STUDIES ON THE MICROBIOLOGY OF 'NMADA'
A MALTED MAIZE BEVERAGE
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SUMMARY

The main ingredients used in the production of Nmada were examined to test their microbiological quality and to determine the source of microbial load in the finished product.

The moulds found on the raw and malted maize grains were Penicillium spp, Aspergillus spp. and Rhizopus spp. Bacterial types isolated include Bacillus spp., a few Gram-positive cocci and Gram-negative rods. Six out of the total number of 10 maize malt samples had total bacterial counts over 100,000 per g. The remaining 4 samples had counts under 50,000 per g. Total bacterial counts for sugar samples ranged from 12 to 46 per 10g. Salt samples had counts of 1,200 to 2,300 per 10g.

Two samples of malt flour prepared in the laboratory showed a better microbiological quality as compared to thesample from the two producers. Total bacterial counts were 510 per g and 720 per g respectively. Yeast and moulds counts were of the order 1,260 per g and 1,460 per g. Analysis of fresh samples of 'Nmada' made from maize malt prepared in the laboratory gave no counts for both bacteria and yeasts and moulds. The fresh samples of 'Nmada' from the two producers had totabl bacterial counts ranging from 80 to 860 per ml and yeast and moulds counts of 5 to 780 per millilitre. Five major groups of organisms which were identified as Bacillus spp. were found to dominate the microflora of the spoilt beverage. It was also observed that using a malt with low microbial load resulted in a beverage of better microbiological quality.

INTRODUCTION

'Nmada' is the Ga-Adangme name for the traditional maize beverage prepared and consumed by the people of the Greater Accra Region in the Southern part of Ghana. Other names for the beverage in the area are 'Ahei' (Fanti, Central Region) and 'Liha' (Ewe, Volta Region) Although Whitby (1968) described it as a fermented beverage it can more appropriately be described as a cooked extract of maize malt prepared by germination of maize. It is non-alhoholic but rich in the B vitamins particularly ribflavin. The beverage is normally served as a refreshing drink at traditional ceremonies such as out-doorings and funerals.

Whitby (1968) in a report on a Nutritional Survey of Ghana recommended the consumption of 'Nmada' because of its importance as a supplemental source of riboflavin.

In spite of the importance of this beverage, Halm (1976) as a result of a special survey indicated that little or no work had been done on its production, microbiology and nutritional value. Christian (1966) described in detail the traditional method of producing pito, a popular West African fermented alcoholic beverage but very little was recorded on 'Nmada'. The only work carried out to some depth was that of Laryea-Brown (1975) who described the traditional method of 'Ahei' the Fanti version of 'Nmada'. He also studied the biochemical changes that take place during steeping and malting of the maize grains over a 5 day period but this work did not include the microbiological aspects.

A survey carried out by Halm and Plahar (1976) to determine the traditional processing proceedures indicated that the beverage has a short storage life of 3 to 6 days and that production is normally carried under poorly controlled unhygienic conditions. The chances of microbial contamination are therefore considerable and this reflects in the storage potential of the product.

The production and distribution of a stable bottled product under standardized conditions will offer a unique industrial opportunity to the consumer. In order to make this possible, there is need to carry out investigations into the production technology, microbiology and nutritional value of 'Nmada'.

This study is set up to investigate the nature and production of 'Nmada' and to determine the spoilage pattern of the beverage as well as the source of spoilage organisms. The study is approached through examination of the microflora of the production ingredients ie. maize malt, salt and sugar and of comparative microbiological analysis of the finished product - both traditional and laboratory type.

MATERIALS AND METHODS

Maize grains, maize malt, salt, sugar and already prepared 'Nmada' were obtained from traditional brewers in Accra.

MEDIA: The following media were used for the isolation and enumeration of micro-organisms from the samples. Their composition is expressed as %, W/V. All agar media were solidified with 2% Agar.

TOTAL PLATE COUNT AGAR: - Tryptone, 0.5; yeast extract; 0.25; glucose 0.1 distilled water, pH 7.0

OVER BROTH MEDIUM:- 500g of finely ground beef Liver were added to 1 litre distilled water and boiled for 1 hour. The pH was adjusted to 7.0 and boiling was continued for another 10 minutes. The mixture was pressed through cheese cloth to remove liver particles and the liquid was made up to one litre with distilled water. Ten grammes of peptone and 1g dipotasium phosphate were added and the solution adjusted to pH 7.0. About $\frac{1}{2}$ in of liver particles from pressed cake were put in the bottom of the tubes (5/8 x 6") and covered with the broth.

NUTRIENT AGAR:- Beef extract, 0.3; Peptone 0.5; distilled water, pH 6.8

DEXTROSE TRYPTONE AGAR:- Tryptone, 1.0; dextrose 0.5; distilled water pH 6.8.

DEXTROSE TRYPTONE BROMOCRESOL PURPLE/AGAR:- Tryptone, 1.0; dextrose, 0.5; bromocresol purple, 0.004; distilled water, pH 6.8

SULPHITE AGAR:- Tryptone, 1.0; Sodium sulphite, 0.1; distilled water.

AMOS AND KENT JONES MEDIUM: Peptone, 1.0; beef extract, 0.54, sodium chloride 0.9; distilled water pH 7.2

DESOXYCHOLATE LACTOSE AGAR:- Peptone 1.0 yeast extract 0.25 gelatin 3.0; d-mannitol 1.0; lactose , 0.2; sodium chloride 7.5; dipotassium phosphate 1.0; sodium acid selenite 0.4; distilled water pH 7.0

SELENITE BROTH:- Tryptone 0.5; lactose 0.4; disodium phosphate 1.0; sodium acid selenite 0.41; distilled water pH 7.0

BISMUTH SULPHITE AGAR: Beef extract , 0.5; peptone 1.0; dextrose 0.5; disodium phosphate 0.4; ferrous sulphate 0.03; bismuth sulphite indicator, 0.8; brilliant green, 0.0025; distilled water pH 7.7

NUTRIENT AGAR + 15% Na Cl:- Beef extract, 0.3 peptone 0.5; sodium chloride 15.0; distilled water.

MAIZE EXTRACT AGAR:- 50g germinating maize grains were ground. 250ml distilled water added and the mixture boiled for 30 minutes and filtered. The filtrate was made up to a litre with distilled water, pH 5.0

POTATO DEXTROSE AGAR: Infusion from white potatoes, 200ml; dextrose 2.0; distilled water, pH 3.5.

METHODS

The samples of raw maize grains, malted maize flour, sugar, salt and already prepared 'Nmada' were all treated as follows:-

(i) Raw Maize Grains

The micro-organisms present on the surface of the maize grains were isolated by placing three grains centrally on sterile media in petri dishes and in petri dishes lined with moistened sterile fiter paper, and incubating for 7 days at room temperature. Another set of grains were surface sterilised with 0.5% mercuric chloride and rinsed with sterile distilled water before plating.

(ii) Malted Maize Flour

From the representative sample taken out of the flour used in the production of the beverage the following determinations were made using methods as given in "Recommended Methods for the Microbiological Examination of Foods" (1960); total aerobic and anaerobic counts at 32°C using plate count Agar; aerobic total thermophilic counts at 55°C using plate Count Agar; anaerobic total thermophilic counts at 55°C using liver Broth Medium; total aerobic thermophilic spore count using Dextrose Tryptone Agar; anaerobic total spores producing hydrogen sulphite using Liver Broth Medium; "rope" spore count using Amos and Kent-Jones Medium; enumeration of coliforms using Deoxycholate Agar. Staphyloccus Medium No.110 was used for the enumeration of staphylococci. Salmonella determination was made by enrichment in 0.8% Tryptone Broth for 24 hours at 35°C and subsequent plating of Bismuth Sulfite Agar and incubating for 48 hours at 35°C.

(iii) S a l t

Ten gram of salt taken from the lot used in the preparation of the beverage was dissolved in 99 ml of Ringers Solution Serial dilutions were then prepared from the solution. Total counts were determined using Total Plate Count Agar and Nutrient Agar plus 15% sodium chloride. Yeasts and moulds were estimated using Potato Dextrose Agar.

(iv) Sugar:- One hundred grammes of sugar were weighed aseptically into a sterile container and diluted with 102.5ml sterile Ringers Solution (Each 2.1m containing 1.25g of sugar). 2.1ml were dispensed into each of 6 petri plates. 2 plates were poured with Total Plate Count Agar and 4 with Potato Dextrose Agar for yeasts and moulds.

(v.) Already Prepared 'Nmada' :- Serial dilutions of the product were prepared with Ringers solution and the Pour Plate Method used for counts of micro-organisms.

For the total viable bacterial counts, Total Plate Count Agar was used and for counts of yeasts and moulds Potato Dextrose Agar was used. After counting, a number of representative, colories were picked from the plates put into Nutrient Broth and incubated at 32°C for 2 days. They were then subcultured on Nutrient Agar Slopes and incubated for 24 hours.

Bacteriological Methods Used in Examining the Cultures

Microscopical Examination:- Organisms grown on Nutrient Agar were Gram-stained and examined microscopically. The size of vegetative rods and of spores was not usually measured. The position and shape of the spre, the thickness of the spore-wall and the shape of the sporangium were noted.

Macroscopical Examination:- The colonial appearance on Nutrient Agar and the nature of growth on Nutrient Agar, Glucose, Nutrient Agar and in Nutrient Broth were observed.

Physiological Reactions:- Strains were examined for the production of acid and gas from glucose, arabinose and manitol. The sugar medium used was a liquid medium in which the sole source of nitrogen was ammonia with 1.2% agar. This medium was inoculated with loopful of nutrient agar culture.

Hydrolysis of casein, gelatin and starch was determined by the Plate Method of Smith et al. (1946). The Urea Medium of Christensen (1946) containing phenol red indicator was used for the detection of urease.

The voges-Proskuver (VP) reaction (acetylmethyl-carbinol production) was determined by the method of Smith et al (1946) after incubation at 28°C for 2 and 5 days, except in the case of Bacillus coagulans when incubation was carried out at 45°C.

Reduction of nitrate to nitrite, utilization of citrate, and growth on Nutrient Agar at pH 6.0 were all examined. All strains were tested for production of gas from glucose under the semi-anaerobic conditions of the Gibson & Abdel-Malek (1945) test.

Test for lecithinases visibly affecting egg-yolk emulsion were made on Nutrient Agar Plates containing 10.0% (v/v) of egg-yolk emulsion (Macfarlane, Oakley & Anderson, 1947).

Determination of Alcohol Content of Nmada

Nmada samples of 100ml were neutralised by titrating against 0.1 N NaOH using phanolphthalein as indicator. The solution was distilled and 50ml collected. The specific gravity of the distillate was determined using the specific gravity bottle method. The corresponding percentage alcohol by volume in the distillate at room temperature was obtained from reference table in official methods of analysis by the Association of Official Agricultural Chemists (1960)

Determination of Lactic and Acetic Acids:- By titration with 0.1N sodium hydroxide and calculating the acidity as (lactic acid and using 1ml 0.1N = 0.0090g) lactic acid.

Acidity as acetic acid was determined by titrating 10ml of sample with 0.5N sodium hydroxide and calculating acidity using the formular.

Titre value (ml 0.5N) x 0.3 = % w/v as acetic acid.

Results

Traditional process for the production of Nmada

The traditional methods used in all three regions of Ghana where the beverage is produced, follow the main processing steps of malting of maize, mashing of malted maize, extraction and boiling of extract. Differences occur from one region to another and among individual producers in the processing steps. The method described here is that used by one section of the Ga tribe.

Maize grains are steeped in water for 24 hours after which they are removed and spread on a cement floor to germinate. A soaked sack is placed over the grains to prevent surface dessication. Water is sprinkled on the grains each morning for 4 to 5 days. By the end of this period the acrospire is about 6cm in length. The germinated grain is sundried on aluminium roofing sheets or on polythene sheets spread on the ground for 3 days to ensure thorough drying. The malted grains are milled coarsely and the flour obtained is mixed with cold water to form a thin slurry. Salt is added to the slurry and the mixture is boiled for 3 hours with constant stirring. The precipitate is allowed to settle and the liquid portion is decanted and cooked again for a further 2 hours. Sugar and caramel are added to this product which is called Nmada.

Examination of raw and malted maize

Raw and malted maize grains were examined for the types of microorganisms present on them. Pure cultures of the micro-organisms were obtained and organisms were identified as far as possible. The most predominant moulds were *Penicilluim spp.* *Aspergillus spp.* and *Rhizopus spp.* Of bacterial types *Bacillus sp.* a few Gram-positive cocci and Gram-negative rods were found on the grains. No micro-organisms were isolated from the grains surface sterilized by rinsing with 0.5% mercuric chloride and distilled water. All the organisms thus appear to have been on the grains and develop when conditions are favourable for their growth.

Examination of Malt Flour

The results of the determinations of malt flour are shown in Table 1. Of the 5 samples of Brand A only 1 had total bacterial count of less than 100,000 per gram. Brand B had 2 samples with counts of over 100,000 per gram.

TABLE I

MICROBIOLOGICAL EXAMINATION OF MALT FLOUR

PRODUCER	BACTERIA PER GRAM OF SAMPLE									
	TOTAL PLATE COUNT 32°C		THERMOPHILIC COUNT 55°C		THERMOPHILIC SPORE COUNT 55°C			ROPE SPORES	COLI FORMS	STAPHYLOCOCCI
	O_2	An O_2	O_2	An O_2	O_2	FLAT SOUR TYPE	An O_2 PRODUCING H_2S			
A1	110,000	10,000	20,000	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	29,000	10,000	22,000	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	275,000	9,500	1,060	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	176,000	180,000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	355,000	137,000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B 1	103,000	212,000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	375,000	127,000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
3	10,600	153,000	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	14,600	3,500	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
5	53,200	4,300	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
C 1	510	250	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2	720	380	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

TABLE 2

MICROBIOLOGICAL EXAMINATION OF FRESHLY MADE NMADA

PRODUCER	BACTERIA (PER ML)			PRESENCE OF SALMONELLA	YEAST AND MOULDS (Per ml)
	TOTAL COUNT	COLIFORM	STAPHYLOCOCCI		
A 1	760	0	0	-	600
2	104	0	0	-	100
3	103	0	0	-	780
4	580	0	0	-	300
5	860	0	0	-	80
B 1	710	0	0	-	3
2	830	0	0	-	6
3	135	0	0	-	5
4	80	0	0	-	20
5	600	0	0	-	15
C 1	0	0	0	-	0
2	0	0	0	-	0

The lowest counts were recorded for Brand C which had counts of 510 to 720 per gram.* Out of the 12 samples only 3 samples from Brand A had thermophillic aerobic counts at 55°C. Though the total counts were high for samples of Brands A and B they did not demonstrate the presence of any thermophillic spores, coliforms, staphylococci and salmonella. The results from Brand C indicated a higher microbiological quality as compared to A and B. Yeasts and moulds count for Brand C were 1,260 and 1,460 per gram respectively whilst only one sample from A and B had count of less than 10,000 per gram. Only one sample of Brand B had a count of 80 rope spores per gram.

Examination of Salt and Sugar

Total bacterial counts for sugar samples ranged from 12 to 46 per 10g of sugar and 29 to 46 yeasts and moulds per 10g. Salt samples had counts of 1,200 to 2,300 per 10g total bacteria and 230 to 300 yeasts and moulds per 10g.

Examination of already prepared 'Nmada'

The results of the microbiological analysis of Nmada from two producers in Accra are presented in Table 2. Brand A and B are the two producers from Accra. Brand C was produced in the laboratory from what can be described as standard maize malt. The total bacterial count for Brand A and B ranged from 80 to 860 per millilitre of Nmada. Of the 10 samples examined, all had total bacterial counts over 80 per millilitre but Brand C had no counts. None of the 12 samples examined had coliforms, staphylococci and salmonella. All 10 samples from Brand A and B had yeast and mould counts ranging from 5 to 780 per millilitre of nmada. Results for B are lower than that of A. All 5 samples of Brand A had yeast and mould count over 20 per millilitre. Brand C showed no counts for Yeast and moulds.

* Determination of alcohol and lactic and acetic acids gave negative results.
Brand C was standard maize malt prepared in the laboratory by steeping maize grains at 28-30°C for 12 to 24 hours followed by germination at the same temperature for 4 to 5 days with sprinkling of water twice daily.

All the samples of Nmada obtained from the producers were microbiologically wholesome. No pathogenic organisms were found and the bacteria types isolated were predominantly mesophilic Bacillus species. Even though the numbers were low ie. 80 to 860 total mesophilic bacteria per gram, under favourable conditions for their growth, these develop to high levels to bring about spoilage. Yeasts and mould counts of the order 2 per millilitre of beverage may not develop product deterioration but may be considered marginal in character. Higher numbers as found in the samples (5 to 780 yeast and mould per ml) is a mark of low quality and they tend to form sediments, discolouration and definite fa.vour changes in the product. Their elimination is principally a matter of proper specification and control of raw materials as well as the use of modern equipment under carefully controlled sanitary and manufacturing methods.

C O N C L U S I O N

'Nmada' is not a fermented beverage as previously reported since any evidence or sign of fermentation is regarded as spoilage by the producer. The microflora of the ingredients are mainly mesophiles but these do not initiate any fermentative action during the processing of nmada.

It is expected that under more asceptic and better controlled conditions of malting, the microbial load of the malt produced can be greatly reduced to give a beverage of a better microbial quality. This is because maize malt produced under controlled conditions in the laboratory, had a much lower microbial load than malt samples produced by the traditional processors.

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