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An investigation on the causes of *Escherichia coli* and coliform contamination of cheddar cheese and how to reduce the problem (A case study at a cheese manufacturing firm in Harare, Zimbabwe)

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Abstract: The aim of this study was to investigate the causes of *E. coli* contamination of Cheddar cheese through the application of Hazard Analysis Critical Control Points (HACCP) principles. Cheese samples were analyzed for *E. coli* and coliforms after production, during the validation stage, as well as at the verification stage. Average *E. coli* and coliform counts were analyzed statistically using the t-test. Results showed that after the implementation of the corrective measures there was a decrease in *E. coli* and coliform counts at the 5% level of significance. Results presented in this study also show that manufacturing Cheddar cheese whole observing high standards of hygiene improves the reduces *E. coli* and coliform contamination of the product, even though the problem is not completely eliminated.

Keywords: Validation, Hazard Analysis Critical Points (HACCP), Pathogenic Microorganisms, Coliform Count

1. Introduction

A company that specializes in the production of dairy products (cheese, ice cream and yoghurt) in Harare was selected for this study. Among the wide range of products that are produced at Kefalos, Cheddar cheese is one of the major income generating products. The production process is labour-intensive and human resources are used extensively for all major processes including raw material acquisition, conversion of raw materials into final product, packaging and dispatch.

The bulk of Cheddar cheese is widely consumed in its natural state (Steffen *et al.,* 1993) while smaller quantities are further processed and consumed as pasteurised process cheese. The cheese is packaged in different forms such as blocks, cuts, slices or shreds to suit the needs of customers. Cheese is a good substrate for the growth of certain adaptive fungal species and other bacteria due to its low pH, elevated salt concentration and low water activity [3]. Growth of microorganisms can occur on cheese during its ripening period or along the distribution chain under refrigerated storage and this can result in a safety and spoilage problem.

Refrigeration alone cannot reduce the number of pathogens, as it has been proven that *Listeria monocytogenes* and other psychrotrophic pathogens are capable of growth at these temperatures. Therefore, lack of pathogen growth in fermented dairy foods may point to effective implementation of control factors such as diligence to good hygiene practices.

During the manufacture of semi-soft, hard and very hard cheese, the cheese is subjected to relatively long exposure periods at temperatures that are ideal for the growth of bacteria [3] . For example, Cheddar and other related varieties are incubated at 31-39°C during manufacture and are hooped at temperatures in the 32-37°C range. Many

Cheddar-type cheeses are cured or aged at temperatures reaching up to 15.6°C. Swiss cheese is held for 4-8 weeks at a temperature of 22.2-23.3°C to develop the characteristic 'eyes' and flavour. If storage of Cheddar and Swiss cheese at room temperature had any inherent detrimental effect on safety of these cheeses, then neither would be safe to consumer [3].

Pathogens such as *Escherichia coli* 0157:H7, *Salmonella, Listeria monocytogenes* and *Staphylococcus aureus* have been found in low as well as high moisture cheese as a result of poor pasteurisation [6]. Post pasteurisation contamination also plays a role in pathogen manifestation in cheese. The main objectives of this study were to investigate the ways by which Cheddar cheese is contaminated during production and processing. Further investigations were to establish ways by which contamination of cheese may be reduced.

2. Materials and Methods

2.1. Scope of Specifications

The Product Design Specifications Sheet for Cheddar manufacturing was drawn from the following requirements: specific standards of the raw milk, the pasteurization and inoculation temperatures, the desired product specifications after the pressing stage, the cultures used and overall sensory characteristics expected. General cleanliness of the machinery, materials, personnel, as well as the quality of water, scale of production and cleaning processes were also some of the requirements.

Cheese and raw milk standards were collected from secondary sources.

Fat, protein, pH and lactose content: Three milk samples from different producers were taken, these were analysed for lactose, protein and fat using a Lactoscan. The samples were warmed to 30°C in a vial before being tested. The determination of fat was also done using the Gerber test.

Somatic cell count (SCC): A somatic cell counter was used to determine the cell counts. This was done by the milk producers themselves.

Bacteria counts: The traditional method of colony forming units (CFU) was used to determine total bacteria counts. *Coliforms and E. coli*: Serial dilution of samples was performed using sterile maximum recovery diluent (MRD) and 1ml aliquots of each sample were plated in 15 ml duplicate MacConkey and Plate count agar pour plates. the selected media was added. Then the cultured plates were incubated at 37°C for 24 hrs and 25°C for 5 days for coliforms.

Plates were incubated at 37°C for 24hrs and colonies enumerated. MRD was also used to collect and subsequently determine coliform and *E. coli* on swabs taken on production personnel as well as equipment used using the same procedure.

Antibiotics: The antibiotic detection test was performed on milk using the ßeta s.t.a.r test (Neogen Corporation, Lansing, MI, USA) according to manufacturer's instructions.

Added water: A lactometer was used to determine if the milk contained any added water.

2.2. Process Audit for the Manufacture of Cheddar Cheese

A process audit for the manufacture of Cheddar cheese was implemented to evaluate efficiency and identify problems, and according to the following regimen.

2.2.1. Prerequisite Plan

Plant construction and equipment programme: this component of the prerequisite plan addressed physical aspects of the primary production or cheese processing facility and their maintenance. It considered the facility's surroundings and the general layout (environmental factors). The design of materials, use of cleaning equipment and utensils was also considered (sanitary factors).

Standard Operating Procedures (SOPs):

Standard Operating Procedures (SOPs) for critical processing operations such as pasteurisation or cooling, instituted with the purpose of addressing a food-borne disease risk factor, were reviewed.

Sanitary Standard Operating Procedures (SSOPs):

Sanitary Standard Operating Procedures (SSOPs) which describe how the facility and equipment must be cleaned and sanitized, were also reviewed.

Personnel hygiene:

This aspect assessed the facility's policy regarding sick workers, the employees' general appearance of cleanliness, the clothing they wore and hand washing rules. It also examined employees' facilities (toilets, hand washing stations, showers, locker rooms and eating areas).

Supplier specifications and control:

Supplier specifications for all raw materials received by the primary operation or processing plant were assessed against set standards.

2.2.2. Microbiological Analysis

Samples were examined for coliform and *E. coli* counts (Section 2.1). The surface (spread) plate technique was applied for enumerating yeasts and moulds.

Glassware such as Petri-dishes, test tubes, pipettes, flasks and bottles were sterilized in a hot oven at 170°C for two hours, whereas all media was autoclaved for 15 min at 121°C.

2.2.3. Chemical Analysis

Total solids content was determined using a moisture analyser and verified using a Lactoscan. Protein content was also determined using the Lactoscan. Fat content was determined by Gerber method and the solids not fat were obtained by subtracting fat from total solids. The pH of cheese samples was determined using an electronic pH meter.

2.2.4. Process Validation

The validation process focused on the collection and evaluation of scientific, technical and observed information to determine whether control measures were capable of achieving their specified purpose in hazard control.

2.2.4.1. The Process Stages which were Audited were as Follows

- Pasteurisation (72-75°C/15sec) and efficiency
- Inoculation temperature (32°C)
- Inoculation
- Curd heating (39°C)
- Whey draining (pH 6.4)
- Cheddaring (pH 5.2)

2.2.4.2. Determination of Pasteurisation and Inoculation Temperatures, Time and Efficiency

Pasteurization and inoculation temperatures and time were collected from secondary sources. This information was essential to evaluate the temperature and time combination which are essential for cheese milk in order to produce a quality product.

2.2.5. Monitoring of Control Measures

Monitoring of control measures was an on-going collection of information at the steps the control measures were applied. The information established that the measures were functioning as intended i.e. within established limits.

2.2.6. Verification

Verification was used to determine whether the control measures had been implemented as intended.

2.2.7. Statistical Analysis

The results of the average coliform and *E. coli* counts on the cheddar cheese after production at validation and verification after the implementation of the corrective measures were analysed using the t-test. Statistical analysis was performed using the SPSS version 9.

3. Results

3.1. Raw Milk

3.1.1. Compositional Analysis

Table 1(a) summarises the compositional analysis results.

Table 1(a). *Compositional analysis of milk from producers.*

	Producer A	Producer B	Producer C
Fat (%)	3.60	3.06	2.68
Protein (%)	3.09	3.12	3.07
Lactose (%)	4.10	3.91	4.00

3.1.2. Somatic Cell Count (SCC)

Somatic cell count was done by the producers due to the unavailability of the somatic cell counter or other means to test for somatic cell.

3.1.3. Bacterial Count

Table 1(b). *Mean bacterial count of the milk samples.*

	Bacterial count (cfu × 10^3/ml)
Producer A	5
Producer B	15
Producer C	12

Bacterial count results were taken from previous results of milk producers and averaged to obtain an estimate (Table 1b).

3.1.4. Coliforms and E. Coli

Table 1 (c) summarises the result of the analysis of three samples.

Table 1(c). *Coliform/E. coli analysis of milk samples*

	Coliforms / *E. coli*
Producer A	-negative
Producer B	+positive
Producer C	-negative

3.1.5. Antibiotics and Added Water

Table 1 (d) shows the results for the antibiotic and determination of added water tests.

Table 1(d). *Antibiotics and added water tests*

Producer	Antibiotics	Added water (%)
A	negative	-
B	negative	-
C	negative	0.002

3.2. Process Audits

3.2.1. Process Validation Results

Table 2(a). *First process audit results for process parameters at validation stage*

Process parameter	Result
Pasteurization (72°C/15sec)	×
Inoculation temperature (32°C)	√
Inoculation	√
Curd heating (39°C)	√
Whey draining (pH 6.4)	√
Cheddaring (pH 5.2)	√

Key:
x specification not met
√ parameter met

Table 2(b). *Process audit results for coliform and E. coli test on cheese production personnel at validation stage*

Process stage of operation	Personnel	Coliform and *E. coli* test
Pasteurisation	Vat attendant	-ve
	Pasteurizer operator	-ve
Addition of colour	Vat attendant	-ve
Addition of $CaCl_2$ and KNO_3	Vat attendant	-ve
Temperature check	Vat attendant	-ve
Inoculation	Vat attendant	-ve
Temperature check	Vat attendant	++ve
Rennet addition	Vat attendant	+ve
Cutting	Vat attendant	-ve
	Production assistant	+ve
Stirring after 15mins from cutting	Vat attendant	-ve
Stirring after 45mins from cutting	Vat attendant	-ve
Stirring after 75mins from cutting	Vat attendant	-ve
Wheying off	Vat attendant	+ve
Process stage of operation	Personnel	Coliform and *E. coli* test
4th turn	Vat attendant	++ve
7thturn	Vat attendant	-ve
Salting and cutting	Vat attendant 1	+ve
	Vat attendant 2	+ve
Shovelling and pressing	Production assistant 1	-ve
	Production assistant 2	+ve
	Production assistant 3	+ve
	Production assistant 4	++ve

Key: -ve negative for coliform and *E.coli* tests, +ve positive for coliform tests only, ++ve positive for both *E. coli* and coliform test

Table 2(c). *Process audit results for coliform and E.coli test on equipment at validation stage*

Process stage of operation	Product	Coliform and *E. coli* test
Pasteurisation	*Vat interior*	-ve
	Agitator blade	-ve
	Speed knob	-ve
	3 bucket system chlorinated water	-ve
Addition of colour	*Measuring cylinder*	-ve
	Dip stick	-ve
Addition of $CaCl_2$ and KNO_3	*20l bucket*	-ve
Temperature check	*Thermometer*	-ve
Inoculation	*Culture sachet*	-ve
	Knife	-ve
Temperature check	*Thermometer*	++ve
Rennet addition	*Measuring cylinder*	-ve
Cutting	*Cheese cutter*	+ve
Stirring after 15mins from cutting	*Thermometer*	-ve
Stirring after 45mins from cutting	*Thermometer*	-ve
	Vial	+ve
Stirring after 75mins from cutting	*Thermometer*	+ve
	Vial	+ve
Wheying off	*Shovel*	++ve
	Strainer	+ve
	Clamps	-ve
Cheddaring 1st turn	*Vat interior*	-ve
	Vial	-ve
	Knife blade	++ve
4th turn	*Vial*	-ve
7thturn	*Vial*	-ve
Salting and cutting	*Cheese knife*	++ve
	5l salt container	-ve
Shovelling and pressing	*Shovel*	++ve
	Working table	-ve
	Cheese cloth	-ve
	Cheddar forms	++ve

Key:
-ve negative for coliform and *E.coli* tests,
+ve positive for coliform tests only,
++ve positive for both *E. coli* and coliform test

Table 2(d). *Coliform and E.coli counts in cheddar cheese during the validation stage*

Sample	Coliform (cfu x 10^3/ml)	*E. coli* (cfu x 10^3/ml)
1	90	23
2	200	37
3	433	42
4	109	29
5	117	31
6	78	11
7	44	3
8	150	34
9	97	20
10	630	44

Table 2 (e). *Observations on plant personnel behaviors during production*

Stage /Activity	Observation	Corrective measure
Milk pasteurisation	*-The pasteuriser operator would set the pasteuriser on forward flow so that in cases when steam would drop the milk continued to flow in the vats without being diverted to the holding tube of the pasteuriser.*	-Pasteuriser operators were trained on how to properly operate the pasteurizer
	-The pasteuriser operator would also give assistance to the vat attendant thus getting in contact with the cheese curd in process.	-Pasteuriser operators were relieved of duties concerning vat attending
Vat cleaning	*-Plant personnel used 20l buckets of hot water to sterilize the vat but in fear to self-injure themselves ineffective vat cleaning occurred*	-Hose pipe which provided water at around 80°C was installed
	-No standard procedure for vat cleaning was followed.	-A proper vat cleaning procedure was developed with appropriate quantities of detergent to be used.
Equipment cleaning and sanitisation	*-Before using equipment during the cheese production, the vat attendant would dip the desired equipment in a vat of boiling water but because the water would be hot and the equipment is metallic the workers would partially deep the piece of equipment so that he/she would not be burnt.*	-Clamps to hold equipment when dipping in hot water were made available -A chlorinated water bath was introduced in the cheese plant
	-Equipment like thermometers and vials were supposed to be sanitized in chlorinated water, however at times this sanitisation procedure was by-passed.	-A policy was made that all equipment should be put in a vat of boiling water after every production cycle to make sure that they are adequately cleaned and ready for the next production.
Hand washing	*-Plant personnel frequently neglected the three bucket system during production and at times would just rinse their hands in chlorinated water.* *-Personnel from other departments would pass through the cheese processing area without washing their hands and would actual greet cheese personnel by handshakes.*	-training sessions were provided for plant personnel -Hand swabs were taken at a frequent basis to make sure that people effectively washing their hands -printed signage were hanged on the walls which showed proper hand washing procedures
	-After taking some breaks, improper hand washing procedure was done by personnel	-personnel trafficking through the cheese production area was restricted for cheese personnel only
	-In fear to cause some bruises on their hands, workers would lightly brush their hands during washing.	-Smooth hand scrubs were provided to substitute the hard brushes

3.2.2. Process Verification Results

Table 3(a). *Process audit results for process parameters at verification stage*

Process parameter	Result
Pasteurisation (72^0C/15sec)	√
Inoculation temperature (32^0C)	√
Inoculation	√
Curd heating (39^0C)	√
Whey draining (pH 6.4)	√
Cheddaring (pH 5.2)	√

Key:
x- parameter not met
√- parameter met

Table 3(b). *Process audit results for coliform and E.coli test on cheese production personnel at verification stage*

Process stage of operation	Personnel	Coliform and *E.coli* test
Pasteurisation	Vat attendant	-ve
	Pasteurizer operator	-ve
Addition of colour	Vat attendant	-ve
Addition of $CaCl_2$ and KNO_3	Vat attendant	-ve
Temperature check	Vat attendant	-ve
Inoculation	Vat attendant	-ve
Temperature check	Vat attendant	++ve
Rennet addition	Vat attendant	+ve
Cutting	Vat attendant	-ve
	Production assistant	+ve
Stirring after 15mins from cutting	Vat attendant	-ve
Stirring after 45mins from cutting	Vat attendant	-ve
Stirring after 75mins from cutting	Vat attendant	-ve
Wheying off	Vat attendant	-ve
Cheddaring 1st turn	Vat attendant	-ve
4th turn	Vat attendant	++ve
7thturn	Vat attendant	-ve
Salting and cutting	Vat attendant 1	-ve
	Vat attendant 2	+ve
Shovelling and pressing	Production assistant 1	-ve
	Production assistant 2	-ve
	Production assistant 3	-ve
	Production assistant 4	-ve

Key:
-ve negative for coliform and *E.coli* test,
+ve positive for coliform tests only,
++ve positive for both *E.coli* and coliform test

Table 3(c). *Process audit results for coliform and E. coli test on equipment at verification stage*

Process stage of operation	Product	Coliform and *E.coli* test
Pasteurisation	Vat interior	-ve
	Agitator blade	-ve
	Speed knob	-ve
	3 bucket system chlorinated water	-ve
Addition of colour	Measuring cylinder	-ve
	Dip stick	-ve
Addition of $CaCl_2$ and KNO_3	20l bucket	-ve
Temperature check	Thermometer	-ve
Inoculation	Culture sachet	-ve
	Knife	-ve
Temperature check	Thermometer	-ve
Rennet addition	Measuring cylinder	-ve
Cutting	Cheese cutter	+ve
Stirring after 15mins from cutting	Thermometer	-ve
Stirring after 45mins from cutting	Thermometer	-ve
	Vial	-ve
Stirring after 75mins from cutting	Thermometer	-ve
	Vial	+ve
Wheying off	Shovel	+ve
	Strainer	-ve
	Clamps	++ve
Cheddaring 1st turn	Vat interior	-ve
	Vial	-ve
	Knife blade	+ve
4th turn	Vial	-ve
7thturn	Vial	-ve
Salting and cutting	Cheese knife	++ve
	5l salt container	-ve
Shovelling and pressing	Shovel	+ve
	Working table	-ve
	Cheese cloth	-ve
	Cheddar forms	-ve

Key:
-ve negative for coliform and *E. coli* tests,

+ve positive for coliform tests only,
++ve positive for both *E. coli* and coliform test

Table 3(d). *Coliform and E. coli counts in cheddar cheese during the verification stage*

Sample	Coliform (cfu x 10^3/ml)	*E. coli* (cfu x 10^3/ml)
1	44	20
2	1	0
3	15	4
4	9	3
5	22	44
6	2	0
7	8	2
8	2	0
9	18	9
10	13	7

4. Discussion

Cattle can harbour the bacterium, *E. coli*, without any ill effects, shedding them in their faeces, and from which they gain entrance into raw milk [22] . Therefore, this gave a reason why raw milk received from various producers tested *E. coli* positive. However, when proper thermal treatment via 'pasteurisation' was applied to the milk, bacterial counts dropped significantly, as expected. The level of hygiene during milking and the cleanliness of the vessels used for storing and transporting the milk are factors which determine the number of spoilage bacteria in raw milk. Raw milk is however, protected from spoilage by inherent natural antibacterial compounds. For the first 2-3 hours after milking, and if the milk is not cooled, these antibacterial substances are degraded resulting in rapid bacterial multiplication [32] .

From the results of three milk samples from the three producers, it was observed that producer A had the best milk which meets all the specification standards.

The phosphatase test was performed to measure the efficiency of the pasteuriser. Phosphatase is a natural milk enzyme [23[, which is heat-sensitive. Some of the results indicated positive phosphatase activity in pasteurised milk implying that pasteurisation was insufficient to destroy the enzyme. This finding prompted further investigation and the process validation report revealed that the pasteuriser was malfunctioning. The milk continued to flow into the vats at times when the pasteuriser steam had dropped, which caused the pasteuriser's temperature to drop as well. Instead of diverting back the milk immediately when the temperatures drop, the forward flow of milk continued until the temperature dropped to 65°C. It was also observed that at times the operator would set the pasteuriser at forward flow allowing raw milk into the vats when the steam was low. Because pasteurisation is credited with dramatically reducing pathogens in milk, improving the shelf life and safety of processed milk [18], this would have meant that contaminated milk may have been used to process cheese. After the pasteuriser was serviced, its efficiency was verified using the phosphatase test and results showed no enzyme activity in milk after pasteurization, thus indicating proper function. The pasteuriser operators also received thorough training on how to operate the machine correctly. *E.coli* and coliform occurrence in Cheddar cheese decreased after this corrective measure was taken.

E.coli should not be present in milk after pasteurisation and the best way to control *E.coli* is through employee education and observation of strict sanitation measures. To minimise contamination of milk from the milk processing environment, all floors of milk reception and processing rooms should be cleaned after a daily operation [25] . The outlined procedure is to first hose down the floor with water to remove milk or the cheese curd remnants, then sweeping with a detergent solution using a stiff broom and finally hosing with hot water.

However, the validation results showed that *E.coli* and coliforms were detected on equipment due to poor sanitation. Assessment of the vat cleaning procedure showed that the vats were poorly sterilized before the beginning of production. Personnel used 20l buckets to pour hot water onto the vats. Due to the precautions taken by the worker in trying not to hurt him/herself when cleaning the vat, insufficient sterilization resulted and there was a high possibility of having surviving coliform bacteria. As a corrective measure, a hot water pipe with water at 80°C was installed and used. This method proved to be more effective and less risky. A detailed vat cleaning procedure was then developed. In addition, a chlorinated water bath for sanitizing equipment during cheese production was also introduced into the cheese plant so that the equipment would be sanitized at frequent intervals.

The assessment of personnel behaviour at the validation stage gave some insight as to how personnel led to the contamination of the curd. They disregarded the three bucket system which is a sequential hand washing procedure in disinfectant, water and sanitizer, and was supposed to be observed frequently (on average after every 10 minutes). Other habits such as hand shaking during cheese-making and the re-use of cleaning brushes without intermittent cleaning are factors identified for cheese contamination by *E. coli* and coliforms. After evaluating the behaviour of plant personnel and conducting one-on-one interviews, it was clear that personnel had received poor training on hygiene issues.

Thus, employees were trained to appreciate and understand the scientific principles upon which such laws are based. Hand washing procedures were clearly demonstrated and signage with recommended chlorine and soap dilutions were displayed in all departments to ensure effective washing and sanitising of hands.

The pasteuriser operators were relieved of all vat attending duties and these were left to the cheese maker to avoid contamination of the pasteurised milk.

After the implementation of corrective measures plant personnel were now following sanitary procedures knowledgeably and with an understanding of the importance of doing so. In addition, signage to restrict plant personnel from other departments from entering the cheese-production area were also put up at every entrance point.

From the statistical analysis, it was shown that there was a decrease in the average *E. coli* and coliform counts on the Cheddar cheese process after the implementation of corrective measures as compared to counts obtained during the validation audits.

5. Conclusion

Poor adherence to sound hygienic practices, incorrect operation of salient processing machinery such as the pasteuriser, as well as uncontrolled people movements within the production area were identified as some of the important causes of cheese contamination. Measures taken to correct these anomalies resulted in a statistically significant decrease in *E. coli* and coliform counts in the cheese.

Note

Name of cheese-making firm withheld according to manufacturer's request.

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