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HAZARD ANALYSES CRITICAL CONTROL POINTS OF FOODS PREPARED BY FAMILIES IN ZARIA, NIGERIA.

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Food safety evaluation was conducted using Hazard Analysis Critical Control Point (HACCP) approach to investigate hazards associated with microbial contamination and critical control points (CCPS) in the preparation and handling of foods in five families in Zaria, Nigeria. Samples of food, water, swabs of food contact surfaces and animal droppings found in or near food preparation areas were collected and tested microbiologically. The formol-ether concentration technique was used for parasites. Enterotoxin production by *B.cereus* and *E.coli* strains was performed on New Zealand white rabbits using the ileal loop technique. All the foods (Tuwo, akamu, vegetable soup, eba) attained temperatures of 65°C – 100°C capable of destroying vegetative forms of food borne pathogens. However, 2-4$\log_{10}$ cells of *B.cereus*, 2-3$\log_{10}$cells of *S.aureus* and 2$\log_{10}$ cells of coliforms were isolated per gram/ml some of the cooked foods. The water samples for drinking, cooking and washing dishes were contaminated with coliforms below 2$\log_{10}$cell/ml. Out of 28 *B.cereus* and 14 *E.coli* strains tested for enterotoxin production, 16(57.1%) *B.cereus* and 3(21.4%) *E.coli* were toxigenic. Though the level of counts are within acceptable limit for food and water, the presence of enterotoxigenic strains of *B.cereus* and *E.coli* and the hazards such as inadequate cleaning of food utensils, high level initial contamination associated with raw foods, food ingredients, food contact surfaces and food handlers call for concern. HACCP is advocated to ensure good personal hygiene and environmental sanitation in order to obtain safe prepared foods.

Keywords: Food safety, Hazards, CCP, Contamination, Enterotoxin, Toxigenic.

INTRODUCTION

Several hazards are known to be associated with food and food ingredients. These include microbiological, nutritional, environmental, natural toxicants, pesticide residues and food additives. The most serious food safety problem however is microbiological hazards considered as unacceptable contamination, growth or survival of microorganism of concern to food safety or of unacceptable production or persistence in food of toxic microbial metabolites (WHO/ICMSF, 1982). The provision of microbiologically safe food demands extra care in preparation and handling practices because microorganisms are ubiquitous and some are harboured by man, animal and food materials. The task of safe food provision is made more difficult in situations of poor sanitary condition and polluted environment which are inherent in most developing countries such as ours. Interestingly, in industrialized countries, from 20 – 40% of the reported outbreaks of foodborne diseases have been traced to mishandling of foods in homes (Bryan, et al 1992). It is expected that this statistics is even higher in Nigeria and other developing countries. This is because of the prevalence in mishandling

of foods, poor storage practices, poor water quality and poor environmental sanitation (Ehiri, et al 2001).

Ensuring the microbiological safety of foods depends on minimizing the initial level of microbial contamination, preventing or limiting the rate of microbial growth or reducing the microbial population. To achieve this, the WHO (1991) suggested a combination of effective food safety infrastructures with an adequate educational programme based on a combination of information on the socio-cultural and economic situation, as well as the technical information relating to food preparation and food habits obtained through the application of hazard analysis critical control point (HACCP) system.

To evaluate food safety practices in Zaria, this study was initiated with the following objectives (1) to develop a better understanding of the microbiological problems associated with the different types of foods prepared in Zaria (2) to identify the critical control points (CCPS) of the different foods (3) measures that could ensure safety of foods were emphasized.

MATERIALS AND METHODS

Description of the region ,population and diet

Zaria represents one of the vital and important urban centres in Kaduna state in northern Nigeria. Zaria urban area covers a land mass of approximately 7542.6 HA, with a population of about 277,187 people. It is located between longitude 90° and 10° and latitude 70° and 50° North-East (ABU, 1975 a, b). Zaria lies on the regional plains of height ranging from 550 and 700 meters. Farming both for subsistence and exchange forms the basis of city's economy. Some of the people are engaged in white collar jobs while others are either fulltime traders, bus drivers or craftsmen (ABU, 1975 a, b). Zaria is provided with primary health care centres with some bore holes and pipe borne water. Wells however, remain the major source of water in most localities.

In general, the diet of persons who live in Zaria consists of a breakfast of akamu (maize, millet or sorghum gruel) and akara/moimoi (Bean cake) or bread and tea. Lunch usually consists of boiled yam/potatoes, rice beans or combinations of these served with stew or prepared as porridge or jollof. Supper often consists of 'tuwo' (corn meal; sorghum meal; yam flour meal (amala); cassava flour meal (lafu) or eba made from garri, a cassava product. The diet is high in carbohydrate and pulses (bean and soybean) which provide the major protein source.

SELECTION AND DESCRIPTION OF FAMILIES SURVEYED

Prior to the hazard analysis was a survey study of Zaria local government area to assess presence of social facilities (hospitals, pipe borne water, good road, market), and family structures.

The entire study area was divided into four clusters namely (1) Zaria walled city (2) Tundun Wada (3) Gaskiya and (4) Wusasa. From the clusters, five families at least one from each cluster were selected based on interest and willingness to participate in the exercise. Consent of the family heads were sought and obtained before commencement of the work.

Family 1 from Wusasa district cluster 4 consisted of mother, father and four children, whose age ranged from 2 to 60 years. The family lived in a three room apartment with an attached store. Food was prepared in the open at a corner of the house. Gas and kerosene were used as sources of energy. Water for food preparation was obtained from tap while drinking water and leftover foods were normally kept in the refrigerator.

Family 2 lived in a large walled compound with four other households in Zaria walled city cluster1. The house is of mud brick with earth and zinc roof. The eleven member family comprising of mother, father and nine children were of ages ranging from 3 to 49 years. Food was prepared in a kitchen about five metres away from the living rooms. Cooking was carried out

on tripod stone stand, fire wood was used as source of energy. Water was obtained from well and tap. Animal droppings were found near food preparation area as animals (chickens, goats and sheep) roam freely in the yard. The cook was observed to keep long and dirty finger nails.

Family 3 from Tundun wada district cluster 2 lived in a room. The family consisted of mother, father and four children of ages 2 – 39 years. Food preparation and diet were similar to those of family 1. Water was obtained from well and tap, and stored in plastic buckets. Animal droppings were found near food preparation area and waste water flows through a shallow gutter about a few metres from the cooking area.

Family 4 from Wusasa cluster 4 consisted of mother, father and six children, whose ages ranged from 3 to 45 years. The family lived in a three bedroom flat. Water was obtained from tap. Drinking water was kept in the refrigerator. Cooking was carried out on an electric stove in the kitchen and sometimes on kerosene stove and firewood if cooking was carried outside within the yard.

Family 5 lived in three rooms and a parlour in Gaskiya district cluster 3. The family consisted of a father, two wives and ten children of ages 2 to 58 years. Cooking was carried out on tripod stone stands outside the kitchen and very close to the toilet and bathroom area. Water was obtained from bore hole and tap. Chickens and goats roamed freely in the yard and the family also had two dogs and a cat. Little children defecated in areas not too far from the cooking area and animal droppings were found near food preparation area.

Food Prepared and Methods of Preparation of the Foods.

Tuwo: Maize flour intended for the meal was sieved in families 1 and 3, this was however, not the case in other families in which grains were decorticated before milling into flour. About half portion of the maize flour was mixed in cold water and poured into a boiling water, stirred with wooden stirrer and then steamed for 5-10 minutes. The remaining half portion of the flour was added and stirred vigorously to form tuwo, which was eaten with vegetable soup (Fig. 1).

Akamu: Akamu is a light gruel prepared from either *Sorghum bicolor* (Guinea corn), *Penisetum typhoides* (Millet) or *Zea may* (Maize). The method of preparation involved steeping of grains overnight, wet milling with spices specifically ginger (*Zingiber officinale*) and sieving to remove the chaff. The sediment was gelled in boiled water to form gruel. Sugar was added to the individual's taste (Fig.2).

Vegetable soup: Soup preparation involved the use of different types of vegetable including bitter leaf as in families 1 and 2; okro as in families 3 and 5, spinach as in family 4. Similar spices and ingredients were added. Meat and/or fish were added if desired and available (Fig. 3). vegetable soup were served with eba or tuwo anytime of the day but more often during lunch and supper.

Eba: This is a product of gari. Eba was prepared by adding about two portions of gari to about three portions of boiling water. This was mixed thoroughly using spoon or wooden stirrer before being served with vegetable soup.

HAZARD ANALYSES:

Each family was visited on six consecutive days, during which studies of methods of food preparation and schematic drawing of each food preparation from beginning to end were made. Potential sources of contamination, likelihood of microbial survival or destruction and of microbial multiplication (growth) were also noted. The Critical Control Points determined during the preparation were temperature, time and pH.

Temperatures of the interior of foods were taken immediately after cooking or during

holding and after reheating. This was carried out by inserting a thermocouple (Type T. Atkins Technical with needle type sensor U.S.A) into the approximate central regions of the food being measured. Time was recorded from a wrist watch, while pH was measured using crimson micro pH meter (model 2000, U.S.A). Samples of foods were taken for analysis at the beginning of cooking, during cooking, after cooking, and during holding for above 30 minutes. Five hundred ml samples of drinking water, water used for food preparation and for washing were collected. Swabs of areas that could serve as sources of food contamination such as hands and fingernails of food handlers, food contact surface (food utensils and equipment) were swabbed by rubbing firmly sterile swabs over areas that were in contact with the food. If the surface was dry, the swab was first moistened in sterile 0.1% peptone water (Oxoid). Other samples collected includes animal droppings found in/or near food preparation area, sewage/waste water, flies and cockroaches, stool samples and sputum from members of families having diarrhoea/stomach pain and cough respectively. Evaluations were also made of hygienic practices and habits that could serve as a means of food contamination such as not washing hands after using the toilet by persons handling foods, picking and blowing nose near food preparation area, keeping long finger nail by cooks and not washing hands before handling finished foods.

Samples were tested for total aerobic mesophilic and coliform counts, *B.cereus* and *S.aureus* counts and the presence of *E.coli*, *Salmonella*, *Shigella*, and for eggs of common protozoans and helminths (*Ascaris lumbricoides; Hook worm; Giardia lamblia; Strongyloides stercoralis; Trichuris trichiuria and Entamoeba histolytica*) depending on the nature of samples and the organisms that would be of hazard.

Sampling procedures

Samples of foods (Maize flour, Tuwo, Gari, Eba, Akamu, Milled beans, Akara, Spices, Vegetable and soup) were collected aseptically into sterile specimen containers with metal spoons used by the family for dishing food. Samples of water were collected with cup in use by the family and poured into sterile screw capped specimen bottles. Swabs of food contact surfaces were transported in sterile specimen containers containing 5ml of sterile 0.1% peptone water (Bryan, et al 1988).

Samples of droppings from animals were picked from the floor with sterile spatula into sterile specimen containers. Persons from which faecal and sputum samples were collected, were each given a wide mouthed screw capped specimen bottles (which were labelled with their names, age, sex and family details). They were instructed to defecate into the container and/or cough into the container and to cover firmly with the screw cap. Samples and specimens were transported to the laboratory on the day of collection in cold pack within 2 hrs and were examined on the same day.

Microbiological Analyses

Enumeration of Microorganisms

Ten gram or 10ml of food samples were homogenized with 90ml of 0.1% peptone water in screw capped flasks by means of horizontal and vertical manual agitation for a few minutes. Serial dilution of 10^{-2} (for cooked foods such as Tuwo, Eba, Akamu, Akara, Soup) and 10^{-3}, 10^{-4} (for raw foods such as maize flour, gari, milled beans, vegetables) of the homogenate were prepared for enumeration. Aliquots of 0.2ml of the serial dilutions of the food samples were spread on duplicate plates using sterile glass spreader. This technique was used for the enumeration of total aerobic viable count, coliform, bacillus and staphylococcal counts on nutrient agar (Difco), eosin methylene blue (EMB) agar (Oxoid), dextrose tryptone agar (Oxoid) supplemented with polymyxin B and egg yolk emulsion and Baird parker agar (Oxoid) supplemented with tellurite and egg yolk emulsion, respectively. All cultures were incubated at 37^0C for 24 hours except for the

coliforms which was incubated at 37^0C and 44^0C for 24 hours. Animal droppings, stool specimens, sputum and swab samples were inoculated directly onto plating media as well as into selective broths for enrichment. One hundred millilitres (100ml) of water samples for drinking, cooking and washing dishes were filtered through 0.45μm pore size membrane filter. The filters were aseptically placed using a sterile pair of forceps on EMB agar plate (Oxoid) and nutrient agar (Oxoid) for coliform and total aerobic viable counts. Incubation was for 24 hours at 44^0C and 37^0C respectively. Media used were prepared according to the manufacturer's instructions.

Characterization of Isolates

Confirmation of coliform organisms was carried out by inoculating colonies into lactose broth with Durham tubes and incubating at 37^0C and 44^0C for 24 hours in the absence of gas production (Speck, 1976). The presence of gas constituted a presumptive test and the broth was streaked out on EMB agar incubated at 37^0C for 24h. Typical colonies on EMB plates appearing bluish black with greenish metallic sheen which are characteristics of *E. coli* or brownish colonies often convex and mucoid which are characteristics of *Enterobacter aerogenes* indicated the presence of coliform organisms. Isolates were stored on nutrient agar slants at 4^0C for further confirmatory tests which included IMViC test, carbohydrate utilization, reaction on TSI, gelatin liquefaction, nitrate reduction, urease production and motility. Large, flat, irregular, wrinkled or smooth ground-glass colonies 4-6mm in diameter were counted as Bacillus spp. Confirmation was based on gelatine liquefaction, citrate utilization, starch hydrolysis, VP test and fermentation of sugars as described by Yusuf, et al (1992). Confirmation of typical colonies of *S. aureus* on Baird-Parker agar was on the basis of the results of catalase, coagulase, phosphatase production, nitrate reduction and carbohydrate utilization (Umoh, et al. 1999). For isolation and confirmation of *Salmonella* and *Shigella*, procedures recommended by speck (1976) were followed. The pre-enriched samples in lactose broth were sub-cultured into selenite F-broth for selective enrichment and on *Salmonella-Shigella* agar (SSA). Typical colonies appearing as colourless (non lactose fermenting), translucent, 2 – 4mm in diameter, convex with an entire edge were Gram-stained and characterized (Speck, 1976).

Protozoa and Helminths Identification

About two gram of faeces (stool, animal droppings and sewage) was emulsified in 10 ml of 10% formol saline. This was stained through a coarse sieve into a centrifuge tube. About 3.5ml aliquot of ether was added, shaken and centrifuged (MSE Minor 35, England) at 2000rpm for 3mins (Cheesbrough 2000). The fatty debris was removed and the supernatant poured off leaving the last bottom sediment to which was then added drops of lugol's iodine as stains (Cheesbrough 2000). After tapping the tube, a drop was transferred to a clean slide, covered with cover slip and examined microscopically using the X10 and X40 objectives.

For identifying protozoa and helminths in vegetable, about 10g vegetable was thoroughly washed in 100ml of sterile water, the water was strained through a coarse sieve into a centrifuge tube and about 3.5ml of 10% of formol saline and equal volume of ether added, shaken and centrifuged (MSE Minor 35, England) at 3000rpm for 5min. The supertant was discarded leaving only a small amount of sediment to which drops of lugol's iodine was added as stain. A drop was then placed on clean slide, covered with a cover slip and examined microscopically under the X10 and X40 objectives. Parasites were identified using descriptions of Cheesbrough (2000).

Ligated Rabbit Ileal Loop test for Screening Enterotoxigenic *B. cereus* and *E. coli*

Enterotoxin production for *B. cereus* was carried out in Brain Heart infusion broth (Oxoid) with 0.1% (w/v) glucose supplement (BHIG). Toxin production for *E. coli* was in Trypticase

soy broth (Difco). The methods as described by Spira and Geopfert (1972) and Turnbull and Kramer (1983) were adopted and these involved subculturing stock cultures on nutrient agar slants into BHIG and Trypticase soy broth, incubation at 37^{0}C for 8 hours and 20hours for *B. cereus* and *E. coli* respectively and on a shaker at 200rpm, with the addition of 1ml of 0.1N NaOH at intervals.

The ligated rabbit ileal loop test was conducted following the procedures of Spira and Geopfert (1972) using New Zealand white rabbits obtained from the National Animal Product Research Institute (NAPRI) Shika Zaria and weighing an average of 1.8kg. New Zealand White rabbits were acclimatized to the laboratory condition for one week before testing. They were examined at Ahmadu Bello University Veterinary Teaching Hospital (ABUVTH) Zaria and were confirmed fit for the test. The rabbits were fasted for 48 hours prior to the surgical operation to externalise the ileum.

The surgical procedures were performed under general and local anaesthesia and with aseptic precautions. Segments of the ileum were tied off to form test loops each approximately 10cm long, separated from one another by 5cm blank loops (Spira and Geopfert, 1972).

Intraluminal injections of 2ml of the enterotoxin extract were made into each of the test loops using 25 guage sterile needle. In each rabbit, one loop was injected with sterile BHIG as a negative control. Ileum were returned to the abdominal cavity and incision stitched. Following recovery from anaesthesia, the rabbit was provided with water but no food and were allowed to live for 6 hours (Spira and Geopfert, 1972).

After the holding period, the rabbits were sacrificed and opened up immediately for examination. The presence or absence of dilatation of the segments was observed and the volume and character of their fluid contents expressed. If either the control loop or any of the blank loops contained fluid, all tests in that rabbit were considered invalid. The length and fluid volume of each test loop was measured. The index of fluid accumulation was derived from the ratio of loop volume (ml) to loop length (cm). Ratio of fluid volume to loop length ranging from 0.2 – 0.4 was considered to be mild; 0.5 – 0.7 as moderate and 0.8 – 1.5 as severe enterotoxin activity (Spira and Geopfert, 1972; Yusuf, et al. 1992).

Statistical Analysis

A soft-ware computer programme SAS was used. Analysis of variance and the Duncan multiple range test were used to compare means of total aerobic, Staphylococcal, coliform and Bacillus counts for food and water samples (Snedecor and Cochran, 1976).

RESULTS

All foods prepared in the families attained boiling or close to boiling temperature during cooking and were usually eaten promptly after cooking thus no temperature-time lapse (abuse) was recorded except, however, a case of soup held overnight at ambient room temperature before reheating in family 2. Table 1 shows the microbial counts of stiff porridge prepared by the respective five families. It reveals that in family 1, tuwo served 30 minutes after preparation at 65°C had total aerobic plate count (TAPC) of 4.66$\log_{10}$ cfu/g. the TAPC is significantly higher ($P<0.05$) than counts obtained from tuwo samples of all the other families. In family 3, tuwo served 35minutes after preparation at 58°C had a *S.aureus* count of 3.40$\log_{10}$ cfu/g. The *B.cereus* and TAPC did not differ from samples of other families. In family 5, a coliform count of 4.04$\log_{10}$ cfu/g was obtained from tuwo. The tuwo had a TAPC and a *B.cereus* counts of 3.39$\log_{10}$cfu/g and 4.83$\log_{10}$ cfu/g respectively when served 30 minutes after preparation at 50°C. Table 1 also shows that tuwo samples from four of the families yielded *B.cereus* with counts 4.1 – 4.8$\log_{10}$ cfu/g. Eba samples from families 2 and 3 had significantly higher ($P<0.05$) *B.cereus* counts. The eba was served 30 minutes after preparation at 40 – 50 °C.

Organisms of concern were not detected (ND) in some of the samples and for lack of permission in some cases, temperature in the internal region were not tested (NT) in some other food samples.

Table 2 reveals the microbial count of akamu a popular breakfast meal. In family 1 akamu served 45 minutes after preparation at 40°C had a coliform count of 2.0log$_{10}$ cfu/ml. Table 2 also reveals that *B.cereus* and *S.aureus* were isolated from samples of akara obtained from families 3 and 4.

Table 3 presents the microbial counts of vegetable soups from five families. Vegetable soup samples had microbial counts of 2.7log$_{10}$ – 3.7log$_{10}$ cfu/ml, except however, soup sample from family 2 with counts 6.7log$_{10}$ cfu/ml. It had a significantly higher TAPC of 6.7log$_{10}$ cfu/ml, *B. cereus* count of 6.11log$_{10}$ cfu/ml and *S. aureus* count 5.47log$_{10}$ cfu/ml. when compared to vegetable soup samples from other families.

Table 4 presents results of ileal loop test. 13 (46.4%) of the *B. cereus* and 2(14.3%) of *E. coli* were mildly toxigenic. No severe reaction was recorded for *E. coli* isolates, only 1(3.6%) of the *B. cereus* showed severe reaction.

Table 1. Mean Microbial Counts log$_{10}$ cfug^{-1} of raw materials and after preparation of the products by five families in Zaria

Organism	Family	Maize flour	Tuwo	Temp.°C* [Time (min)]*	Gari	Eba	Temp.°C * [Time(min)]*
TAPC	1	4.61±4.6	4.66±4.6^{a}	65(30)	1.00±0.2	ND	NT(30)
	2	4.15±4.2	4.20±3.8^{b}	50(30)	1.88±1.5	4.56±3.7	40(30)
	3	4.97±4.7	4.52±4.3^{ab}	58(35)	2.00±1.4	1.69±1.2	50(30)
	4	4.36±4.4	4.00±4.0^{a}	50(30)	2.68±1.2	ND	45(35)
	5	5.46±5.7	3.39±3.9^{b}	50(30)	**	**	**
B. cereus	1	4.57±4.6	4.85±5.0	65(30)	2.40±1.4	ND	NT(30)
	2	4.00±3.8	ND	50(30)	ND	4.56±2.6^{a}	40(30)
	3	4.66±4.6	4.38±4.2	58(35)	2.98±0.8	4.00±1.6^{a}	50(30)
	4	4.20±4.1	4.11±4.2	50(30)	3.65±2.2	2.40±1.9^{b}	45(35)
	5	4.98±4.8	4.83±4.7	50(30)	**	**	**
S. aureus	1	3.00±0.0	ND	65(30)	ND	ND	NT(30)
	2	ND	ND	50(30)	ND	ND	40(30)
	3	3.74±3.8	3.40±0.0	58(35)	ND	ND	50(30)
	4	3.18±0.0	ND	50(30)	1.23±0.8	ND	45(35)
	5	ND	ND	50(30)	**	**	**
Coliform	1	2.88±2.3	ND	65(30)	ND	ND	NT(30)
	2	ND	ND	50(30)	ND	ND	40(30)
	3	ND	ND	58(35)	ND	ND	50(30)
	4	ND	ND	50(30)	ND	ND	45(35)
	5	5.28±4.8	4.04±2.7	50(30)	**	**	**

** = Food not prepared in family

ND = Organism not detected

NT = Not Tested

a,b,c = Means within column with the same letter for same count are not significantly different (P = 0.05)

± = standard deviation

* = Temperature of internal portion (approximate centre) of finished food and holding Time before consumption.

TAPC = Total aerobic Plate Count

Table 2. Mean Microbial Counts (log_{10} $cfug^{-1}$) of breakfast meals prepared by five families in Zaria.

Organism	Family	Milled maize	Akamu	Temp.^{0}C* [Time (min)]*	Milled Beans	Akara	Temp(^{0}C) [Time(min)]
TAPC	1	5.65±5.3	4.32±4.1	40(45)	7.00±6.4	3.00±2.4	NT
	2	5.11±4.1	4.30±0.0	50(40)	ND	2.39±1.8	NT
	3	5.96±5.8	4.60±4.5	49(40)	ND	3.11±2.5	NT
	4	4.97±4.7	4.30±0.0	60(30)	4.74±4.7	2.65±2.6	31(40)
	5	5.81±5.9	4.20±4.0	62(30)	**	**	**
B. cereus	1	5.43±5.2^{bc}	4.64±4.7	40(45)	6.81±5.8	ND	NT
	2	4.18±3.2^{c}	4.18±4.1	50(40)	ND	ND	NT
	3	5.77±5.8^{abc}	4.59±4.3	49(40)	ND	2.39±2.4	NT
	4	6.11±6.0^{a}	4.08±3.4	60(30)	4.58±4.4	2.23±2.1	31(40)
	5	6.18±5.7^{a}	4.95±4.8	62(30)	**	**	**
S. aureus	1	5.08±5.0	ND	40(45)	6.30±6.1	ND	NT
	2	3.69±2.3	ND	50(40)	ND	ND	NT
	3	5.11±5.0	ND	49(40)	ND	2.58±2.6	NT
	4	ND	ND	60(30)	ND	2.00±0.8	31(40)
	5	5.32±5.3	ND	62(30)	**	**	**
Coliform	1	3.72±2.6	2.00±1.8	40(45)	ND	ND	NT
	2	ND	ND	50(40)	ND	ND	NT
	3	ND	ND	49(40)	ND	ND	NT
	4	ND	ND	60(30)	ND	ND	31(40)
	5	4.82±3.8	ND	62(30)	**	**	**

** = Food not prepared in family

ND = Organism not detected

NT = Not Tested

a,b,c = Means within column with the same letter for same count are not significantly different ($P > 0.05$).

± = standard deviation

* = Temperature of internal portion (approximate centre) of finished food and holding time before consumption.

TAPC = Total aerobic plate count

Table 3. Mean Microbial Counts ($\log_{10}$ cfug^{-1}) Vegetable, Soup and spices from five families in Zaria

Organism	Family	Vegetable	Spices	Soup	Temp.°C* [Time min.]*
	1	7.00 ± 0.0^{ab}	6.20 ± 0.0	3.74 ± 0.0^{a}	70(<30)
TAPC	2	7.15 ± 6.7^{a}	6.00 ± 5.9	6.70 ± 6.9^{b}	50(30)
	3	5.92 ± 6.1^{b}	6.20 ± 4.8	3.36 ± 3.5^{a}	55(30)
	4	7.15 ± 6.4^{a}	5.98 ± 5.8	2.76 ± 2.7^{a}	NT
	5	6.70 ± 5.6^{ab}	6.20 ± 4.9	3.34 ± 3.4^{a}	56(30)
<u>B</u>. <u>cereus</u>	1	6.81 ± 0.0^{ab}	5.63 ± 5.4	3.46 ± 3.5^{a}	70(<30)
	2	6.79 ± 6.1^{ab}	6.49 ± 6.6	6.11 ± 6.2^{b}	50(30)
	3	5.81 ± 6.0^{c}	5.72 ± 4.4	3.68 ± 3.8^{a}	55(30)
	4	7.00 ± 6.1^{a}	5.23 ± 5.3	2.80 ± 2.8^{a}	NT
	5	6.32 ± 6.3^{bc}	5.59 ± 5.4	3.23 ± 3.3^{a}	56(30)
<u>S</u>. <u>aureus</u>	1	6.36 ± 0.0	5.18 ± 4.8^{b}	ND	70(<30)
	2	6.18 ± 6.0	4.45 ± 4.0^{c}	5.48 ± 4.0	50(30)
	3	4.63 ± 4.8	5.88 ± 0.0^{a}	ND	55(30)
	4	6.36 ± 5.5	5.11 ± 4.8^{bc}	ND	NT
	5	6.85 ± 6.5	4.92 ± 4.1^{bc}	ND	56(30)
Coliform	1	ND	ND	ND	70(<30)
	2	6.36 ± 0.0^{a}	ND	ND	50(30)
	3	2.93 ± 0.0^{b}	ND	ND	55(30)
	4	6.20 ± 6.2^{a}	5.40 ± 4.6	ND	NT
	5	6.08 ± 5.2^{a}	ND	ND	56(30)

ND = Organism not detected

NT = Not tested

a,b,c = Mean within column with the same letter for same count are not significantly different ($P > 0.05$)

± = standard deviation

* = Temperature of internal portion (approximate centre) of finished food and holding time before consumption

TAPC = Total aerobic plate count

Table 4. Enterotoxigenicity of *B.cereus* and *E.coli* using ileal loop test

Reaction	No of isolates tested (n)/Number (%) of Total	
	B.cereus n = 28	E.coli n = 14
Mild reaction	13 (46.4)	2 (14.3)
Moderate reaction	2 (7.2)	1 (7.1)
Severe reaction	1 (3.6)	-
Total	16 (57.1)	3 (21.4)

n = No. of isolates tested

\- = No reaction

DISCUSSION

The application of HACCP system in the study of household food preparations is based on the fact that it determines quickly and relatively cheaply the critical points in the food preparation, handling and serving processes that are crucial to safety while taking into account local habits and culture. The early identification of potential food hazards and faulty practices will lead to measures that prevent risks to consumers.

Foods (tuwo, akamu, vegetable soup, eba) examined in this study were cooked to 65^0C – 100^0C temperature which should have been high enough to kill large numbers of vegetative cells but not heat resistant spores. The foods were promptly eaten. Thorough cooking of food is a CCP, so too is prompt consumption that prevent holding within temperature range that encourages the germination of spores and the multiplication of resulting vegetative cells as well as microbial contaminants that come in contact with the foods after cooking.

The total aerobic plate count of 1.69$\log_{10}$ cfu/g – 6.70$\log_{10}$cfu/g observed for cooked foods from all the families could be explained either by survival of spores which could have come initially from the ingredients and raw foods (Table 1 – 3) or by reduction of but not total elimination of vegetative cells that propagated on the food stuffs and food ingredients. The significantly higher ($P<0.05$) TAPC of tuwo from families 1 and 3 (table 1) could be associated with the processing method adopted since the cooks (food handlers) in these households sieved their maize flour before cooking as opposed to families 2, 4 and 5 in which grains were dehusked before milling, sieving was therefore not necessary. Dehusking before milling could have reduced contamination by organisms that may reach the flour during and after sieving. The high TAPC (6.70$\log_{10}$ cfu/ml) of soup sample from family 2 (Table 3) could be attributed to germination of spores, recovery of injured cells and multiplication of vegetative cells due to long period of holding at ambient temperature.

Isolation of *B. cereus* from cooked food could be attributed to the ubiquitous distribution of this organism. Presence of *B. cereus* in tuwo and eba (Table 1) and in akamu (Table 2) could be attributed to the method of processing, handling, method of drying of grains or tubers, machine milling and the environment. The limited time/temperature exposure of 5 – 10min which tuwo, eba and akamu undergo during processing (figs. 1 and 2) is equally not enough to destroy *B. cereus* spores. The high level of contamination of vegetables and spices could be responsible for the presence of this organism in soup (table3). The ability of *B. cereus* to form endospores and its known osmo and thermotolerant nature (McKillip, 2000) could account for the survival of *B. cereus* in cooked food. *B. cereus* has been isolated from flour based food (Yusuf et al., 1992) and other food product, (Ryu and Beuchat, 1997). The international commission on microbiological specifications for foods (ICMSF, 1974) suggested the acceptable limit for *B. cereus* in food as 3$\log_{10}$ cell/g with 4$\log_{10}$ cell/g as tolerable and >6$\log_{10}$ cell/g as above the accepted limit. It has also been established that very high number of cells approximately 7$\log_{10}$ cells/g food are required to cause outbreaks of disease, although in young children 5$\log_{10}$ cells/g is enough to result in an outbreak (Mossel *et al.*, 1967; Gilbert, 1999). Although the count of 6.11$\log_{10}$ cell/g obtained from soup sample in family 2 is still below 7$\log_{10}$ cells/g required to cause outbreak of disease, such high count can cause spoilage leading to change of taste. Similarly the soup could pose problem to children, the elderly and immunocompromised.

The isolation of *S. aureus* from cooked foods (tuwo, akara and soup) could be attributed to post – process contamination. The foods had been boiled or heated to near boiling point and that temperature should have been high enough

to kill *S. aureus* cells, so contamination could have occurred afterwards from the food handlers during dispensing or from improperly washed food utensils and equipment. *S. aureus* in hands and finger nails of cooks could have been the source of this organism to food utensils and equipment and to food samples. Hands and finger nails would have been the source of this organism from the food handler's body either by touching the hair, picking and blowing the nose. S. aureus is known to be a common inhabitant of the human body and 40 – 50% of the population carry this organism in their nose and throat (Bergdoll, 1980). The isolation of *S.aureus* from tuwo (table1) is attributed to contamination from utensils, hands and fingers used in wiping off food from wooden stirrer. Similarly, akara is served with bare hands and this could be responsible for *S. aureus* contamination (table 2). *S .aureus* has been isolated from a variety of food and food products (Kwaga and Adesiyan, 1984; Umoh, 1989; Umoh, et al 1990a, b). The ICMSF (1974) suggested a count of $3\log_{10}$ cell/g as the acceptable limit for S. aureus in food with $6\log_{10}$ cells/g as tolerable/marginal limit and above $6\log_{10}$ cells/g as unacceptable limit. It has also been reported that a population at least $6\log_{10}$ cells/g food of enterotoxigenic strains of staphylococci are required to present any risk of intoxication (Bergdoll, 1980). The non – detection of *S. aureus* and coliforms in gari and eba could be attributed to the dry nature (low water activity) of gari which will not encourage the survival and multiplication of vegetative cells.

The isolation of coliform organisms from tuwo and akamu is attributed to post process contamination from water normally used to cool off the fingers when wiping off food from the stirrer and sometimes used for dilution of akamu to required consistency, from improperly cleaned utensils and equipment; persons handling foods and animals present in the environment. The isolation of *E. coli* from animal droppings and sewage collected from near food preparation area is consistent with *E. coli* being a common organism found in the gastrointestinal tracts of warm blooded animals and human beings. Animal droppings could have been the source of coliform to the environment from which the food samples and water were contaminated. *E. coli* and several other coliforms have been isolated from a variety of foods (Barrel and Rowland 1979; Ehiri, et al 2001). It has been reported that a concentration of $3\log_{10} – 7\log_{10}$ cells/g food of enteropathogenic strains of E. coli is required to induce gastroenteritis (Marier *et al.*, 1973).

Food preparation practices, food eating habits and recipes used in cooking were similar in families surveyed, hazards observed in the families were associated with presence of bacteria spores on food and food ingredients, cross contamination from utensils, equipments; raw to cooked foods, handling of foods and time – temperature range of cooked food during holding after cooking. The CCPs for preparation of tuwo and vegetable soup are time – temperature exposure during cooking and holding after cooking. The hazard is that spores survive cooking and following their germination, the resulting vegetable cells can multiply at ambient temperature. CCPs for akamu preparation is time lapse during steeping of grains, pH changes during product fermentation, time – temperature exposure during gelling and holding after gelling. The hazard is that vegetative cells multiply during steeping, spores and vegetative cells survive the fall in pH and the gelling temperature and time of exposure to heat to kill vegetative cells. Following resuscitation of surviving vegetative cells and germination of spores, the resulting cells can multiply at temperatures of holding.

Foods prepared by the respective families during the course of this study were promptly eaten, thus the habit of holding cooked foods for eating several hours after preparation which is a food safety hazard of major concern in developing countries (Michanie *et al.*, 1988) was not observed.

Cooked food were usually covered by lids or bowls or saucers placed over the dishes

that were poorly cleaned. Thus kitchen utensils could play a role in cross contamination and be vehicles by which micro organisms were transferred. From observations made during food preparation, utensil surfaces and water appear to be more of probable sources of food contamination due to inadequate cleaning of utensils and poor water quality.

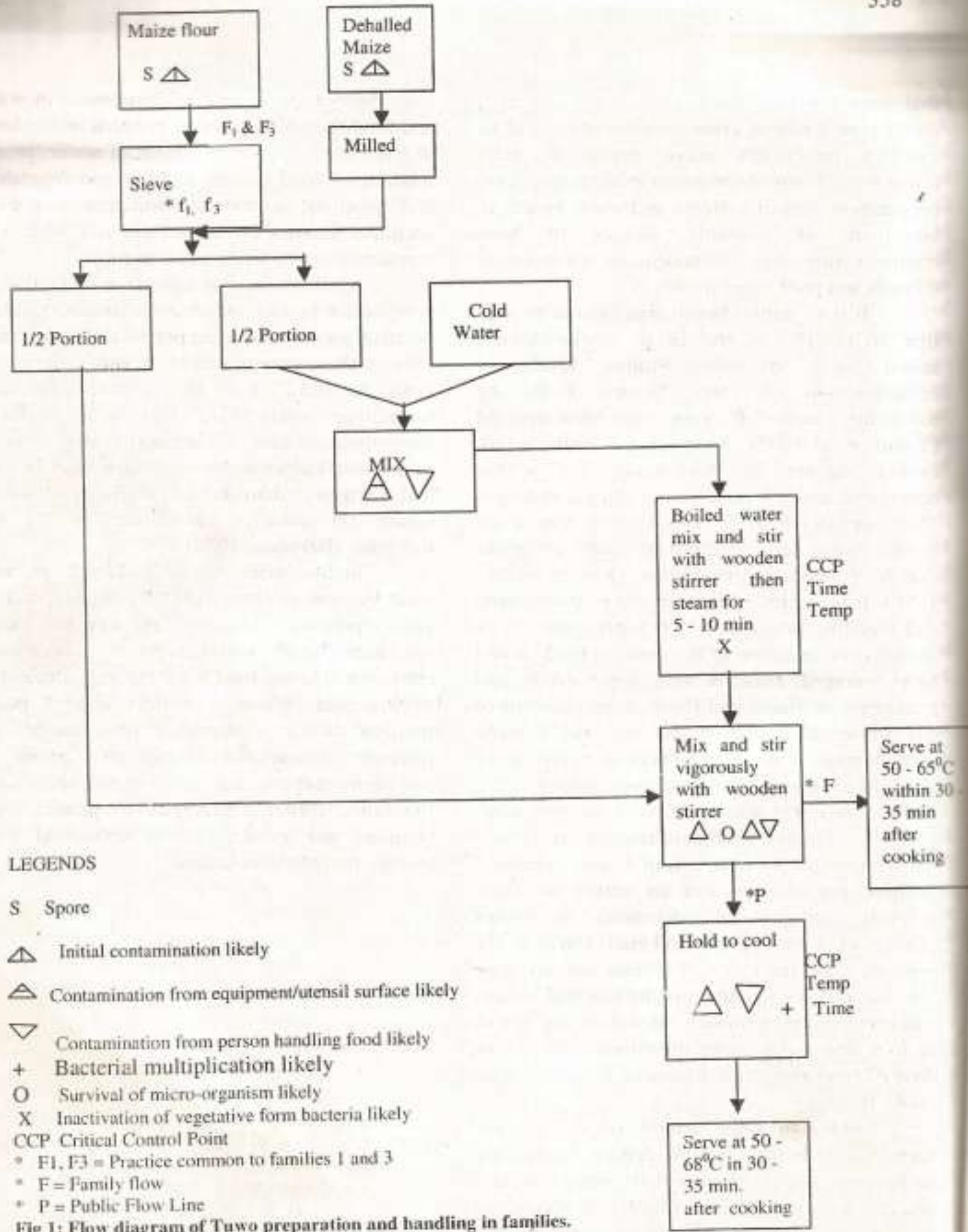
It is of public health significance to note that 16 (57.1%) of the 28 *B. cereus* isolates tested were toxigenic. Similar levels of contamination of some Nigeria foods by toxogenic strains of *B. cereus* have been reported (Yusuf, et al 1992). Although the counts of *B. cereus* isolates in this study lies within acceptable and tolerable limits ($3log_{10}$ cells/g – $5log_{10}$ cells/g) ICMSF (1994), these counts are below $7log_{10}$ cells required to cause outbreaks due to enterotoxin production (Mossel *et al.*, 1976). It is suggested that improved preparation and handling practices which further prevent or reduce contamination of B. cereus in food should be encouraged. These include proper drying and packaging of grains and flours, high standard of environmental sanitation and personal hygiene and increased time – temperature exposure of foods like tuwo and akamu during cooking.

Three (21.4% of E. coli isolates were toxigenic. Studies have confirmed a correlation between the ability of strains of *E. coli* to cause a positive gut reaction and its ability to cause infantile enteritis and diarrhoea in babies (Taylor, et al 1961; Smith and Hall, 1967). In the present study, the count of *E. coli* isolates were low, but of health concern is the fact that infants and immunocompromised individuals are at risk of food and water borne infections even at low dose of enterotoxigenic strains of *E. coli* (Nataro *et al.*, 1998).

Although some strains of *E. coli* are harmless to human health, certain strains are pathogenic in man, causing both septic infection and diarrhoea (food borne illness). A number of food borne infection due to this organism has been associated with water, human, animal and animal by products (Nataro *et al.*, 1998). *E coli* have their origin in faeces, its presence in water and food therefore, calls for concern on the level of hygiene. Adequate treatment of water, proper washing of food contact surfaces and vegetables and good environmental sanitation would be adequate measures to reduce the risk of *E. coli* contamination of foods and water.

All the *S. aureus* isolates were coagulase positive for human and sheep plasma. Coagulase positive staphylococci are potentially pathogenic. This is therefore of serious health significance since 30 (68.2%) of the isolates were β – haemolytic while 12(27.3%) were Alpha – haemolytic. Alpha – haemolytic strains of *S. aureus* are known to be more of human biotype and toxigenic than Beta – haemolytic strains which are more of animal strains and less toxigenic (Bergdoll, 1980).

In conclusion, the best means to promote food hygiene is using HACCP strategy such as good personal hygiene and environmental sanitation which will prevent or reduce food contamination via food handlers and surrounding environment, adequate critical control points monitor such as temperature, time and pH and through educational campaign aimed at informing and stimulating the public specifically the home makers. Strategies to protect water supplies and promote proper household water storage must be encouraged.



LEGENDS

S Spore

Initial contamination likely

Contamination from equipment/utensil surface likely

Contamination from person handling food likely

\+ Bacterial multiplication likely

O Survival of micro-organism likely

X Inactivation of vegetative form bacteria likely

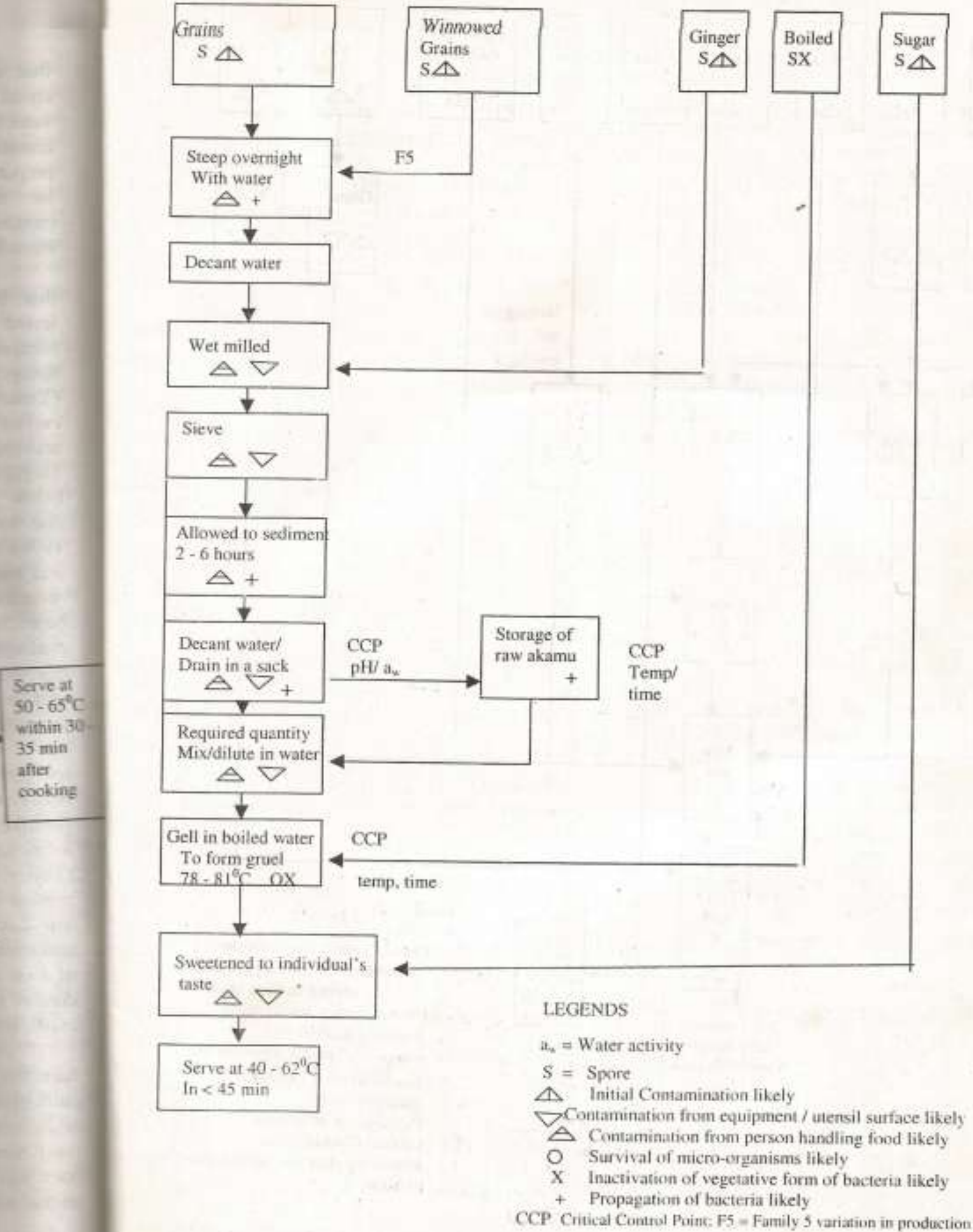
CCP Critical Control Point

* F1, F3 = Practice common to families 1 and 3

* F = Family flow

* P = Public Flow Line

Fig 1: Flow diagram of Tuwo preparation and handling in families.



2. Flow diagram of Akamu preparation and handling in households.

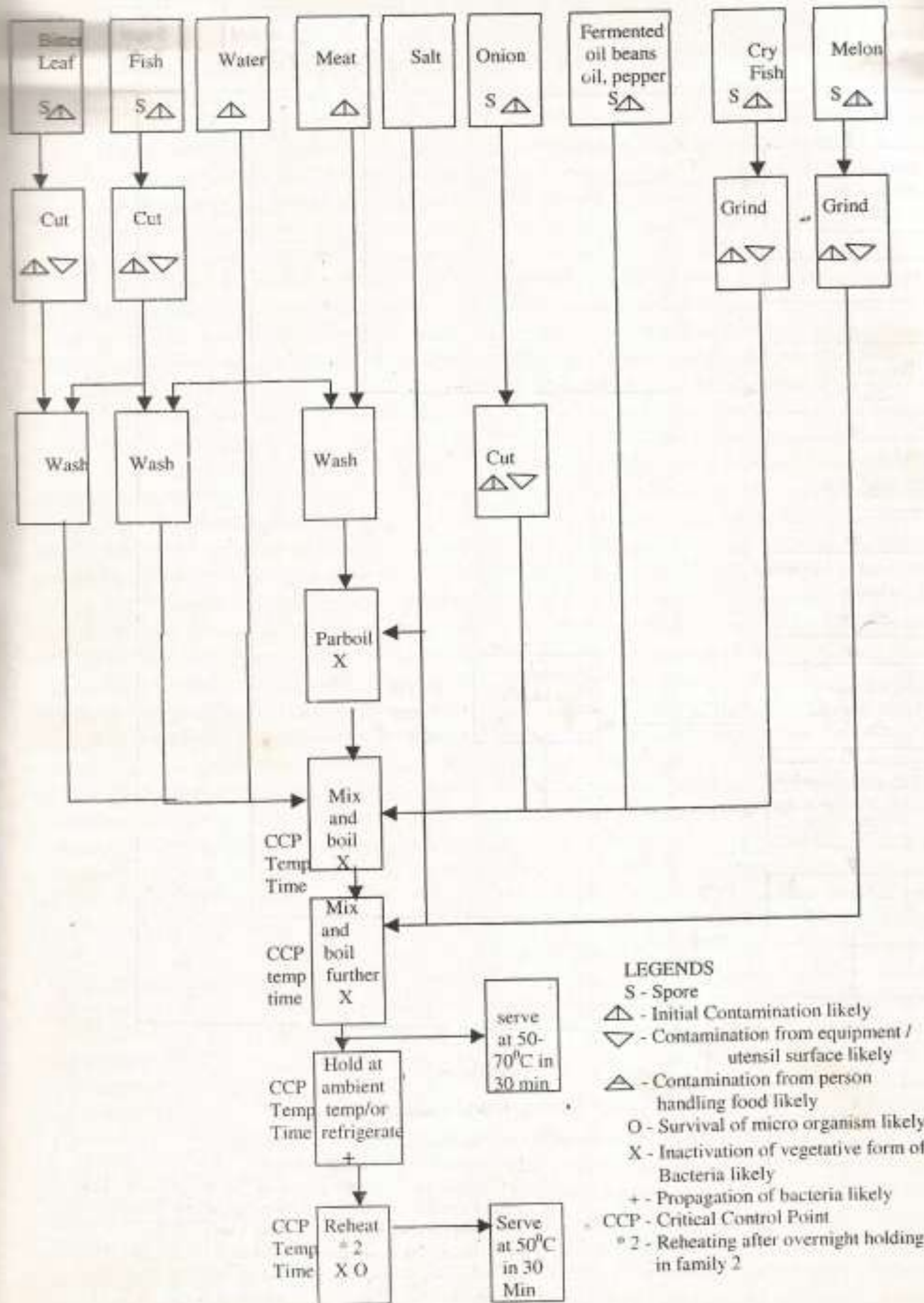


Fig 3. Flow diagram of vegetable soup preparation and handling in households.

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