophane packaged samples had significantly higher Staphylococcal count compared to bottled packaged samples ($P < 0.05$). It also revealed that there was a significant difference in the mean total aerobic plate count, mean total staphylococcal count and mean total coliform count of commercial and laboratory produced samples. There was no significant difference in mean total Bacillus count of commercial and laboratory samples except for bulk packaged samples with a count of $1.0x10^4$ cfu/ml.

Table 2 shows that there were significant differences in microbial contamination of the products from the producers compared

to the laboratory product. It also reveal temperature drop with progressive increase in microbial load during hold up. Temperature of 70-74°C used for gelling and time intervals during holding up before pitching and the PH drop during overnight holding up before product is ready for consumption appear to be the major critical control points for all three producers.

The flow chart showing hazards and critical control points during preparation of "kunun zaki" for the three different producers are presented in Figs. 3, 4 and 5. Fig. 3 revealed that producer A sieved product before gelling while Figs. 4 and 5 revealed that producers B and C sieved product after gelling with hot water. Producers A and B added wet milled sweet potato at the point of pitching while producer C steeped potato along with grains and spices. In all three producers, a small portion of the wet milled mixture of grains and spices was used for pitching. The graph in Fig. 6 illustrate the temperature time exposure of "kunun zaki" for the different producers. There were appreciable temperature drops within 1 hr for producers B and C as compared to producer A and laboratory processing, probably because both producers left product uncovered after gelling.

Table 3 shows the possible sources of contamination common to the three producers to include presence of animals in or near house, water vessels subject to contamination and contamination from raw materials and environment.

Neither Salmonella nor Shigella was isolated from products of the 3 producers immediately after gelling, during holding and when

packaged and ready for serving. B. cereus was isolated from all the three producers, S. aureus from producers B and C and E. coli from only producer B.

There was a significant difference in the preserving abilities or preservative effect of the different preservatives used. These are illustrated in Fig. 7. From the figure, it is evident that sodium benzoate, sodium citrate/sodium benzoate, citric acid/sodium benzoate and sodium benzoate/citric acid had better preservative effect. Fig. 7 also revealed that citric acid had no preservative effect on "kunun zaki" and that citric acid and sodium citrate may have hindering effect on the preservative ability of sodium benzoate, considering the fact that sodium benzoate alone had better preservative effect than its combinations with sodium citrate and citric acid. An initial sharp rise in microbial load was observed during the 1st day (0.14 wk) followed by a period of almost no increase in microbial load for 1 wk except in the control. A sharp drop in microbial load till the 4th week was observed except in control and citric acid treated samples in which the drop was not sharp. This was followed by a sharp increase in microbial load till 16th week except for the sodium benzoate, sodium benzoate/sodium citrate, citric acid/sodium benzoate and citric acid/sodium citrate/sodium benzoate treated samples. However, control and citric acid treated samples maintained higher microbial count than other treated samples.

Table 4 shows the result of a four point hedonic rating test scored by six panelists to determine the extent of the differences

in the sensory attributes of the commercial, control and treated samples. At zero hour, laboratory produced samples without preservatives (control) was given very similar mean ratings to that of commercial samples between 3-3.83 (good- V.good) except for sourness which rated as poor (2.35-2.5). Texture rated as V. good (3.50-3.83) while odour and flavour rated as good (3-3.48). At 24 hr, treated samples did not differ significantly from control and commercial samples except for citric acid, citric acid/sodium benzoate, and citric acid/sodium citrate treated samples which differ significantly in sweetness, sourness, flavour and overall acceptance when compared to control, commercial and other treated samples. Acceptability of the control fell significantly to poor (2.2) at 2 weeks declining rapidly thereafter (Appendix II). Except for citric acid/sodium benzoate treated samples, the overall acceptability of sodium benzoate treated samples and samples treated with mixed preservative containing sodium benzoate remained relatively better throughout the storage period when compared to other treated samples.

Table 5 presents the biochemical, physiological and morphological data for characterization of microbial isolates obtained from "kunun zaki". The pathogenic bacterial organisms isolated include alpha and beta haemolytic S. aureus, B. cereus, E. coli and Salmonella spp. Out of the 10 B. cereus isolates screened for enterotoxin production using the rabbit ileal loop test 5 (50%) produced toxin. Bulk packaged and bottled samples had 2 (20%) toxic strains each, while cellophane packaged samples had 1 (10%).

Of the 5, B. cereus isolates with positive enterotoxin response, 2 had mild and moderate reactions respectively with fluid/length ratio between 0.2 to 0.4 (mild) and 0.5 to 0.7 (moderate) while 1 gave severe reaction with fluid/length ratio between 0.8 to 1.5. Except for one isolate that produced bloody fluid, all others were straw coloured (Table 6). Plate 1 is a photograph showing the results of the rabbit ligated ileal loop test.

Six (60%) of the 10 S. aureus isolates were of alpha (00 haemolytic pattern and coagulated human and sheep plasma, while 4 (40%) gave beta haemolysis.

Table 7 presents the result of the antimicrobial susceptibility test. Of the 37 isolates tested, many were resistant to the commonly used antibiotics. Out of the 15 E. coli tested, 13 (86.6%) were resistant to cephalexin, 12 (79.9%) for cefuroxime, and 8 (53.3%) resistant to ampicillin and streptomycin each. All the isolates were sensitive to floxacin. Of the 10 S. aureus isolates (70%) were resistant to penicillin and streptomycin, (60%) to sulphafuraxole, none was resistant to chloramphenicol and gentamycin. One S. aureus isolate was sensitive to all the broad spectrum antimicrobial agents used for both Gram positive and Gram negative organisms. Of the 11 B. cereus isolates 3 (27.3%) were resistant to each of erythromycin and penicillin and 2 (18.2%) to cloxacillin, ampicillin and streptomycin respectively. All were sensitive to tetracycline. The only Salmonella strain isolated was resistant to 3 of the antibiotics and sensitive to 6 of the antimicrobial agents.

Table 8 shows the % resistance of the bacterial isolates to the different antimicrobial agents as classified by Gram staining reaction to depict the normal practice use of antibiotics in hospitals for chemotherapy (Bryan 1982). While the Gram negative strains showed high sensitivity to nitrofurantoin and floxacilin, the Gram positive strains showed high resistance to tetracycline and chloramphenicol. Both groups showed high resistance to ampicillin, streptomycin and sulphafurazole. These are the normal drugs of choice in the treatment of common infections. Except for tetracycline and chloramphenicol, there was no significant difference in resistance of Gram positive and Gram negative isolates to the broad spectrum antibiotics used for both groups.

The predominant multiple antimicrobial resistance pattern is given in Table 9. One isolate was resistant to the antimicrobial agents that are usually employed in the therapy of both Gram positive and Gram negative organisms. The most common patterns for *E. coli* were streptomycin - ampicillin (40%), streptomycin - ampicillin - sulphafurazole (40%), sulphafurazole - ampicillin (33.3%), sulphafurazole - streptomycin (20%). The common patterns for *S. aureus* were sulphafurazole - streptomycin (60%), penicillin - sulphafurazole (50%), penicillin - ampicillin (30%), ampicillin - cloxacillin (20%). And the most common patterns for *B. cereus* were penicillin - ampicillin (18.2%) and penicillin - ampicillin - cloxacillin (18.2%).

Proximate analysis revealed that there were no significant difference ($P > 0.05$) between the composition of the commercial and

laboratory products except for lead (Pb) content which was significantly higher ($P < 0.05$) in the commercial product. The laboratory product had slightly higher % crude protein, carbohydrate and lower lipid and moisture content (Table 10).

Correlation analysis using rank correlation method revealed that crude protein, carbohydrate and microbial load during the storage period were positively correlated while pH, lipid and titrable acidity were inversely correlated to microbial load. There were no correlations between microbial load and lead content (Appendix III).

At a temperature of 28±2°C which is the normal economical state of storage, the proximate composition of "kunun zaki" showed no appreciable changes during the entire storage period of 24 weeks, except for percentage crude protein which differ in the control, citric acid treated, acetic acid treated, benzoic acid treated and citric acid/sodium citrate treated samples when compared to other samples (Appendix IV). There was a progressive decrease in moisture content, pH and lipid content of "kunun zaki" throughout the storage period. The crude protein and total solid increased during the storage period. Appendix IV also reveal fluctuation in carbohydrate content during the storage period. The sodium (Na), potassium (K) and lead (Pb) contents remained almost constant. There were variations in Na, K, Pb contents and pH and titrable acidity of treated, untreated and commercial samples.

TABLE 1

MEAN AND RANGE OF AEROBIC PLATE COUNT, STAPHYLOCOCCAL COUNT, COLIFORM COUNT AND BACILLUS COUNT OF COMMERCIAL* AND LABORATORY** PRODUCED FURUN TARI

Sample	Total aerobic plate count (TAPC)		Staphylococcal Count		Coliform count		Bacillus count	
Commercial*	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Bulk n=20	1.5×10^4 – 6.2×10^6	2.5×10^6 a	0.8×10^1 – 2.5×10^3	7.3×10^2 a	0.4×10^1 – 4.0×10^2	1.5×10^2 a	2.2×10^2 – 4.1×10^4	1.0×10^4 c
Cellophane n=130	3.4×10^4 – 4.2×10^6	1.5×10^6 a	6.0×10^1 – 1.0×10^3	4.6×10^2 a	0.5×10^1 – 1.8×10^4	3.6×10^3 b	2.0×10^2 – 2.1×10^4	5.2×10^3 d
bottle n=90	$4.5.10^4$ – 3.2×10^6	9.7×10^5 b	0.4×10^1 – 2.0×10^2	6.0×10^1 b	0.0×10^1 – 4.0×10^3	1.8×10^3 b	1.4×10^2 – 2.5×10^3	1.0×10^3 d
Lab Product**								
UNTREATED	$1.0.10^2$ – 4.3×10^5	6.3×10^4 c	<10	<10c	<10	<10c	6.0×10^1 – 8.0×10^3	4.2×10^3 d
Acetic acid (A.A)	$1.0.10^2$ – 4.0×10^4	9.2×10^3 d	<10	<10c	<10	<10c.	4.0×10^1 – 4.2×10^3	2.0×10^3 d
Benzoic acid (B.A)	$1.0.10^2$ – 2.4×10^4	5.9×10^3 d	<10	<10c	<10	<10c	2.0×10^1 – 3.4×10^3	1.1×10^3 d
Sodium benzoate (S.B)	$1.0.10^2$ – 1.1×10^3	4.2×10^3 e	<10	<10c	<10	<10c	2.0×10^1 – 4.2×10^2	2.0×10^2 e
Sodium citrate (S.C)	$1.0.10^2$ – 2.0×10^3	1.5×10^3 d	<10	<10c	<10	<10c	4.0×10^1 – 1.8×10^3	9.4×10^2 d
Citric acid (C.A)	$1.0.10^2$ – 2.0×10^5	6.2×10^4 c	<10	<10c	<10	<10c	6.0×10^1 – 6.6×10^3	3.9×10^3 d
CA+SB	$1.0.10^2$ – 1.8×10^3	5.9×10^2 e	<10	<10c	<10	<10c	2.1×10^1 – 4.3×10^2	2.5×10^2 e
CA+SC	$1.0.10^2$ – 2.1×10^4	5.3×10^3 d	<10	<10c	<10	<10c	4.0×10^1 – 1.9×10^3	9.2×10^2 d
CA+SB+SC	$1.0.10^2$ – 2.0×10^3	6.5×10^2 e	<10	<10c	<10	<10c	2.2×10^1 – 4.3×10^2	2.4×10^2 e
SB+SC	$1.0.10^2$ – 1.4×10^3	5.3×10^2 e	<10	<10c	<10	<10c	2.2×10^1 – 4.0×10^2	2.3×10^2 e

abcde : Means within rows with different superscripts are significantly different

* Mean and Range of Count obtained from 10 randomly selected samples

** Mean and Range of count obtained over the storage period

<10 No significant growth. (Not isolated)

TABLE 2

Bacteria Count at Different Stages of Kunun Zaki Preparation

Producer	Periodof collection	Temp. (°c)	Total aerobic plate (cfu/ml)	Coliform count (cfu/ml)	Staphy-lococcal count (cfu/ml)	Bacillus count (cfu/ml)
A	Immediately aftergelling	72	$4.3\times10^{3}_{d}$	-	-	$1.1\times10^{2}_{c}$
	Duringhold-up afterpitching	45	$4.2\times10^{4}_{c}$	<10	1.0×10^{3}	$3.3\times10^{2}_{c}$
	Afterovernight holding	29	$8.2\times10^{8}_{b}$	8.0×10^{3}	9.6×10^{3}	$1.0\times10^{8}_{b}$
	Packagedand readyforserve	29	$6.4\times10^{8}_{b}$	8.0×10^{3}	1.0×10^{2}	$1.3\times10^{8}_{b}$
B	Immediately aftergelling	74	$1.2\times10^{4}_{c}$	<10	-	$8.1\times10^{3}_{d}$
	Duringhold-up afterpitching	38	$6.1\times10^{4}_{c}$	1.5×10^{3}	7.2×10^{3}	$2.5\times10^{2}_{c}$
	Afterovernight holding	28	$1.2\times10^{8}_{a}$	1.0×10^{2}	1.3×10^{2}	$2.2\times10^{4}_{a}$
	Packagedand readyforserve	28	$2.0\times10^{8}_{a}$	1.8×10^{2}	1.3×10^{2}	$2.3\times10^{4}_{a}$
C	Immediately aftergelling	70	$2.1\times10^{4}_{c}$	2.0×10^{3}	-	$4.9\times10^{3}_{d}$
	Duringhold-up afterpitching	44	$3.5\times10^{8}_{b}$	8.2×10^{2}	<10	$1.0\times10^{2}_{c}$
	Afterovernight holding	29	$2.4\times10^{8}_{a}$	1.7×10^{2}	8.0×10^{3}	$4.0\times10^{8}_{b}$
	Packagedand readyforserve	29	$3.5\times10^{8}_{a}$	1.2×10^{2}	8.0×10^{2}	4.0×10^{8b}
D	Immediately aftergelling	93	$6.0\times10^{4}_{f}$	-	-	$3.0\times10^{3}_{d}$
	afterovernight holding	28	$1.0\times10^{8}_{e}$	-	-	$7.3\times10^{3}_{d}$
	Packagedand readyforserve	28	$1.0\times10^{8}_{e}$	-	-	$7.3\times10^{3}_{d}$

TABLE 3

Potential Sources of Contamination Observed During Hazard Analyses of Producers

Analysis of Producers

Source	Producer 1	2	3	Laboratory
FARCES				
Chickens in or near house	x	x	x	o
Dogs/Goats/Sheep in or near house	o	x	x	o
ENTERIC MICROORGANISMS FROM WATER (UNPROTECTED)				
Well water	o	o	x	o
Water vessels subject to contamination	x	x	x	x
SPORES FROM RAW MATERIALS/ENVIRONMENT				
Cereals (Sorghum, Millet)	x	x	x	x
Spices used	x	x	x	x
Utensils/equipments used	x	x	x	x
Sweetening agent(s) used	x	x	x	x
Refuse dump/waste paper basket nearby	x	x	x	x
Nearby workshop(s)/market	x	x	o	o
Nearby major busy road	x	x	x	x
Motor parks/Bus stop nearby	x	x	o	o

x = sources present.

TABLE 4

Results of four Point Descriptive Tests for Sensory Attributes of Untreated, Treated and Commercial "Kunun Zaki"

Sample	Zero Hour Sensory Attributes and Mean Scores						
	Sweetness	Odour	Texture	Sourness	Flavour	Colour	Overall acceptance
Untreated (control)*	3.50^{a}	3.33	3.50^{a}	2.33^{b}	3.40^{b}	2.67	2.67^{b}
Commercial**	3.67^{a}	3.50	2.83^{b}	3.83^{c}	3.67^{b}	2.33	3.67^{c}
	24 Hours						
Untreated (control)	3.50^{a}	3.32	3.50	2.34^{b}	3.40^{b}	2.67	2.67^{b}
Acetic Acid (AA)	3.50^{a}	3.32	3.50	2.34^{b}	3.40^{b}	2.67	2.67^{b}
Benzoic Acid (BA)	3.50^{a}	3.30	3.50	2.36^{b}	3.40^{b}	2.67	2.67^{b}
Sodium benzoate (SB)	3.50^{a}	3.33	3.50	2.34^{b}	3.40^{b}	2.67	2.67^{b}
Sodium citrate (SC)	3.50^{a}	3.32	3.46	3.34^{b}	3.40^{b}	2.66	2.67^{b}
Citric Acid (CA)	1.50^{b}	3.30	3.50	1.30^{a}	1.82^{a}	2.67	1.60^{a}
CA + SB	1.52^{b}	3.30	3.50	1.40^{a}	1.83^{a}	2.65	1.80^{a}
CA + SC	1.54^{b}	3.31	3.51	1.40^{a}	1.80^{a}	2.67	2.00^{a}
CA + SB + SC	2.50^{c}	3.30	3.50	2.32^{b}	3.38^{b}	2.64	2.40^{b}
SB + SC	3.50^{a}	3.32	3.50	2.34^{b}	3.40^{b}	2.67	2.67^{b}

* Mean of scores at zero hour

** Mean of scores of 12 different samples

a,b,c Means along the row with different superscripts are significantly different.

TABLE 5

MORPHOLOGICAL, PHYSIOLOGICAL, BIOCHEMICAL CHARACTERISTICS AND IDENTITY OF BACTERIA ISOLATES OF KUNUN ZAKI

Gram Reaction	tve rod	-ve rod	-rod	+ve cocci
Spore Shape	CND -	- -	- -	- -
Catalase	+	+	+	+
Coagulase	0	0	0	+
Phosphatase	0	0	0	+
Indole	-	+	-	+
Methyl Red	+	+	+	0
V.P.	+	-	-	-
Citrate	+	-	+	-
Urease	-	-	-	+
Motility	+	+	+	-
Nitrate	+	+	+	+
TSI				
Slant	0	Y	R	0
Butt	0	Y	Y	0
Lactose	0	+	-	0
Sucrose	0	+	+	0
Glucose	0	+	+	0
Gas	0	+	-	0
H_2S	-	-	+	0
Starch HYD	+	0	0	+
Gelatin LIQ	+	-	-	-
Lithmus Milk	+	0	0	0
Lecithinase	+	0	0	-
Haemolysis	+B	0	0	+&B
Carbohydrate Utilization				
Glucose	+A	+AG	+	+A
Mannitol	-	+	+	+A
Lactose	0	+AG	+	+A
Maltose	+	+	+	+A
Xylose	-	+	+	-
Identity	Bacillus Cereus	Escherechia Coli	Salmonella Species	Staphylococcus aureus

\+ - Positive response
\- - Negative response
0 - Not applicable
R - Red (Alkaline reaction)
Y - Yellow (acidic reaction)
A - Acid Production
G - Gas Production

TABLE 6

Enterotoxigenic Strains, Amount of Fluid Accumulation and Colour during Ileal Loop Test

Isolates	Volume of Fluid (cm)	Volume/Length Ratio	Characteristic Fluid colour
26_{14} BU	5.0	0.50	Straw Coloured
29_{16} BU	6.1	0.61	Straw Coloured
31_{10} CR	3.0	0.30	Straw Coloured
33_{24} BO	8.6	0.86	Bloody Coloured
34_{50} BO	3.9	0.39	Straw Coloured

TABLE 7

Antibiogram of S. aureus, E. coli and B. cereus isolated from Kunun Zaki

Isolates

Antimicrobial Agent (Concentration)	E. coli number (%) resistant to	S. aureus number (%) resistant to	B. cereus number (%) resistant to
Chloramphenicol (10ug)	5 (33.3)	0 (0.0)	1 (9.1)
Erythromycin (10ug)	7 (46.6)	3 (30.0)	3 (27.3)
Sulphafurazole (100ug)	6 (40.0)	6 (60.0)	1 (9.1)
Cloxacillin (5ug)	-	2 (20.0)	2 (18.2)
Penicillin (1.5ug)	-	7 (70.0)	3 (27.3)
Ampicillin (2ug)	8 (53.3)	3 (30.0)	2 (18.2)
Streptomycin (10ug)	8 (53.3)	7 (70.0)	2 (18.2)
Tetracycline (10ug)	5 (33.3)	1 (10.0)	0 (0.0)
Cephalexin (25ug)	13 (86.6)		
Cefuroxime (30ug)	12 (79.9)		
Gentamicin (10ug)	3 (20.0)	0(0.0)	1 (9.1)
Floxacin (10ug)	0 (0.0)	0(0.0)	0 (0.0)
Nitrofurantoin (200ug)	2 (13.3)		
Co-Trimoxazole (50ug)	4 (26.6)		
Ampicillin (25ug)	6 (40.0)		
Tetracycline (50ug)	4 (26.6)		

A total of 15 E. coli, 10 S. aureus and 11 B. cereus were tested against 16 commonly used antimicrobial agents.

TABLE 8

Percentage Resistance of Gram Positive and Negative Isolates from Kimam Zaki to Antimicrobial Agents

Antimicrobial Agents (Concentration)		No. & Percentage (%) of Grams negative isolates resistant to (n = 16)	No. & Percentage (%) of Grams positive isolates resistant to (n = 21)	Total & Percentage (%) of isolates resistant to (n = 37)
Cephalaxin	(25µ)	14 (87.5)^{a}	**	**
Caturoxin	(30µg)	13 (81.3)^{a}	**	**
Penicillin	(1.5µg)	**	10 (47.6)^{b}	**
Cloxacillin	(5µg)	**	4 (19.0)^{c}	**
Streptomycin	(10µg)	9 (56.3)^{b}	9 (42.8)^{b}	18 (48.6)
Ampicillin	(2µg)	9 (56.3)^{b}	5 (23.8)^{c}	14 (37.8)
Erythromycin	(10µg)	8 (50.0)^{b}	6 (28.5)^{c}	14 (37.8)
Ampicillin	(25µg)	7 (43.8)^{c}	**	**
Sulphaturazole	(100µg)	6 (37.5)^{c}	7 (33.3)^{c}	13 (35.1)
Tetracycline	(10µg)	6 (37.5)^{c}	1 (4.7)^{d}	7 (18.9)
Chloramphenicol	(10µg)	5 (31.3)^{c}	1 (4.7)^{d}	6 (16.2)
Co-trimoxazole	(50µg)	4 (25.0)^{d}	**	**
Tetracycline	(50µg)	4 (25.0)^{d}	**	**
Gentamicin	(10µg)	3 (18.8)^{d}	0(0.0)	3(8.1)
Nitrofurantoin	(200µg)	2 (12.5)^{d}	**	**
Pioxacin	(10µg)	0 (0.0)^{a}	0(0.0	0(0.0)

** = Not applicable

n = number of isolates

abcde = percent values in rows with different superscripts are significantly different

TABLE 9

Predominant Patterns of Multiple Antimicrobial Resistance of *E. coli*, *S. aureus* and *B. cereus* Isolated from Kimam Zaki

Resistance Pattern	*E. coli* number (%) Resistance to n = 15	*S. aureus* number (%) Resistance to n = 10	*B. cereus* number (%) Resistance to n = 11
S,AMP	6 (40)	1 (10.0)	1 (9.1)
S,AMP, Sf	6 (40)	1 (10.1)	1 (9.1)
S,AMP	5 (33.3)	2 (20.0)	0 (0.0)
P, Amp,	**	3 (30.0)	2 (18.2)
P, AMP, Ox	**	2 (20.0)	2 (18.2))
P, Sf,	5 (33.3)	5 (50.0)	1 (9.1)
Sf, S	3 (20.0)	6 (60.0)	0 (0.0)
Te,E	5 (33.3)	1 (10.0)	0 (0.0)
P, AMP, Sf	4 (26.7)	1 (10.0)	0 (0.0)
Resistant to All	1 (6.7)	0 (0.0)	0 (0.0)
Sensitive to All	0 (0.0)	1 (10.0)	0 (0.0)

P = Penicillin, Sf = Sulphafurazole, Amp = Ampicillin, S = Streptomycin

Te = Tetracycline, Ox = Cloxacillin, E = Erythromycin

TABLE 10

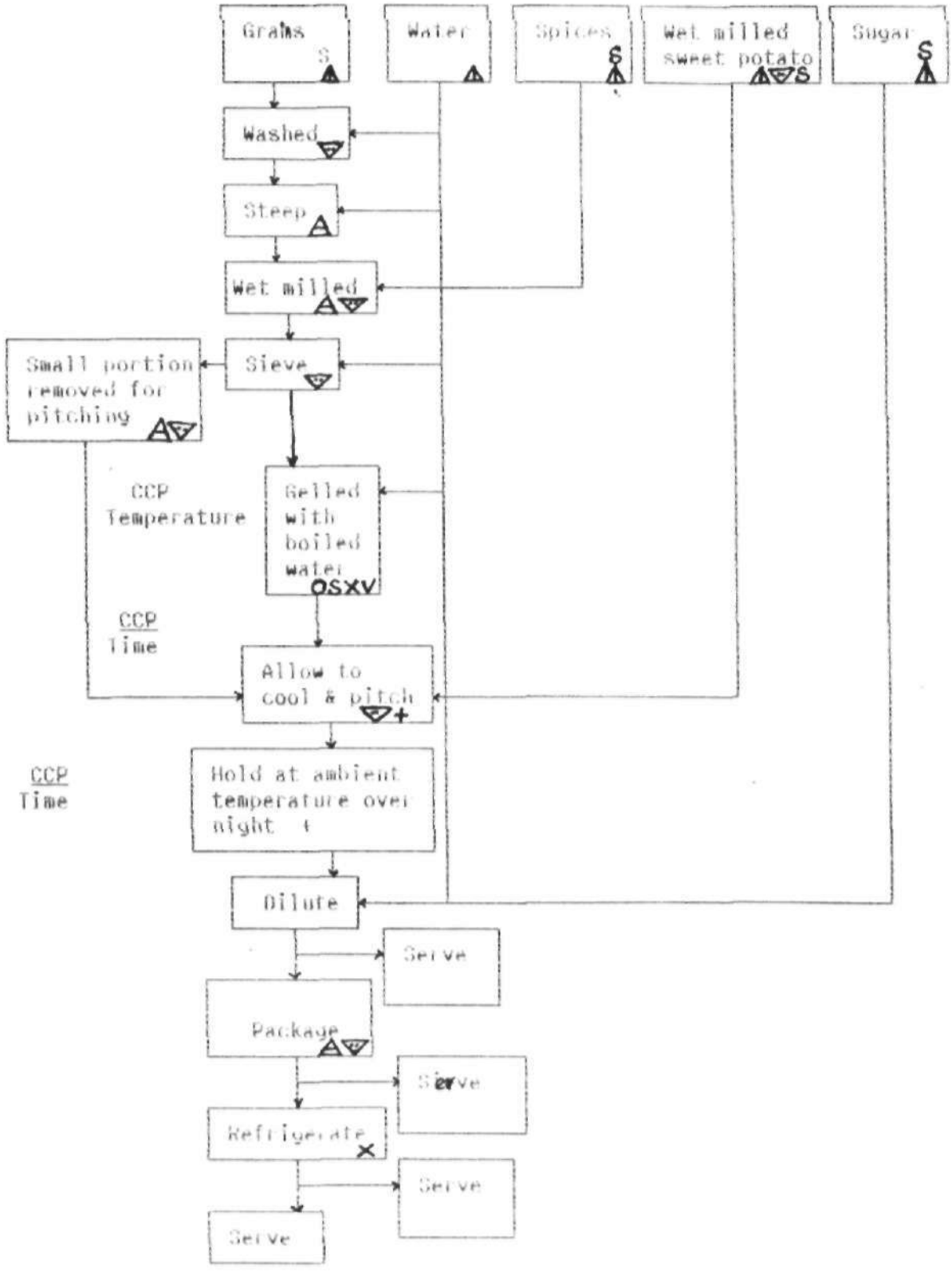
Proximate Composition of Commercial and Laboratory Produced Kunun Zaki

Sample	% Nitrogen	% Crude Protein	% Lipid	Carbohydrate	% Moisture	% Ash	% Total Solid	% Organic Matter	Na (ppm)	% Na	K (ppm)	%K	Pb (ppm)	% Pb	Titrable Acid	PH
Laboratory	0.058	0.363	0.77	5.16	93.55	0.16	6.45	6.293	76.88	0.007688	187.50	0.018750	0.110	0.00001	0.21	4.80
Commercial	0.050	0.312	0.79	3.21	95.55	0.14	4.46	4.312	78.98	0.007898	172.95	0.017295	2x.808	0.00268	0.25	4.7x

* = Values obtained from zero hour analysis

** = Values obtained from mean of twelve pooled samples

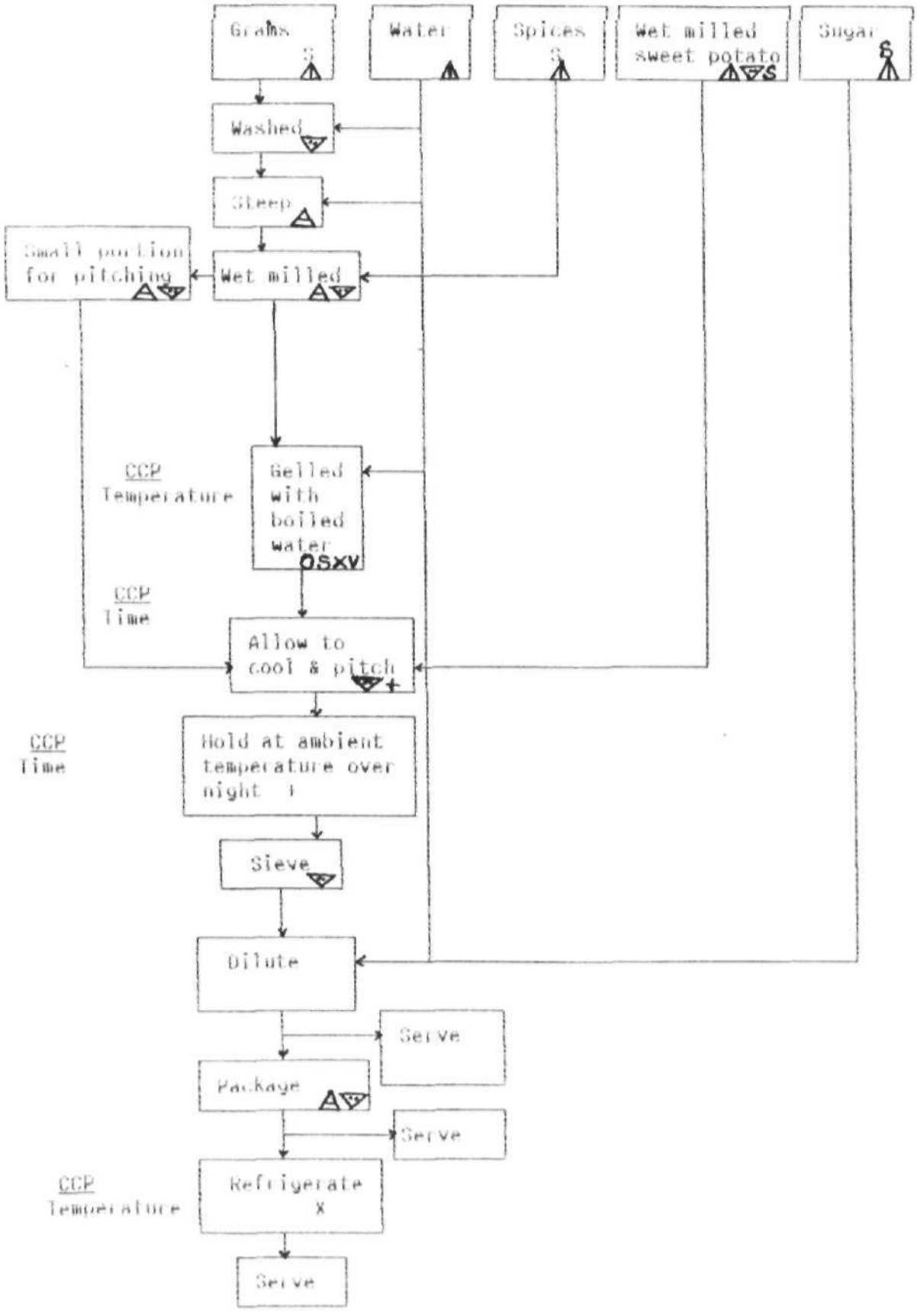
ab = means within rows with different superscripts are significantly different


Grains
Water
Spices
Wet milled sweet potato
Sugar
Washed
Steep
Wet milled
Small portion removed for pitching
Sieve
CCP
Temperature
Gelled with boiled water
OSXV
CCP
Time
Allow to cool & pitch
CCP
Time
Hold at ambient temperature over night
Dilute
Serve
Package
Serve
Refrigerate
Serve
Serve

KEY:

	initial contamination
	contamination from equipment/utensil surfaces likely
	contamination by persons handling kunu likely
CCP	critical control point
0	survival of vegetative form of bacteria likely
X	inactivation of vegetative form of bacteria likely
+	propagation of bacteria likely
S	spores
V	vegetative cells.

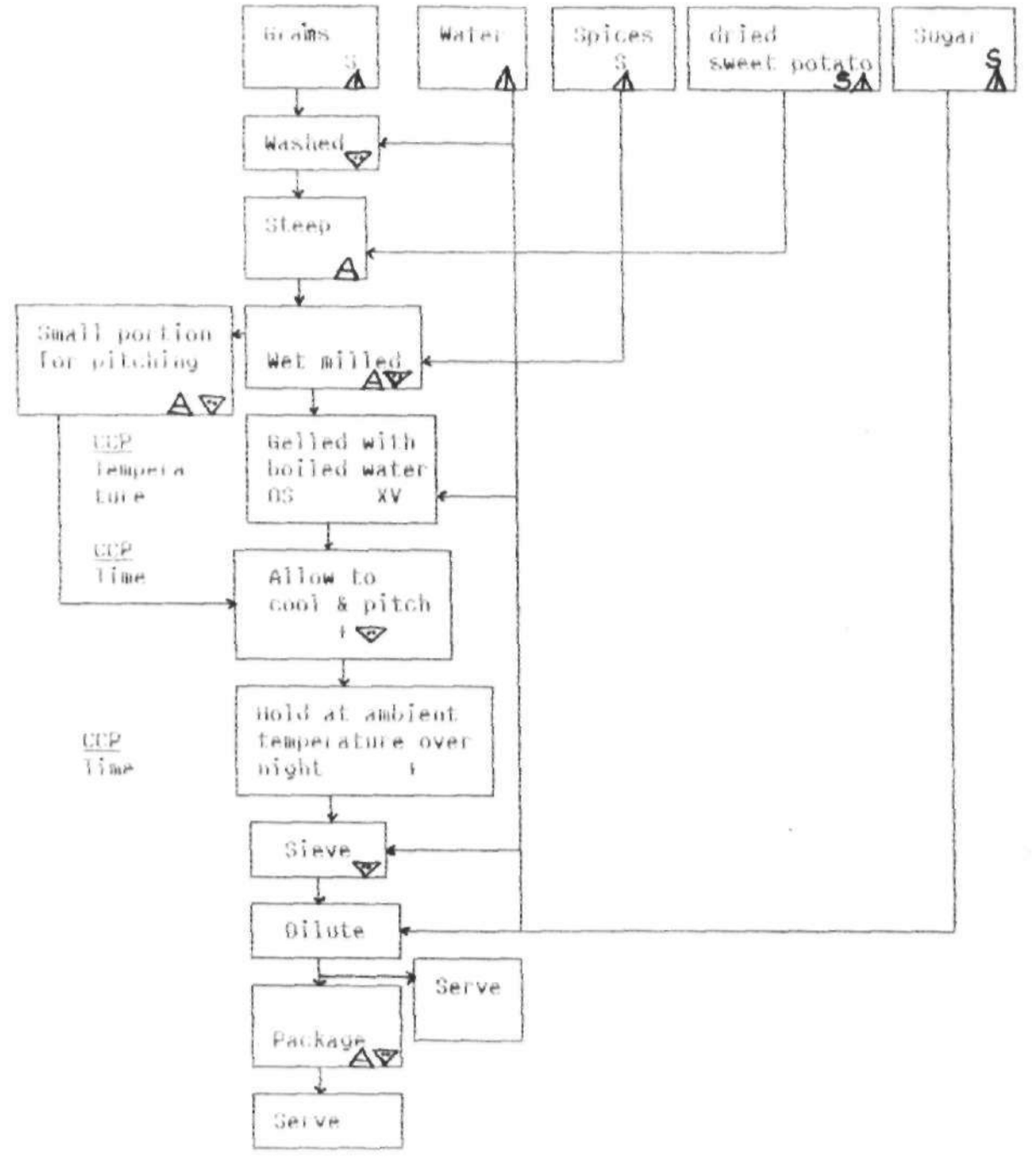
Fig. 3: Preparation of Kunun Zaki by Producer A


Grains
Water
Spices
Wet milled sweet potato
Sugar
Washed
Steep
Small portion for pitching
Wet milled
CCP Temperature
Gelled with boiled water
CCP Time
Allow to cool & pitch
CCP Time
Hold at ambient temperature over night
Sieve
Dilute
Serve
Package
Serve
CCP Temperature
Refrigerate
Serve

KEY:

	initial contamination likely
	contamination from equipment/utensil surfaces likely
	contamination by persons handling kuni likely
CCP	critical control point
Ω	survival of vegetative form of bacteria likely
X	inactivation of vegetative form of bacteria likely
+	propagation of bacteria likely
S	spores
V	vegetative cells

Fig. 4: Preparation of Kimin Zaki by Producer B



initial contamination likely

contamination from equipment/utensil surfaces likely

contamination by persons handling kunu likely

critical control point

survival of vegetative form of bacteria likely

inactivation of vegetative form of bacteria likely

proliferation of bacteria likely

spores

vegetative cells

Fig. 5 Preparation of 'Kunun Zaki by Producer C

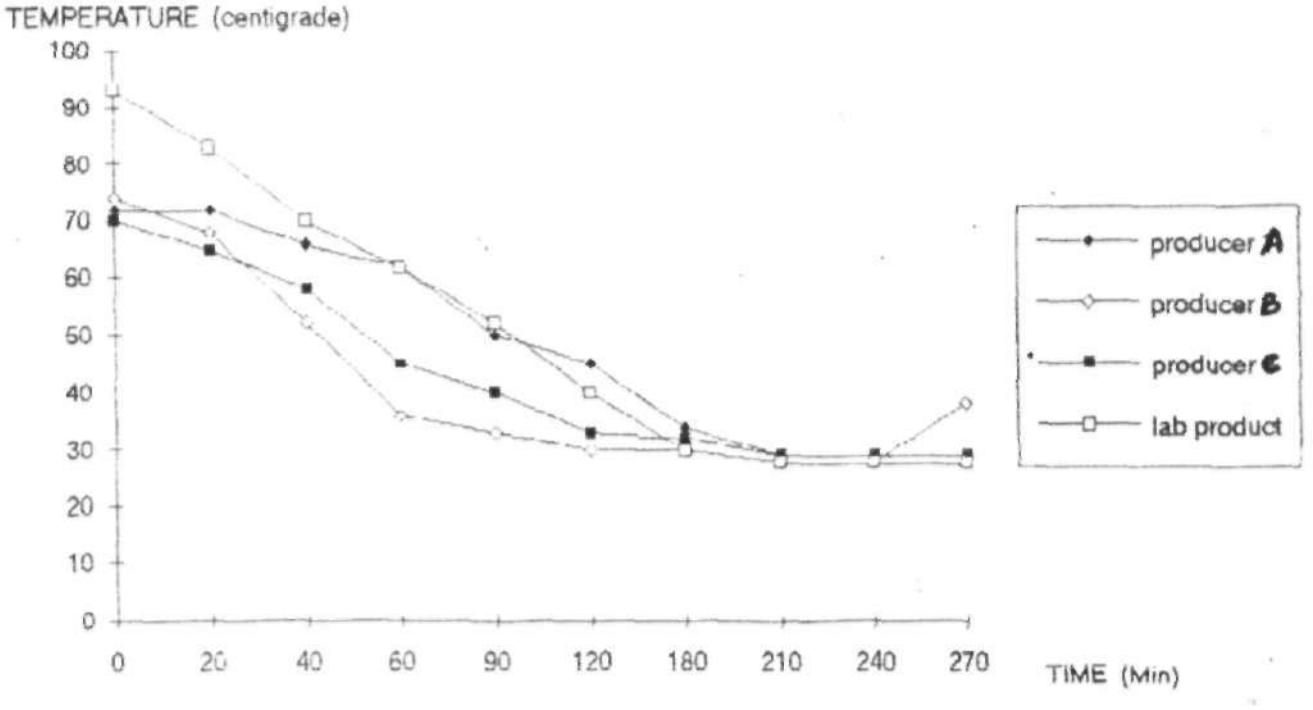


Fig. 6: Temperature Vs Time graph for the 3 producers and laboratory processing from immediately after getting to when ready for serving.

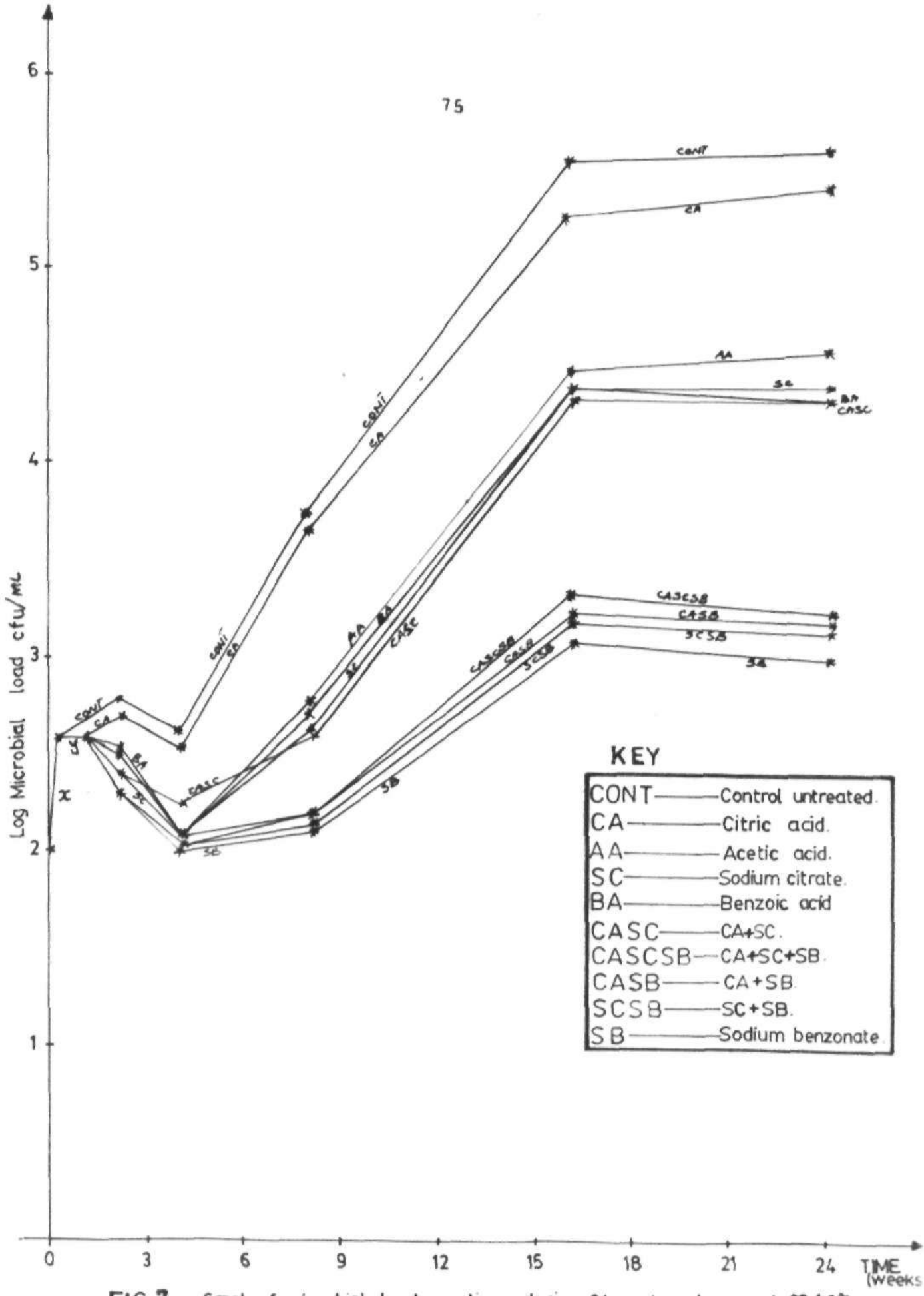


FIG. 7 Graph of microbial load vs time during 24 weeks storage at 28 ± 2°C.



Plate 1: Result of Rabit Ileal loop Test

Letter N - Negative response

P - Positive response (**Fluid accumulation**)

C - Control loop

B - Blank loop.

CHAPTER 5

DISCUSSION

All the 240 (100%) "kunun zaki"samples were contaminated with Bacillus ssp while 221 (92.1%) were with coliforms, 216 (90.0%) with Staphylococcus ssp and only 1 (0.4%) was contaminated with Salmonella.

The highest mean aerobic plate count was $2.5x10^6$ cfu/ml, Bacillus count was $1.0x10^4$ cfu/ml, Coliform count was $3.6x10^3$ cfu/ml and Staphylococcal count was $7.3x10^2$ cfu/ml. Based on standards set up by Standard Organisation of Nigeria (SON, 1985) and the ICMSF (1974), it could be deduced that the "kunun zaki" samples were highly contaminated. The high value for aerobic plate count and contamination rates with Bacillus, Coliform and Staphylococcus may be attributed to the ubiquitous distribution of these organisms, the method of processing, handling, (machine) milling, raw materials, water and the environment. The materials used for packaging could also play a role. The Staphylococcus and Coliform counts obtained for the products at the point of preparation and sales outlet are similar to the values previously reported in this environment (Omoirah et al, 1985).

The Standard Organisation of Nigeria (SON) suggested that Coliform bacteria and pathogenic micro-organisms should be absent from soft drinks (SON, 1985). It has also been reported that very high cell count of B. cereus $1x10^7$ cells/g food (ICMSF, 1974) and a population of at least 10^6 cfu/g food of enterotoxigenic strains

of Staphylococci (Bergdoll, (1979) are required to present any risk of intoxication. A count of 10^4 cfu/g has been suggested as the acceptable limit for total bacterial in food, with 10^4-10^6/g as marginal and above 10^6 cells per g as unacceptable limit (ICMSF, 1974). However, a single cell of Salmonella in food calls for a serious concern (Litchfield, 1980).

The higher prevalence of Staphylococcus and Bacillus in bulk and cellophane packaged samples compared to bottled kunun zaki could be attributed to the mode of handling of the product during packaging in cellophane and constant exposure of bulk packaged products during sales. This constant exposure of bulk kunun zaki coupled with the fact that buyers sometimes handled the product during self-service could equally have accounted for the isolation of Salmonella in only the bulk packaged product.

It is of public health interest to observe that species of E. coli, S. aureus, B. cereus and Salmonella were detected in kunun zaki even though in low counts. The isolation of E. coli from kunun zaki is not surprising since this organism has been shown to survive high temperature (Braocett et al, 1994), and acidic conditions (Arocha et al,1992; Cutter and Sirogusa, 1994; Donald and John, 1995). E. coli and Staphylococcus aureus have equally been shown to thrive in kunun zaki (Onuorah et al, 1987). The high water activity (a_w) of kunun zaki has been found to equally prove adequate for S. aureus survival (Sonia et al, 1993). The ability of Bacillus spp to form endospores and its known Osmo and thermotolerant nature (Frazier and Westhoff, 1978; Odunfa, 1987;

Oranusi et al, 1994) are points that could account for its survival in kunun zaki after gelling at temperature of 70-93°C and storage with preservatives.

The isolation of Salmonella from one sample of bulk packaged kunun zaki is in agreement with the report of Obosanya and Whong (1991), who reported low prevalence of isolation of Salmonella spp from this product which could be attributed to post-processing contamination due to poor sanitary condition.

The low counts of E. coli and S. aureus in kunun zaki recorded in this study may be attributed to the inhibitory effect of cloves and the low PH (4.76-4.80)of kunun zaki (Onuorah et al, 1987). There is likely to be an inhibitory effect of the extracts of these spices on B. cereus, Salmonella, Shigella and other pathogens (Hardman, 1972). The thermal effect of gelling kunun zaki may have also contributed to the low count of the pathogens isolated since organisms exposed to thermal processing or other adverse conditions like low PH as experienced in kunun zaki often require some resuscitation prior to plating on selective media. The sharp increase in microbial load during storage from 0 - 0.14 wk (Fig. 7 could be attributed to activation of spores by heat shock during gelling (Sinha and Srivastava, 1988). The relative period of significant increase in microbial load during storage from 0.14 - 1-0 wk (Fig 7) could be attributed to extended lag phase posibly by the preservative effect and anaerobic environment created by bottling (Desrosfer and Desrosier, 1977).

The diauxic kind of growth curve obtained during the storage

period could be attributed to the interrelationship that usually occur between microorganisms in such mixed culture as seen in kunun zaki (Oyeyiola 1991) and also to the availability of different utilizable nutrients metabolized at different periods by the microorganisms due to the catabolite repression of induced enzyme synthesis (Sinha and Srivastava 1983).

The significant difference in texture between laboratory and commercial samples could be associated to the use of larger diameter sieve pores by commercial producers, which would allow for more bulk in the product. Commercial products rated better for sweetness than laboratory produced drinks probably because commercial producers may use more effective sweeteners such as saccharine. Commercial samples rated better for sourness compared to laboratory samples, it could be possible that there was little or no fermentation in the laboratory product compared to commercial products as the yeasts which are the major fermenters present in the commercial product may have been destroyed during the more controlled laboratory processing. The significant difference in flavour and overall acceptance of sodium benzoate treated samples compared to the citric acid treated samples could be because citric acid presented no preservative effect and as such deteriorated faster and also to the natural sour taste of citric acid.

The fact that microbial load remained within acceptable limit throughout the storage period, suggests that deterioration in some samples revealed by sensory analysis could be attributed to chemical and biochemical changes in the product such as rancidity,

pH changes and changes caused by products of microbial metabolism.

At the end of 24 weeks of storage, no detectable deterioration was observed for sodium benzoate, citric acid/sodium benzoate, citric acid/sodium benzoate/sodium citrate and sodium benzoate/sodium citrate treated samples. However, only sodium benzoate and sodium benzoate/sodium citrate treated samples maintained their acceptability at the end of the storage period. The presence of citric acid in the other preservative mixtures may have contributed to the defect in the others. This is because it was the only difference observed that could be responsible for the difference in acceptability of the products.

Heat treatment of food (e.g cooking) not only improves the taste, smell, appearance and digestibility, it also render food less toxic, improve the keeping qualities by killing moulds, yeasts and bacteria that promote spoilage and infection. Thus heat treatment is a practice aimed at improving the overall safety of food and therefore, which makes it a critical control point (CCP).

Kunun zaki prepared by the three producers and from the laboratory attained an immediately after gelling temperature of 70 - 93^{0}C (Table 2) that should be sufficiently high to kill large numbers of vegetative cells, but not heat resistant spores. The high total aerobic plate count of 10^3-10^4 cfu/ml recorded immediately after gelling from the three producers could be explained either by survival of spores which could have come initially from the grains, spices, sweet potato or sweeting agents or by reduction of but not total elimination of very large number

of vegetative cells that propagated during steeping. The practice of pitching with fresh portion of the wet milled grains, spices and sweet potato, appear the likely point for contamination with coliform, Bacillus and Staphycoccus.

Heating during gelling and holding at ambient temperature during the interval between gelling and serving appear to be the major critical control point of kunun zaki. Increases in total aerobic plate count, Coliform count, Staphylococcal count and Bacillus count were observed as the duration of holding was prolonged, indicating that considerable microbial multiplication occurred (Table 1). During the interval of holding, spores that survived gelling could germinate and injured vegetative cells can resuscitate. The high counts found at point of serving obtained for products from producers B and C could be associated to the processing method adopted, considering the fact that these producers sieved their product after gelling as opposed to producer A and laboratory processing in which sieving was done before gelling. Seiving before gelling could have reduced contamination by pathogenic bacteria that may reach the products during and after sieving. The environment may have tremendously added to the contamination rate considering the presence of animals in and around the premises of producers B and C.

The low count in the laboratory product could also be attributed to the higher temperature (93^{0}C) of gelling compared to temperatures of 70, 72 and 74^{0}C employed by producers C, A and B respectively. The low count could also be due to the introduction

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Duncan et al (1968) is in opposition to this report as distention was not observed.

Six (60%) of the S. aureus were alpha (ó) haemolytic and coagulated human and sheep plasma, while 4 (40%) were beta (ß) haemolytic. This raises serious public health concern since ó-haemolytic strains are likely to be human biotypes and more enterotoxigenic than animal biotype which are often beta haemolytic (Bergdoll, 1979). This finding is in agreement with that of Onuorah et al (1987).

The resistance to antimicrobial agents of various food-borne pathogenic bacteria from this location have been well documented. Kwaga and Adesiyun (1984) and Umoh et al (1990), reported the antibiotic resistance pattern of S. aureus from ready-to-eat foods and from milk and milk products respectively. Hin-Chung et al (1988) and Yusuf (1990) recorded the antimicrobial resistance of B. cereus from diary products and flour based foods. Resistance of E. coli to antimicrobial were documented by Ojo and Moomow (1972). Pocurull et al (1971); Natha and Lakshman (1995) reported multiple drug resistance of Salmonella from animal, fish and crustaceans respectively. The antibiotic resistance of Staphylococci obtained in this study are higher than values from the result of earlier studies recorded by Kwaga and Adesiyun (1984) except for ampicillin, chloramphenicol and tetracycline. The higher prevalence of resistance to penicillin however, compare favourably with 97.8% reported by Paul et al (1982). Similarly, the % resistance of Staphylococci from milk and milk products recorded by

Umoh et al (1990) are lower compared to the result of the present study except for the values of tetracycline, chloramphenicol and ampicillin, which are comparable. These differences could be attributed to the difference in the source of isolates, drug resistance transfer and the indiscriminate and widespread use of antibiotics in the environment.

The antibiograms of B. cereus isolates from kunun zaki obtained in the present study is in agreement with the previous report of Yusuf (1990) on the susceptibility patterns of B. cereus from flour based products.

The finding that resistance to penicillin, ampicillin and sulphafurazole was most prevalent compared to the antimicrobial agents is in agreement with those of previous studies in which a higher % of resistance to these agents were recorded (Adekeye 1979; Kwaga and Adesiyun 1984; Umoh et al 1990. The patterns of resistance to antimicrobial agents recorded in the present study differ from those reported by Yusuf (1990). The multiple resistance patterns of S. aureus recorded by Kwaga and Adesiyun (1984) and Umoh et al (1990) is similar to the results of the present study. The high % of strains found in this study showing resistance to the commonly used antimicrobial agents could be due to their misuse in sublethal doses in medical and veterinary practice to prevent or treat infections and also due to their use in feeds to promote growth. Umoh et al (1990) attributed the high prevalence of resistance to sulphafurazole among other antimicrobial agents to the fact that this drug is commonly added

to drinking water in veterinary practice to prevent or treat infection in cattle. This is not surprising because outside of the hospital environment, the general population has easy access to various antibiotics at any drug store without prior prescription by a medical or veterinary practitioner. Bugler and Sherris (1968) have earlier shown that the effectiveness of any antibiotic decreases in the absence of a rational utilization policy, which unfortunately is virtually non-existent in Nigeria. (Kwaga and Adesiyun 1984).

Chemical analysis of kunun zaki for crude protein, carbohydrate and lipid revealed that it possessed lower nutrient content than those of the raw materials in this case, the sorghum and millet grains as reported by other workers (Olatunji et al, 1980; Hulse et al, 1980; Thekoronye and Ngoddy, 1985). Similar nutritional losses of maize, sorghum or millet in processing of ogi have been reported by several workers (Oke 1967; Akinrele 1970; Benigo and Muller 1972; Umoh and Fields 1981.

The proximate composition of kunun zaki obtained in the present study differ from previous report of Edward et al (1988), except for total solid, moisture content and pH which are similar. The percentage protein and fat for kunun zaki recorded in this study is similar to the report of Sopade and Kassum (1992), except for the percentage carbohydrate and ash which differ in both reports. Differences may partly be attributed to possible differences in hybrid of grains used, quantity of other raw materials and rate of contamination by impurities. In the present

study, no statistically significant difference in the proximate composition of the laboratory product and the commercial products was found except for lead (Pb) content which was significantly higher in the commercial products ($P < 0.05$ Anova). This may be attributed to the use of petrol in the grinding machine. Some of these petrol contain tetraethyl lead used as anti-knock agent. The significant variation in Na, K, Pb, titrable acidity, and PH of treated, untreated and commercial samples may be attributed to the preservatives used, since some of them are highly acidic and may contain the ions as impurities or component parts.

The progressive decrease in moisture of kunun zaki during the storage period could be attributed to evaporation to the head space and utilization by micro-organisms for growth. Variation obtained in carbohydrate composition could be attributed to the method of difference used in its determination. This could be because all other components of the medium other than crude protein, ash and lipid, such as organic acid, volatile components and other products of microbial and chemical degradation were regarded as carbohydrate. The increase in crude protein, total solid, relative decrease in pH may indicate increased microbial activity and spoilage in the sample (Okoli and Ezenwaka 1990). However, the increase in crude protein without detectable increase in microbial load as observed during the initial storage process could be attributed to increase in size without obvious increase in number of organisms. The relative decrease in lipid could be due to microbial utilization, rancidity caused by either moisture,

microbial activities or other chemical and biochemical changes in the product (Brian and Allan, 1980).

CHAPTER 6

CONCLUSION AND RECOMMENDATION

The high total aerobic plate counts, and the isolation of *E. coli* are indications of poor sanitation by the producers and contamination from the environment, while the occurrence of ó and ß haemolytic *S. aureus*, enterotoxigenic *B. cereus* and *Salmonella* spp in "kunun zaki" point to the danger of food-borne infection that may result from kunun zaki consumption. This is more so judging from the rich nutritional environment and the storage temperature suitable for growth of these organisms in kunun zaki. It is suggested here that proper education of producers will aid towards the production of kunun zaki of better hygienic standard.

Packaging materials and methods appear to contribute to microbial contamination. In the light of the findings of this study, it is advocated that bulk packaging be discouraged considering the high risk of contamination associated with this packaging method. Cellophane packaging should equally be discouraged considering possible mishandling by retail stores and kunu producers. It is also likely that toxic chemicals used in cellophane treatment such as organic peroxides, esters of phosphoric, ricinoleic and phthalic acids, antioxidants, organo-tin-salts or calcium salts of fatty acids and microbiocides can leach into kunun zaki and which can be dangerous if consumed over a period of time. Bottle packaging should be encouraged with education of producers on adequate cleaning and disinfection of bottles before use and hygienic bottling methods.

Of interest was the finding that untreated kunun zaki could keep well for 2 weeks. This confirms the advantage of good sanitation and low initial number of microorganisms that could cause spoilage. The good keeping quality of sodium benzoate treated sample for up to 24 weeks at ambient temperature is an indication that this work could form the basis for a pilot production of bottled kunun zaki. It is observed that the elimination of the raw portion of wet milled raw materials used for pitching which encourages microbial contamination and spoilage helped to enhanced the keeping quality of the lab products. However, the elimination of this step in kunun zaki production resulted in kunun zaki without the required sour taste. It is proposed that a starter culture be introduced in the controlled fermentation of the product to impart the needed sour taste. The consumption and industrial production of kunun zaki is advocated because it is cheaper to produce due to availability of raw materials. This will help in conserving foreign exchange and creating job opportunities.

The fact that higher resistance to common used antibiotics were recorded in this study than was recorded by earlier reports, may point to the increased misuse and abuse of these drugs over the year. It is therefore of great concern since these drugs are used frequently in both medical and veterinary practices. There is therefore the need for a stricter control possibly by restricting the prescription and dispensing of antibiotics and other drugs to only qualified professionals. Education of the public on the

dangers of indiscriminate purchase and use of drugs especially antibiotics is also imperative in the control of drug misuse.

Nutritional losses in preserved/stored kunun zaki recorded in this study and in similar previous studies call for fortifying kunun zaki with nutrients from rich natural or artificial sources in order to raise its nutritive value either to the level of that of its primary raw materials or even higher.

Further work should be carried out on the fungal, protozoal and viral safety of kunun zaki since only the bacterial safety was determined in this study. Efforts should also be directed at fortification of kunun zaki and possibly at extending the shelf life of kunun zaki for a longer period since only 24 weeks was monitored in this study.

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APPENDIX I

Four Point Descriptive Scale for Sensory Scoring

Name..... Sample Code..... Date.....

Please taste each sample and mark (x) against the one that best describes the attribute of the kunun zaki.

Rinse your mouth with water provided after testing each sample. Answer the questions that follow too.

1. Colour

   4 - Milky white
   3 - Milky with brownish ting
   2 - Light brown
   1 - Brown

2. Odour

   4 - Odourless
   3 - Neither odourless nor foul smelling
   2 - Foul smelling
   1 - Rotten smell

3. Texture

   4 - Very smooth
   3 - Slightly smooth
   2 - Slightly coarse
   1 - Very coarse

4. Sourness

   4 - Moderately sour
   3 - Slightly soar
   2 - Not sour
   1 - Very soar

5. Flavour

   4 - Slightly spicy
   3 - Moderately spicy
   2 - Very spicy
   1 - Not spicy

6. Sweetness

   4 - Moderately sweet
   3 - Slightly sweet
   2 - Not sweet
   1 - Bitter

7. Overall Acceptance

   4 - Very good
   3 - Good
   2 - Poor
   1 - Very poor

A. Which sample(s) best meets your taste of a good kunun zaki?

B. Which sample(s) least meets your taste of a good kunun zaki?

APPENDIX II

Results of four Point Descriptive Tests for Sensory Attributes of Untreated, Treated and Commercial "Kunun Zaki"

Sample	Sensory Attributes and Mean Scores						
	Colour	Overall	Sweetness	Odour	Texture	Sourness	Flavour acceptance
Untreated							
(control)	3.50	2.28	3.52	2.36	3.10	2.68	2.51
Acetic Acid (AA)			3.50	3.30	3.51	2.34	3.40	2.64	2.67
Benzoic Acid (BA)	3.49		3.30	3.53	2.32	3.41	2.60	2.68	
Sodium benzoate (SB)			3.50	3.31	3.50	2.31	3.42	2.64	2.68
Sodium citrate (SC)	3.50		3.29	3.54	2.35	3.40	2.60	2.70	
Citric Acid (CA)			1.43	3.06	3.55	1.22	1.74	2.67	1.35
CA + SB	1.55		3.31	3.51	1.30	1.78	2.67	1.78	
CA + SC	1.53		3.22	3.53	1.51	1.81	2.66	1.80	
CA + SB + SC	2.51		3.25	3.48	2.28	3.38	2.60	2.65	
SB + SC	3.50		3.33	3.48	2.35	3.41	2.68	2.70	
2 Weeks							
Untreated	2.81		2.24	3.61	2.50	2.36	2.63	2.21	
Acetic Acid (AA)			3.48	3.30	3.53	2.34	3.42	2.50	2.71
Benzoic Acid (BA)	3.49		3.29	3.53	2.31	3.22	2.66	2.80	
Sodium benzoate (SB)			3.51	3.30	3.51	2.30	3.44	2.60	2.69
Sodium citrate (SC)	3.44		3.34	3.55	2.32	3.28	2.53	2.57	
Citric Acid (CA)			1.20	2.82	3.62	1.16	1.60	2.64	1.30
CA + SB	1.56		3.30	3.50	1.30	1.79	2.68	1.77	
CA + SC	1.51		3.22	3.55	1.49	1.62	2.61	1.56	
CA + SB + SC	2.51		3.28	3.50	2.26	3.14	2.42	2.40	
SB + SC	3.50		3.33	3.50	2.38	3.44	2.71	2.66	
4 Weeks							
Untreated	2.50		2.13	3.63	2.53	2.30	2.70	2.01	
Acetic Acid (AA)			3.42	3.28	3.55	2.32	3.40	3.21	2.75
Benzoic Acid (BA)2	3.42		3.25	3.60	2.33	3.18	3.22	2.80	
Sodium benzoate (B)	3.49		3.50	3.53	2.30	3.42	3.11	3.41	
Sodium citrate (SC)	3.42		3.41	3.61	2.34	3.21	3.33	2.72	
Citric Acid (CA)			1.20	2.60	3.63	1.15	1.50	2.94	1.21
CA + SB	1.45		3.33	3.54	1.30	1.80	2.69	1.52	
CA + SC	1.48		3.10	3.60	1.48	1.54	3.37	1.50	
CA + SB + SC	2.52		3.44	3.61	2.25	3.30	3.38	2.42	
SB + SC	3.53		3.52	3.53	2.38	3.46	3.16	3.00	

Sample	Zero Hour							
	Sensory Attributes and Mean Scores							
	Colour	Overall	Sweetness	Odour	Texture	Sourness	Flavour	acceptance
	16 Weeks							
Untreated	2.10	1.88	3.65	2.57	2.20	2.40	1.22	
Acetic Acid (AA)		3.00	2.86	3.66	2.50	3.00	2.20	2.21
Benzoic Acid (BA)	3.00	3.32	3.71	2.51	2.94	2.18	2.23	
Sodium benzoate (SB)		3.48	3.47	3.73	2.32	3.29	2.50	2.52
Sodium citrate (SC)	3.01	3.08	3.74	2.64	3.00	2.14	2.21	
Citric Acid (CA)		1.00	2.24	3.70	1.06	1.48	2.12	1.04
CA + SB	1.34	3.00	3.69	1.08	1.68	2.20	1.21	
CA + SC	1.30	2.57	3.64	1.11	1.54	2.15	1.21	
CA + SB + SC	2.41	3.16	3.67	2.57	3.13	2.18	2.40	
SB + SC	2.35	3.42	3.74	2.23	3.41	2.19	2.50	
	24 Weeks							
Untreated	1.50	1.70	3.70	2.69	1.88	2.09	1.02	
Acetic Acid (AA)		2.80	2.30	3.83	2.60	2.10	2.00	2.12
Benzoic Acid (BA)	2.81	3.21	3.80	2.60	2.03	2.00	2.10	
Sodium benzoate (SB)		3.46	3.46	3.84	2.34	3.37	2.32	2.48
Sodium citrate (SC)	2.47	3.01	3.81	2.92	2.87	2.01	2.11	
Citric Acid (CA)		1.00	2.14	3.84	1.00	1.32	2.01	1.00
CA + SB	1.16	2.90	3.74	1.00	1.60	2.11	1.05	
CA + SC	1.13	2.15	3.74	1.00	1.54	2.04	1.00	
CA + SB + SC	2.22	3.09	3.84	2.83	3.12	2.10	2.17	
SB + SC	3.16	3.38	3.83	2.26	3.39	2.13	2.47	

APPENDIX III

Key to Correlation Analysis

Column	1	Microbial Load
Column	2	Percentage Crude Protein
Column	3	Percentage Lipid
Column	4	Percentage Carbohydrate
Column	5	Percentage Moisture
Column	6	Ash %
Column	7	Total Solid %
Column	8	(ppm) Sodium (Na)
Column	9	(ppm) Potasium (k)
Column	10	(ppm) Lead (Pb)
Column	11	Titrable acid
Column	12	PH

APPENDIX III

Correlation analysis of proximate Composition and Microbial load of Laboratory prepared kunun zaki

Acetic Acid (AA) treated sample

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Column 1	1											
Column 2	0.271877	1										
Column 3	-0.81129	-0.48555	1									
Column 4	0.544989	0.314777	-0.79207	1								
Column 5	-0.27659	-0.92296	0.560649	-0.60131	1							
Column 6	0.03856	-0.60283	0.450605	-0.58557	0.767594	1						
Column 7	0.281217	0.92191	-0.56308	0.604254	-0.99989	-0.7655	1					
Column 8	0.260115	0.935986	-0.54109	0.567292	-0.99905	-0.75365	0.998831	1				
Column 9	-0.43924	-0.78679	0.746093	-0.82259	0.945767	0.78872	-0.94672	-0.93227	1			
Column 10	6.39E-17	-1.3E-16	3.65E-16	6.56E-15	1.32E-14	-1.5E-15	-1.6E-15	1.3E-16	-4.8E-16	1		
Column 11	0.00112	0.899533	-0.35159	0.42576	-0.94146	-0.72291	0.940728	0.946374	-0.82534	-5E-16	1	
Column 12	-0.27734	-0.9315	0.557764	-0.58566	0.999682	0.763514	-0.99942	-0.99964	0.940235	3.71E-16	-0.94126	1

Benzoic acid (BA) treated sample

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Column 1	1											
Column 2	0.191478	1										
Column 3	-0.75432	-0.13021	1									
Column 4	0.259421	0.328305	-0.28074	1								
Column 5	-0.25471	-0.91034	0.271555	-0.6694	1							
Column 6	-0.46034	-0.34531	0.815687	-0.47503	0.52756	1						
Column 7	0.272556	0.90903	-0.28639	0.675646	-0.99955	-0.53132	1					
Column 8	0.159288	0.939639	-0.18099	0.582584	-0.9858	-0.50065	0.982063	1				
Column 9	-0.327	-0.89056	0.475131	-0.41088	0.90962	0.689207	-0.90723	-0.91549	1			
Column 10	0	0	9.77E-15	-6.3E-15	1.41E-15	1.74E-16	1.67E-15	-1.4E-16	-7.4E-17	1		
Column 11	0.358769	0.792717	-0.61461	0.495763	-0.84951	-0.77131	0.856518	0.827045	-0.91832	-8.2E-16	1	
Column 12	-0.4734	-0.8671	0.527206	-0.59279	0.922489	0.663949	-0.93143	-0.89049	0.90462	1.56E-15	-0.95246	1

Sodium benzoate (SB) treated sample

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Column 1	1											
Column 2	0.339063	1										
Column 3	-0.69077	-0.51506	1									
Column 4	0.274794	0.203313	-0.79889	1								
Column 5	-0.41178	-0.93599	0.694878	-0.47041	1							
Column 6	0.090632	-0.15566	-0.10965	0.42073	0.00702	1						
Column 7	0.38797	0.937031	-0.68933	0.465128	-0.99921	-0.02931	1					
Column 8	0.328361	0.97228	-0.48986	0.186557	-0.95389	-0.1527	0.956106	1				
Column 9	-0.34816	-0.9717	0.495562	-0.17786	0.951051	0.13967	-0.95296	-0.99906	1			
Column 10	0	4.47E-16	1.19E-14	1.74E-15	-5.9E-15	-4.7E-16	4.65E-16	-9.7E-17	-2.4E-16	1		
Column 11	-0.04418	-0.82805	0.004778	0.250718	0.701392	0.216556	-0.70434	-0.87155	0.858713	3.1E-16	1	
Column 12	-0.5231	-0.00312	0.604228	-0.25269	0.049392	0.840353	-0.05798	0.045702	-0.02819	1.62E-15	-0.39198	1

Sodium Citrate (SC) treated sample

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Column 1	1											
Column 2	0.437974	1										
Column 3	-0.63816	-0.91961	1									
Column 4	0.27064	0.090752	-0.23589	1								
Column 5	-0.38513	-0.78606	0.681537	-0.53555	1							
Column 6	-0.57959	-0.21622	0.446174	0.179314	-0.03475	1						
Column 7	0.320364	0.749909	-0.62639	0.55264	-0.99438	0.095552	1					
Column 8	0.256307	0.827278	-0.61373	0.160417	-0.91138	-0.00664	0.909857	1				
Column 9	-0.33408	-0.79622	0.654966	-0.4784	0.995427	-0.09108	-0.99252	-0.93525	1			
Column 10	0.278064	0.841684	-0.6331	0.148986	-0.90853	-0.02785	0.904963	0.999547	-0.93176	1		
Column 11	-0.28771	-0.42753	0.358727	-0.71196	0.806081	-0.35598	-0.79533	-0.58058	0.799531	-0.568	1	
Column 12	0.592195	-0.38447	0.173258	0.425685	0.041768	-0.23926	-0.05981	-0.24279	0.099182	-0.25193	-0.21596	1

Appendix III cont.

Citric acid (CA) treated sample

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Column 1	1											
Column 2	0.648564	1										
Column 3	-0.85177	-0.6283	1									
Column 4	0.671298	0.824021	-0.88792	1								
Column 5	-0.30822	-0.83411	0.561161	-0.83472	1							
Column 6	-0.68238	-0.47731	0.870927	-0.83352	0.424332	1						
Column 7	0.306009	0.833625	-0.55919	0.834214	-0.99998	-0.4236	1					
Column 8	0.072281	0.755068	-0.30836	0.651351	-0.94978	-0.18289	0.949826	1				
Column 9	-0.13696	-0.65187	0.438272	-0.67048	0.911573	0.199188	-0.91207	-0.88916	1			
Column 10	0.25454	0.815232	-0.5205	0.806566	-0.99403	-0.37813	0.994077	0.966884	-0.9331	1		
Column 11	-0.44251	-0.40642	0.570929	-0.5377	0.55474	0.361069	-0.55418	-0.35246	0.504568	-0.47973	1	
Column 12	-0.58582	-0.42562	0.885134	-0.7617	0.579448	0.658207	-0.57746	-0.3671	0.621233	-0.56264	0.629409	1

CA + SB treated sample

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Column 1	1											
Column 2	0.323013	1										
Column 3	0.109235	0.245593	1									
Column 4	0.45604	0.684954	-0.29235	1								
Column 5	-0.31766	-0.72461	0.275699	-0.94165	1							
Column 6	-0.37248	0.055814	-0.68344	0.189893	-0.37235	1						
Column 7	0.331692	0.725488	-0.27236	0.947218	-0.99981	0.357707	1					
Column 8	0.509241	0.825152	-0.15487	0.939087	-0.9631	0.232503	0.965855	1				
Column 9	-0.36684	-0.71447	-0.02435	-0.84713	0.91823	-0.05259	-0.92023	-0.89887	1			
Column 10	0.30285	0.722209	-0.27347	0.93817	-0.99979	0.38082	0.999321	0.958926	-0.91723	1		
Column 11	-0.33502	-0.76427	-0.41002	-0.72573	0.708969	0.242981	-0.71438	-0.73552	0.840074	-0.71109	1	
Column 12	-0.38509	-0.66885	0.325844	-0.95331	0.992662	-0.34179	-0.99391	-0.95977	0.913593	-0.9908	0.674947	1

CA + SC treated sample

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Column 1	1											
Column 2	0.585244	1										
Column 3	-0.66186	-0.7114	1									
Column 4	0.478459	0.848632	-0.84297	1								
Column 5	-0.29593	-0.7357	0.624638	-0.93867	1							
Column 6	-0.14532	0.295045	-0.08211	0.534135	-0.77074	1						
Column 7	0.298563	0.734986	-0.62574	0.938825	-0.99999	0.770357	1					
Column 8	0.636213	0.866972	-0.53725	0.502401	-0.30238	-0.15461	0.301535	1				
Column 9	-0.64301	-0.79867	0.78394	-0.93394	0.88777	-0.44277	-0.88888	-0.48897	1			
Column 10	0.253949	0.696818	-0.57318	0.912126	-0.99748	0.807938	0.997457	0.250256	-0.86498	1		
Column 11	0.077125	-0.69049	0.462935	-0.68876	0.661725	-0.33593	-0.65914	-0.46461	0.528119	-0.64294	1	
Column 12	-0.3394	-0.79432	0.587339	-0.92444	0.98696	-0.7737	-0.9858	-0.39598	0.878693	-0.9835	0.64279	1

CA + SB + SC treated sample

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Column 1	1											
Column 2	0.443976	1										
Column 3	0.177184	-0.14462	1									
Column 4	-0.17	-0.86511	0.250843	1								
Column 5	-0.12241	-0.58737	-0.06535	0.55863	1							
Column 6	0.083759	0.371907	0.062499	-0.47459	-0.15357	1						
Column 7	0.353607	0.96778	-0.18302	-0.91595	-0.73507	0.390323	1					
Column 8	0.390893	0.961204	-0.20354	-0.88731	-0.72496	0.462623	0.991006	1				
Column 9	-0.07197	-0.81823	0.412056	0.952589	0.629771	-0.2442	-0.89752	-0.85556	1			
Column 10	0.293217	0.951867	-0.25482	-0.96109	-0.67538	0.413071	0.989428	0.974724	-0.94084	1		
Column 11	-0.41292	-0.72459	-0.32449	0.594413	0.86187	-0.05488	-0.77763	-0.73918	0.600696	-0.70825	1	
Column 12	-0.12933	-0.5447	0.442513	0.741986	-0.08295	-0.62428	-0.51794	-0.52787	0.613501	-0.61628	-0.04268	1

Appendix III Cont.

SB + SC treated sample

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Column 1	1											
Column 2	0.509613	1										
Column 3	-0.64392	-0.6095	1									
Column 4	-0.22024	-0.82281	0.297548	1								
Column 5	-0.1941	-0.75472	0.292188	0.477355	1							
Column 6	0.209418	-0.31291	0.289338	0.27738	0.353992	1						
Column 7	0.254317	0.710121	-0.61793	-0.28848	-0.85963	-0.36647	1					
Column 8	0.303401	0.959965	-0.56	-0.85217	-0.79812	-0.40788	0.74593	1				
Column 9	-0.31558	-0.95842	0.586905	0.858276	0.771776	0.405467	-0.73454	-0.99878	1			
Column 10	0.136461	0.888782	-0.45275	-0.90857	-0.59333	-0.38783	0.563652	0.934918	-0.93919	1		
Column 11	-0.07279	-0.6787	-0.0579	0.695762	0.83512	0.139395	-0.48099	-0.73822	0.711998	-0.62318	1	
Column 12	-0.89859	-0.62766	0.788807	0.402581	0.114974	0.013925	-0.27286	-0.44038	0.463204	-0.39102	-0.02411	1

Control sample

	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7	Column 8	Column 9	Column 10	Column 11	Column 12
Column 1	1											
Column 2	0.535713	1										
Column 3	-0.78633	-0.77443	1									
Column 4	-0.29581	-0.89583	0.645911	1								
Column 5	-0.32978	-0.80008	0.424775	0.527587	1							
Column 6	-0.45038	-0.92679	0.683678	0.707811	0.935774	1						
Column 7	0.144219	0.513002	-0.13873	-0.17687	-0.9223	-0.75745	1					
Column 8	-0.04013	-0.52617	0.433367	0.597747	0.255092	0.389277	-0.08134	1				
Column 9	0.110547	-0.27683	0.095688	0.134122	0.425897	0.364326	-0.49659	0.759462	1			
Column 10	0	0	0	1.24E-16	-1.9E-14	-1.2E-16	-6.4E-16	-5.9E-14	4.92E-13	1		
Column 11	-0.22513	-0.83926	0.372663	0.67314	0.948829	0.910433	-0.81808	0.276618	0.327355	3.8E-16	1	
Column 12	-0.25242	-0.1634	0.201477	-0.25334	0.594095	0.496496	-0.76871	-0.17021	0.329338	-1.9E-15	0.367071	1

APPENDIX IV

Proximate Composition of Untreated (control) and Treated "Kunun Zaki" During 24 Weeks Storage

Time (Week)	% crude protein	% Lipid	% carbo-hydrate	% Moisture	% Ash	% Total solid	Na (PPM)	K (PPM)	Pb (PPM)	Titrable acid	pH
Control Sample											
0	0.363	0.770	8.160	93.55	0.160	6.450	76.88	187.5	0.100	0.230	4.800
1	1.550	0.720	5.700	92.68	0.080	7.320	76.85	187.5	0.100	0.110	4.770
2	1.031	0.768	8.020	92.55	0.080	7.900	76.85	187.4	0.100	0.130	3.900
4	1.612	0.580	8.770	92.70	0.070	7.300	76.50	187.4	0.100	0.140	4.500
8	1.612	0.510	5.740	92.80	0.070	7.200	76.50	187.4	0.100	0.130	4.550
16	1.800	0.450	7.950	92.64	0.070	7.360	76.54	187.4	0.100	0.130	4.540
24	1.813	0.300	6.080	92.61	0.050	7.360	76.85	187.5	0.100	0.130	4.150
Acetic acid (AA) Treated Samples											
1	1.131	0.750	8.140	89.72	0.150	10.280	435.0	140.0	0.100	0.350	3.840
2	0.825	0.700	8.810	89.53	0.140	10.470	435.0	115.0	0.100	0.340	3.820
4	1.031	0.670	8.470	89.63	0.140	10.310	434.8	125.0	0.100	0.340	3.821
8	0.940	0.600	8.660	89.62	0.135	10.380	434.0	117.0	0.100	0.350	3.830
16	1.006	0.570	8.660	89.62	0.140	10.380	434.9	116.0	0.100	0.310	3.820
24	1.013	0.520	8.860	89.54	0.150	10.460	434.0	110.0	0.100	3.330	3.810
Benzoic acid (BA) Treated Samples											
1	0.925	0.770	9.110	89.07	0.130	10.93	435.0	130.0	0.100	0.260	0.980
2	1.238	0.776	8.330	89.57	0.160	10.49	435.0	120.0	0.100	0.260	3.880
4	1.031	0.762	8.460	89.63	0.100	10.31	433.0	100.0	0.100	0.267	3.900
8	1.000	0.761	8.560	89.55	0.130	10.45	390.0	110.0	0.100	0.270	3.890
16	1.001	0.758	8.640	89.45	0.124	10.55	398.0	110.0	0.100	0.260	3.860
24	1.019	0.750	8.730	89.50	0.100	10.80	390.0	110.0	0.100	0.280	3.510
Sodium benzoate (SB) Treated Samples											
1	0.775	0.768	7.750	70.41	0.150	9.59	525.0	115.0	0.100	0.150	4.870
2	-	-	-	-	-	-	-	-	-	-	-
4	0.925	0.760	8.780	89.51	0.170	10.49	523.0	115.0	0.100	0.170	4.910
8	0.840	0.760	8.770	89.48	0.150	10.52	524.0	118.0	0.100	0.170	4.900
16	0.856	0.757	8.620	89.48	0.168	10.36	524.0	115.0	0.100	0.170	4.890
24	0.869	0.750	8.730	89.46	0.150	10.54	520.0	115.0	0.100	0.190	4.440
Sodium citrate (SC) Treated Samples											
1	0.925	0.700	6.880	91.15	0.140	8.850	640.0	140.0	2.200	0.230	2.460
2	0.619	0.764	9.070	89.53	0.220	10.670	630.0	115.0	2.200	0.160	4.880
4	0.081	0.650	8.660	89.36	0.250	10.620	645.0	110.0	2.200	0.160	4.430
8	1.023	0.630	8.840	89.36	0.120	10.640	640.0	115.0	2.200	0.190	4.490
16	1.044	0.620	8.520	89.34	0.106	10.290	640.0	114.0	2.200	0.160	4.890
24	1.049	0.610	8.960	89.34	0.120	10.660	640.0	115.0	0.190	0.190	4.860

Appendix IV cont.

Time (Week)	% crude protein	% lipid	% carbo-hydrate	% Moisture	% Ash	% Total solid	Na (PPM)	K (PPM)	Pb (PPM)	Titrable acid	pH
Citric acid (CA) Treated Samples											
1	1.544	0.720	9.070	88.51	0.160	11.490	510.0	125.0	10.000	0.210	2.400
2	1.650	0.680	9.310	88.83	0.150	11.170	525.0	125.0	10.400	0.260	4.310
4	1.156	0.610	9.130	88.94	0.160	11.060	450.0	100.0	9.980	0.207	2.460
8	1.069	0.510	9.620	88.66	0.130	11.330	450.0	125.0	10.000	0.220	2.410
16	1.781	0.450	9.540	88.68	0.144	11.290	465.0	125.0	10.100	0.220	2.400
24	1.968	0.340	10.150	88.43	0.120	11.570	390.0	125.0	10.100	0.180	2.220
CA + SR Treated Samples											
1	1.238	0.767	9.140	88.68	0.180	11.320	570.0	125.0	13.40	0.200	2.620
2	0.618	0.660	9.190	88.75	0.200	11.250	465.0	140.0	13.20	0.240	2.400
4	0.788	0.761	9.010	88.77	0.170	11.238	465.0	105.0	13.20	0.200	2.500
8	0.809	0.757	9.210	88.75	0.180	11.270	465.0	125.0	13.40	0.190	2.580
16	0.856	0.754	9.130	88.73	0.176	11.300	570.0	120.0	13.20	0.210	2.390
24	0.916	0.750	9.480	88.70	0.150	11.400	570.0	115.0	13.20	0.190	2.330
CA + SC Treated Samples											
1	1.031	0.710	9.110	88.96	0.190	11.040	599.0	130.0	30.50	0.180	2.760
2	1.031	0.764	8.970	89.03	0.210	10.970	600.0	145.0	30.60	0.226	2.500
4	1.081	0.650	9.250	88.87	0.200	11.130	600.0	145.0	30.60	0.190	2.700
8	1.086	0.630	9.380	88.79	0.190	11.210	600.0	130.0	30.50	0.190	2.700
16	1.031	0.620	9.300	88.87	0.190	11.150	601.0	115.0	30.40	0.240	2.680
24	1.802	0.600	9.530	88.71	0.180	11.290	599.5	115.0	30.10	0.180	2.350
CA + SR + SC Treated Samples											
1	0.825	0.769	7.170	88.05	0.190	8.950	600.0	125.0	26.00	0.140	4.660
2	0.835	0.670	7.050	91.31	0.190	8.690	555.0	115.0	26.40	0.220	2.600
4	0.825	0.763	6.850	91.37	0.200	8.630	480.0	115.0	26.20	0.180	2.700
8	0.849	0.761	7.040	91.21	0.240	8.790	595.0	135.0	26.00	0.200	2.620
16	0.868	0.760	7.050	91.00	0.264	8.800	598.0	134.0	26.00	0.180	2.650
24	0.947	0.760	7.240	91.05	0.160	8.900	595.0	134.0	26.00	0.160	2.840
CA + SC Treated Samples											
1	0.825	0.770	5.610	91.65	0.150	7.350	540.0	65.00	23.50	0.110	4.660
2	0.825	0.765	6.870	91.39	0.150	8.610	540.0	65.00	23.70	0.160	4.640
4	0.818	0.765	5.450	92.80	0.150	7.200	536.0	60.00	32.20	0.190	4.350
8	0.742	0.762	6.350	92.01	0.140	8.000	540.0	60.00	22.50	0.180	4.620
16	0.875	0.761	6.120	92.14	0.168	7.830	535.8	60.00	22.20	0.170	3.960
24	0.892	0.760	6.310	92.14	0.140	7.860	525.0	65.00	22.50	0.200	3.840