Microbiological Hazard Analysis and Critical Control Points of Locally Processed Milk in Kano Metropolis

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Abstract

This study was carried out on microbial hazard analysis and determination of critical control points during local milk processing in Ungogo and Kumbotso Local Government Areas of Kano metropolis. Four stages were analysed which included the fresh milk (FM), mixed milk (MM), unskimmed fermented milk (USM) and skimmed fermented milk (SM). The analysis consisted of aerobic plate count (APC), coliform count (CC), Staphylococcal count (SC), fungal count (FC), detection of *E. coli, Salmonella* and *Staphylococcus aureus* following standard procedures. From Ungogo Local Government Area, the mean aerobic plate count of fresh milk (FM), mixed milk (MM), unskimmed fermented milk (USM) and skimmed fermented milk (SM) samples were found to be $2.05x10^6$, $5.23x10^7$, $1.25x10^8$ and $1.43x10^8$cfu/ml, Staphylococcal count had a mean counts of $1.94x10^5$, $2.26x10^6$, $4.00x10^5$ and $6.00x10^6$cfu/ml, fungal count had mean counts of $1.99x10^7$, $8.30x10^7$, $1.07x10^8$ and $1.39x10^8$cfu/ml respectively. Coliform counts ranged from 44 to >2400MPN/ml. For Kumbotso Local Government Area, the mean aerobic plate counts of fresh milk (FM), mixed milk (MM), unskimmed fermented milk (USM) and skimmed fermented milk (SM) samples were found to be $1.07x10^7$, $3.57x10^7$, $1.31x10^8$ and $9.84x10^7$cfu/ml, Staphylococcal count had mean counts of $7.29x10^5$, $2.46x10^6$, $3.84x10^5$ and $1.94x10^6$cfu/ml, fungal mean counts were $5.20x10^5$, $7.12x10^7$, $8.36x10^7$ and $2.43x10^8$cfu/ml respectively. Coliform count ranged from 36 to >2400MPN/ml. The counts were above the acceptable limit of $1.0x10^5$cfu/ml. Also, *E. coli, Salmonella* and *Staphylococcus aureus* were isolated which are indicators of contamination. The mean pH and temperature from all the samples which ranged from 4.71 to 6.55 and 19.76^0C to 33.16^oC respectively which are ideal for the growth of spoilage organisms. The study expressed the need of ensuring personal and environmental hygiene as well as the use of clean utensils which could help in eliminating or reducing the hazards to an acceptable level.

Key words: Hazard, Kumbotso, Ungogo, Critical Control Point, Milk, Hygiene

Introduction

Hazard is any biological, physical or chemical agent that is reasonably likely enough to cause illness or harm in the absence of its control. Microbiological hazard is any microbiological agent that is reasonably likely to cause illness or infection in the absence of its control. This includes disease causing bacteria, viruses and parasites that occur naturally in the environment. A critical control point (CCP) is a point, step or procedure in which hazard exist and control can be applied and a food safety hazard can be prevented, eliminated or reduced to acceptable level.[1]

Milk is a white fluid secreted by female mammals for the sole purpose of rearing their offspring. It consists of small globules of fat suspended in watery solution containing protein, sugar and minerals.[2] Milk is sterile at secretion in the udder but is contaminated by bacteria even before it leaves the udder. Except in the case of mastitis (an inflammation of the udder as a result of bacterial infection), the bacteria at this point are harmless and few in number. Further contamination of the milk by micro organisms can take place during milking, handling, storage, and other pre-processing activities.

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The safety of dairy products with respect to food-borne diseases is of great concern around the world. This is especially true in developing countries where production of milk and various milk products takes place under unsanitary conditions and poor production practices.[3] The microbial load of milk is a major factor in determining its quality. It indicates the hygienic level exercised during milking, that is, cleanliness of the milking utensils, condition of storage, manner of transport as well as the cleanliness of the udder of the individual animal.[2] Milk produced under hygienic conditions from healthy animals should not contain more than 1.00 x 10^5cfu/ml.[4]

Kano is the capital of Kano State in Northern Nigeria. Its metropolitan population is the second largest in Nigeria after Lagos. The Kano urban area covers 137sq.km and comprises six Local Government Areas (LGAs) - Kano Municipal, Fagge, Dala, Gwale, Tarauni and Nassarawa - with a population of 2,163,225 at the 2006 Nigerian census. The Metropolitan Area covers 499 sq.km and comprises eight LGAs - the six mentioned above plus Ungogo and Kumbotso - with a population of 2,828,861 at the 2006 Nigerian census. The research study areas are Ungogo and Kumbotso Local Government that are part of the eight metropolitan Local Government areas.[5]

Sample Size and Sample Collection

Two hundred (200) samples were collected from our local milk processors. One hundred samples from Ungogo and Kumbotso Local Government. From each Local government, five cows were selected each from five *Fulani* herds. From each cow, four samples which included the fresh milk (FM), mixed milk (MM), skimmed fermented (SM) and unskimmed fermented (USM) milk samples were collected. It was then transported immediately to the laboratory in a box containing ice for microbiological analysis to investigate organisms likely to cause hazard at each step of the milk production. At each step of sample collection, pH and temperature were measured and recorded.

Sample Preparation and Serial Dilution

Method of FAO[6] was used for this research. For each milk sample, 25ml was homogenized in 225ml of sterile buffered peptone water. This was labelled 10^{-1}dilution. A quantity (1.0ml) of the homogenate was pipetted into a tube containing 9ml of the buffered peptone water. This was mixed carefully by aspirating 10 times with a pipette, and labelled as 10^{-2}dilution. The procedure was repeated up to 10^{-6}.

Enumeration of Coliform Using (MPN)

The lactose broth was prepared and 9ml was dispensed into each of the nine (9) test tubes each containing an inverted Durham tube. The whole test tubes were sterilized in autoclave at 121^{o}C for 15minutes. After cooling down, 1.0ml of the diluted sample from 10^{-1} was transferred into each of three test tubes of lactose broth; 1.0ml from 10^{-2} dilution was also transferred into another set of three test tubes of lactose broth then 1.0ml from 10^{-3} dilution was transferred into another set of three test tubes. These test tubes were incubated at 37^{o}C for 24hrs. Tubes that produced gas were counted and the number was compared with the most probable number (MPN) table to obtain the counts in MPN/ml.[7]

Aerobic Mesophilic Bacterial Count

One mililitre (1ml) from each dilution of the sample was pipetted into each of the labelled duplicate Petridishes. This was followed by pouring aseptically, molten nutrient agar. The inoculated Petridishes were incubated at 37^{o}C for 24hrs. After incubation, all plates with 30 to 300 colonies were counted and the numbers obtained were multiplied by the inverse of the dilution factor to get the number of colony forming unit (cfu/ml).[7]

Detection of *Escherichia coli*

A loopful of inoculum from gas positive tubes were streaked on to plates of Levine's eosine methylene blue (L-EMB) and the plates were incubated at 37^{o}C for 24hrs. Following incubation, bluish black colonies with green metallic sheen were preserved as suspected of *Escherichia coli* while pinkish colonies were suspected *Enterobacter aerogenes.* Suspected colonies of *E. coli* were later confirmed by a series of biochemical tests.[6]

Detection of *Salmonella*

A loopful of inoculum from the dilution tubes were streaked on MacConkey agar and Deoxycholate Agar (DCA). After incubation at 37^{0}C for 24hrs, pale coloured colonies on MacConkey and colourless colonies usually with black centre on DCA were suspected to be *Salmonella* species.

Suspected colonies of *Salmonella* species were later confirmed by a series of biochemical tests.[6]

Enumeration of Fungi

One mililitre (1ml) from each dilution tube was transferred into each of the appropriately labelled duplicate Petridishes followed by pouring malt extract agar. After solidification, plates were incubated at room temperature for 3 - 5 days. When there is excessive growth, colonies were counted first after 3 days and then again after 5 days. The number obtained was multiplied by the inverse of the dilution factor to get cfu/ml.[6]

Isolation and Enumeration of *Staphylococcus aureus*

From the serially diluted tubes, 0.5ml was transferred into appropriately labelled Petridishes. This was followed by pouring aseptically molten Baird Parker agar (BPA). The plates were then incubated at 37°C for 24hrs. Following incubation, plates were checked for colonies with dark centre. The number of colonies were counted and multiplied by two (2) and then by the inverse of the dilution factor to get the colony forming unit (cfu/ml). Suspected colonies of *Staphylococcus aureus* were later confirmed by a series of biochemical tests.[8]

Detection of Mould and Yeast

Based on physical appearance and reverse pigmentation from the Malt Extract Agar (MEA), and also microscopic examination of the isolates after five days of incubation at room temperature, different types of mould were examined as described FAO.[6]

Determination of Temperature of the Sample

Temperature of the samples was measured by putting mercury in glass thermometer into the milk at the time of collection and the value was recorded.[6]

Determination of pH of the sample

Acidity and alkalinity of the milk was measured using mobile battery operated pH meter manufactured by Entryway Industries Limited. The result was recorded as described by FAO.[6]

Determination of the Critical Control Points

All the steps involved in the local milk processing were carefully studied. The result obtained from the microbiological analysis of each step were compared with that of critical control point (CCP) decision tree to establish whether that step is a critical control point or not.[9]

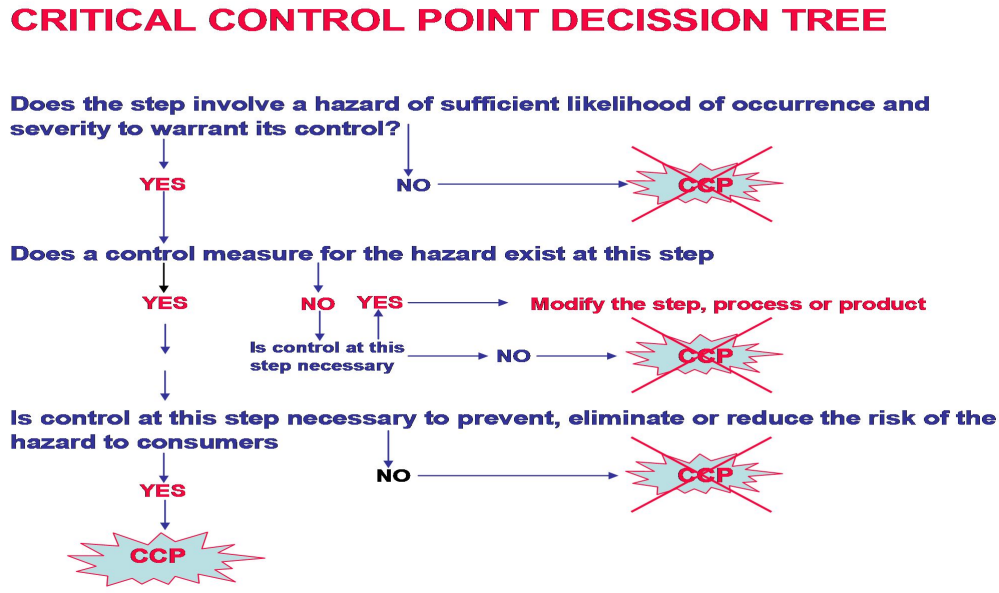

Figure 2 Critical Control Point Decision Tree.
Source: (NACMCF, 1997).

Statistical Analysis

The statistical tool used for this research was SPSS 16.0 vision 2007 model. Raw data were transformed to mean values for analysis. A significance level of .05 (95%) level of confidence was used for all tests. For the Aerobic Plate Counts (APC), Staphylococcal Counts (SC), and Fungal Counts (FC), an analysis of variance (ANOVA) was used to analyse 4 stages of local milk processing as for the fresh milk (FM), mixed milk (MM), unskimmed fermented milk (USM) and skimmed fermented milk (SM). No statistical tests were conducted for the *E. coli*, *S. aureus* and *Salmonella* spp detection. For comparism of APC, SC and FC of Ungogo and Kumbotso Local Governments, student t test was used.

Table 1: Mean microbial counts of sample from Ungogo Local Government

Sample				
N=25	Mean APC(cfu/ml)	CC(MPN/ml)	Mean SC(cfu/ml)	Mean FC(cfu/ml)
FM	$2.05x10^{6a}$	150	$1.94x10^{5a}$	$1.99x10^{7a}$
MM	$5.23x10^{7a}$	>2,400	$2.26x10^{6a}$	$8.29x10^{7a}$
USM	$1.25x10^{8ns}$	44	$3.98x10^{5a}$	$1.07x10^{8ns}$
SM	$1.43x10^{8ns}$	44	$6.03x10^{6ns}$	$1.39x10^{8ns}$

Key:

APC = Aerobic plate count
FM = Fresh milk sample
CC = Coliform count
MM = Mixed milk sample
SC = Staphylococcal count
USM = Unskimmed fermented milk sample
FC=Fungal count
SM = Skimmed fermented milk sample

[a]Transformed mean followed by the same letter means there is significant difference at P>0.05 between the four stages of milk processing.
[ns]Means no significant difference (P<0.05)

Table 2. Mean Microbial Counts of Samples from Kumbotso Local Government.

Sample				
N=25	Mean APC(cfu/ml)	CC(MPN/ml)	Mean SC(cfu/ml)	Mean FC(cfu/ml)
FM	$1.07x10^{7ns}$	36	$7.29x10^{5ns}$	$5.20x10^{5a}$
MM	$3.57x10^{7ns}$	>2,400	$2.46x10^{6ns}$	$7.12x10^{7ns}$
USM	$1.31x10^{8a}$	>2,400	$3.84x10^{5ns}$	$8.36x10^{7ns}$
SM	$9.84x10^{7a}$	36	$1.94x10^{6ns}$	$2.43x10^{8a}$

Key:

APC = Aerobic plate count
FM = Fresh milk sample
CC = Coliform count
MM = Mixed milk sample
SC = Staphylococcal count
USM = Unskimmed fermented milk sample
FC=Fungal count
S = Skimmed fermented milk sample
N = Number of sample

[a]Transformed mean followed by the same letter means there is significant difference at P>0.5 between the four stages of milk processing.
[ns]Means no significant difference

Table 3. Frequency of Contamination of Samples with Pathogens in Ungogo Local Government

Sample N=25	*E. coli* FD(%)	*S. aureus* FD (%)	*Salmonella* spp FD (%)	*Aspergillus* spp. FD (%)	*Fusarium* spp FD (%)	*Candida* spp FD (%)	*Pen.*Spp FD (%)
FM	02(8)	04(16)	04(16)	09(36)	04(16)	08(32)	07(28)
MM	05(20)	23(92)	06(24)	12(48)	06(24)	12(48)	10(40)
USM	02(8)	07(28)	08(32)	09(36)	09(36)	12(48)	13(52)
SM	04(16)	20(80)	07(28)	10(40)	12(48)	15(60)	17(68)

Key

FM = fresh milk sample
MM = mixed milk sample
USM =Unskimmed fermented milk
SM = Skimmed fermented milk
N = Number of sample
E. coli= Escherichia coli
S. aureus = Staphylococcus aureus
Spp = Species
Pen. = *Penicillium*% = Percentage
FD= frequency distribution

Table 4. Frequency of Contamination of Samples with Pathogens in Kumbotso Local Government

Sample N=25	*E. coli* FD(%)	*S. aureus* FD(%)	*Salmonella* spp FD(%)	*Aspergillus* spp FD(%)	*Fusarium* spp FD(%)	*Candida* spp FD(%)	*Pen.* Spp FD(%)
FM	00(00)	10(40)	02(8)	08(32)	09(36)	08(32)	10(40)
MM	04(16)	21(84)	07(28)	12(48)	12(48)	09(36)	12(48)
USM	05(20)	13(52)	07(28)	12(48)	10(40)	15(60)	08(32)
SM	01(4)	21(84)	06(24)	15(60)	13(52)	12(48)	10(40)

Key

FM = fresh milk sample
MM = mixed milk sample
USM= Unskimmed fermented milk
SM = Skimmed fermented milk
N = Number of sample
E. coli = Escherichia coli
S. aureus = Staphylococcus aureus
Spp = Species
Pen. = *Penicillium*FD =Frequency distribution
% = Percentage

Table 5. Mean Standard Deviation of pH And Temperature Of Milk Samples From Ungogo And Kumbotso Local Governments.

Sample	**Ungogo**		**Kumbotso**	
N=25	pH	Temperature (^oC)	pH	Temperature (^oC)
FM	6.52 ± 0.11^{ns}	33.16 ± 2.69^{ns}	6.53±0.05ns	27.08±6.04ns
MM	6.54 ± 0.09^{a}	32.08 ± 3.23^{ns}	6.55±0.03a	26.60±5.90ns
USM	5.67 ± 0.18^{a}	26.56 ± 1.76^{a}	5.45±0.60a	19.76±5.50a
SM	4.71 ± 0.43^{a}	25.40 ± 2.10^{a}	5.09±0.43a	20.64±6.22a

Key

FM Fresh milk sample
MM Mixed milk sample
SM Skimmed fermented milk
USM Unskimmed fermented milk
N Number of samples ± plus or minus

[a]Transformed mean followed by the same letter means there is significant difference at (P>0.5) between the four stages of milk processing. [ns]Means no significant difference

Table 6. Comparison of Mean Microbial Counts Between Ungogo and Kumbotso Local Government Samples.

1	Aerobic plate count	Mean Difference	Q P value		Comparison
FM		-8639760	0.6366	ns p>0.05	U vs K
MM		$1.69x10^7$	1.248	ns p>0.05	" "
USM		-5872000	0.4326	ns p>0.05	" "
SM		$4.46x10^7$	3.304	ns p>0.05	" "
2	**Staphylococcal Count**				
FM		-535231	0.4518	ns p>0.05	" "
MM		-199413	0.2316	ns p>0.05	" "
USM		13430	0.01213	ns p>0.05	" "
SM		4093809	5.031	[a] P<0.05	" "
3	**Fungal Count**				
FM		-6792400	0.1806	ns p>0.05	" "
MM		$1.176x10^7$	0.3127	ns p>0.05	" "
USM		$2.38x10^7$	0.6337	ns p>0.05	" "
SM		$1.05x10^8$	2.796	ns p>0.05	" "

Key:
FM= Fresh milk sample
MM= Mixed milk sample
USM=Unskimmed fermented milk
SM= Skimmed fermented milk
P=Probability,
ns= No significant difference,
[a]= Indicate significant difference,
U=Ungogo,
K=Kumbotso,
q=Level of confidence

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Discussion

The major microbiological hazard is the presence of high microbial population in the fresh milk (FM), mixed milk (MM), unskimmed fermented milk and skimmed fermented milk. This is really a hazard and therefore unacceptable because according to O'Connor (1995), milk produced under hygienic conditions from healthy animals should not contain bacterial counts in number greater than $1.0x10^5$ cfu/ml. Presence of high Staphylococcal count which ranged from $1.94x10^5$ to $6.03x10^6$cfu/ml is also a point of concern and hence hazardous, because *Staphylococcus* is among the most important cause of food poisoning owing to their ability to produce potent enterotoxins and therefore, the count of 10^6cfu/ml is enough to produce enterotoxins in food.[9]

Generally, coliform bacteria are known to be indicators of some degree of potentially hazardous contamination. According to standard by Harrignan,[10] coliform in milk and related products with less than 10, greater than 100 and range of 500 – 2,500 are regarded as satisfactory, unsatisfactory and dubious respectively.

The results of this study showed three (3) bacterial genera (*Escherichia coli, Salmonella and Staphylococcus aureus*) and four (4) fungal genera (*Aspergillus* spp, *Candida* spp, *Penicellium* spp and *Fusarium* spp) identified in all samples obtained from Ungogo and Kumbotso Local Government Areas of Kano metropolis. Umoh[11] also isolated *Staph*ylococcus *aureus*, *E .coli,* and *Salmonella* in contamination of *fura da nono*. Of the bacterial genera isolated in this work, *S. aureus* is predominant in all samples tested. This study is supported by reports of Eka[12] that also isolated *S. aureus*. The contamination of the samples with these organisms might have originated from the animals coughing or via contact with hands and other body parts of the processors since the organisms like *S. aureus* is among normal body flora of human and

animals.[13] The presence of *E. coli* and *Salmonella* is an indication of contamination that may be traced to fecal origin and this is also a hazard and unacceptable because according to FAO[6], *E. coli* should not be recovered. The incidence of these organisms in both milk and milk products have been reported by Bryan.[14]

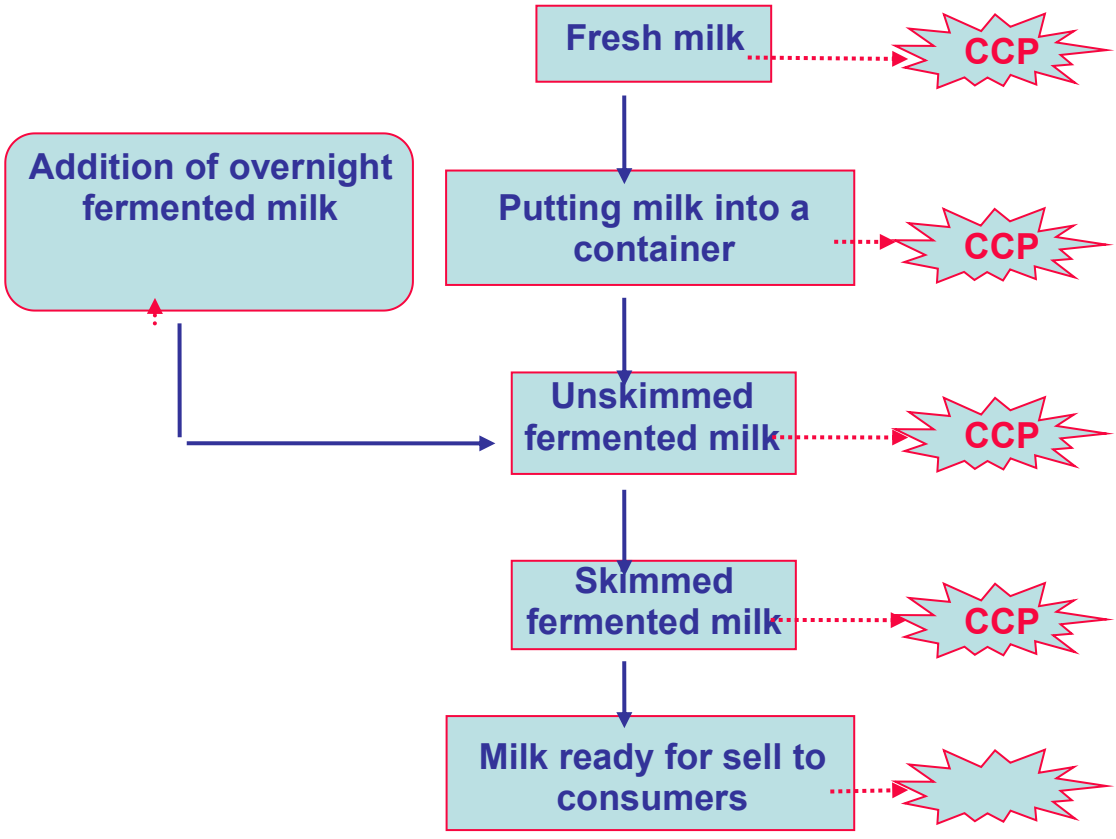
The genera of fungi that were isolated and identified in this work using both cultural and microscopic characteristics were *Aspergillus* spp, *Candida* spp, *Penicelium* spp and *Fusarium* spp. Their presence in milk products may result in immediate or cumulative health hazard, because fungi especially mould secrete some extracellular toxic metabolites called mycotoxins which results in serious intoxication in man and other animals including birds.[15] For instance *Aspergillus flavus* produces aflatoxins. When a lactating cow consumes aflatoxin B contaminated feed, part of the toxin is hydroxylated and excreted in milk as aflatoxin M.[14]

The comparison of mean aerobic plate counts, staphylococcal counts and fungal counts of fresh, mixed, unskimmed fermented and skimmed fermented milk samples between Ungogo and Kumbotso local government showed no significant differences between microbial counts of Ungogo and Kumbotso local government sample except in skimmed fermented milk samples of Staphylococcal counts showed a significant difference at $P>0.05$.

In addition, the mean pH and temperature from all the samples which range between 4.71 to 6.55 and 19.76^{o}C to 33.16^{o}C is ideal environment for the growth of many microorganisms that can cause spoilage of dairy products because according to Cousin,[16] it only takes one psychrotroph per container of milk to cause spoilage.

Identified Critical Control Points in Local Milk Processing

Based on the results obtained in the four stages of local milk processing analysed (fresh, mixed, unskimmed fermented and skimmed fermented milk) and using the critical control point decision tree provided by the United States National Advisory Committee on Microbiological Criteria for Foods[9], chart flow below is the modified flow diagram of local milk processing and the identified critical control points.



Flow Chart of Local Milk Processing with the Identified Critical Control Point CCP. (Ccp) = Critical Control Point.

Step One (Fresh milk sample) A fresh milk sample in this research is the one collected directly from the cow udder into the sterile sample bottle. This step answer all the three steps of critical control point decision tree because the hazard occurs at this step by the presence of indicator organisms and counts above the acceptable limit, control measure exist and is necessary in order to prevent, reduce or eliminate the hazard to an acceptable limit. This is by cleaning the udder, hand of the processor and reducing the holding time and temperature after collection for processing.

Step Two (Mixed milk sample) This milk is taken from the processors container after milking all the cows. This step or point is a critical control point because of the high mean microbial counts and presence of indicator organisms. This hazard can be controlled by cleaning the container and hand of the processor. This control is necessary so as to reduce, prevent or eliminate this hazard to an acceptable level.

Step Three (Unskimmed fermented milk *'Kindirmo'*) As defined by Umoh[11], unskimmed fermented milk is locally uncontrolled fermented cow milk. It is milk at which overnight fermented milk is added as a starter culture and the inoculated fresh milk is left overnight at room temperature for fermentation to get sour milk known as '*Kindirmo*'. In this step, the hazard exits because of the high microbial count and presence of indicator organisms, the hazard can be controlled by pasteurization and controlling the fermentation time and temperature. Control at this step is necessary since many consumers consume the product at this step.

Step Four (Skimmed fermented milk sample *'Nono'*) This milk has the same process as above but differs because there is removal of fat and addition of large volume of water to the curled milk which is then stirred with T shaped stick to get a liquid of fine consistency which give rise to the skimmed fermented milk.[17] This point or step is also a critical control point because the hazard exists and can be

controlled by holding the pH, time and temperature of the milk at 40^{O}C and also use of potable water. This will prevent, reduce or eliminate the hazard to an acceptable limit.

Conclusion

There are high microbial counts at different stages of the samples isolated in both Ungogo and Kumbotso local government which are above the acceptable value of 1.0x10^{5}cfu/ml.

The detection of indicator organisms was carried out and organisms detected included *Escherichia coli, Salmonella* species and *Staphylococcus aureus*. The present study showed that the quality of milk processed in the study area was unsatisfactory. This was evident from the high values of mean aerobic plate count (APC), mean fungal count (FC), coliform count (CC) and presence of indicator organisms. All the various stages examined are found to be critical control point (CCP) based on the CCP decision tree mentioned earlier. These stages are CCP because they involve hazards, control measures exist and the measures must be applied in order to eliminate or reduce the hazards.

Recommendations

It is therefore recommended that:-

- The local milk processors should exercise personnel hygiene in all processing stages.
- Use of hygienic milking, improving milk and milk handling environment.
- The local milk processors should also be encouraged to pasteurize the milk before fermentation so as to reduce the microbial load.
- The poor bacteriological quality observed in the present study requires further investigation of the status of the animals' health, especially mastitis and the significance of the effect of containers to ascertain their contribution on microbial quality.

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