MICROBIOLOGICAL QUALITY CONTROL OF ARTISANAL CHEESES

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Summary

Fresh cheeses are the most preferred by consumers for their shape, texture and flavor, however, they can also be contaminated with pathogenic microorganisms, due to the characteristics of their composition that favors microbial proliferation, so any deficiency in production, handling, conservation, transport and marketing can turn these dairy products into sources of foodborne diseases (STDs) (Soria, 2020).

The Center for Disease Control and Prevention (CDC) reported from 1993 to 2006 in the United States and Canada there were 121 outbreaks of illness linked to the consumption of liquid milk and pasteurized and unpasteurized cheeses, resulting in 4413 infections, 239 hospitalizations and3 deaths (Nyachuba, 2023).

In 2008, the Ministry of Public Health (MSP) registered 10,000 cases of food poisoning, but it is not recorded what type of food or microorganism caused these poisonings. Milk and its derivatives for various reasons may be contaminated with pathogenic microorganisms that represent a danger to the population, so these products are related to the registration of these poisonings (Bayas, 2021)

Keywords: MICROBIOLOGICAL QUALITY CONTROL, ARTISANAL CHEESES

The safety of the microbiological quality of cheeses begins with the processing that is given to the milk since it is the raw material for its elaboration. Pasteurization is one of the processes that helps prevent health problems related to these products, because this process consists of subjecting the milk to temperature changes to destroy pathogenic microorganisms such as: *Staphylococcus aureus*, *Escherichia coli* and *Enterobacteria*, which present a danger to the population, however, it is important to control and monitor all stages of cheese processing, as there could be the re-entry of pathogens into the final product (Ayala, 2015).

Introduction

In the city of Riobamba, a wide variety of cheeses are marketed, from microenterprises in the area, which are made in an artisanal way and in many cases do not have the quality controls required by the International Organization for Standardization (ISO) and regulated by the National Agency for Regulation, Control and Sanitary Surveillance (ARCSA).

Food safety is a very important concept within the food sector, defined as "the set of conditions and necessary measures taken during the production, storage, distribution and preparation of food so that once ingested, it does not represent a risk to health" (Kaur, te al. 2022). Thus, the ISO 22000: 2005 standard relates to food safety as the assurance by the producer so that the food (fresh cheese) produced does not cause harm or disease to consumers (Calugullín, 2017).

The conditions for the production of artisanal cheeses are often not the best and constitute a source of danger during production, because they can contaminate the cheese with pathogenic microorganisms (Vargas, 2018).

Definition of Milk

Milk is a secretion of mammals from their mammary glands, used for feeding their young, as it contains carbohydrates, proteins, fats, vitamins and mineral salts beneficial for their growth. The milk of several mammals domesticated by man is intendedfor consumption (Grosch, 2008).

Cow's milk from a legal point of view is defined as a fresh and integral product from the milking of one or more cows that must be healthy and well fed (Pineda, 2014). In Ecuador, a production of 5.60 million liters of milk was recorded in 2014, the highest production was

registered in the Sierra region, of this total more than a third is destined to the production of cheese of different varieties (Vargas, 2018).

Definition of Cheese

Since ancient times cheese has been the way to preserve the main nutrients of milk these include proteins, minerals, calcium, phosphorus, fats and vitamins. Cheese belongs to the fermented dairy family (McSweeney, et al. 2017). According to the *Codex Alimentarius*, cheese is the solid, semi-solid, fresh or ripe product, which is obtained by coagulation either partially or totally of milk proteins, which is produced by the action of rennet or other coagulants and ends with a draining of whey that is also produced by the coagulation of milk **(**Ramírez & Vélez, 2012).

Composition

Fresh cheese, being a derivative of milk, shares largely the same nutritional properties, except lactose. The components are in the following proportions, water 50%, fat 24%, protein 21%, carbohydrates 2%, mineral salts 2%, calcium 477mgs, phosphorus 292mgs and vitamin A, B, D, E and K in 1%. In addition, this product contains a humidity of 60% and a pH of 4.5. (Pachar, 2020).

Cheese Making

Making most varieties of cheese involves the combination of three ingredients; milk, rennet and salt, which are processed by a series of steps and variations in ingredient mixing and subsequent processing have led to the production of a number of cheese varieties (Beresford, 2011).

The milk, being the raw material for the production of cheeses, must be of very good quality, so it cannot be altered or adulterated, milking must be in hygienic conditions and also the cows must be healthy and stress-free.

A correct elaboration of the cheese consists of several steps that are detailed below:

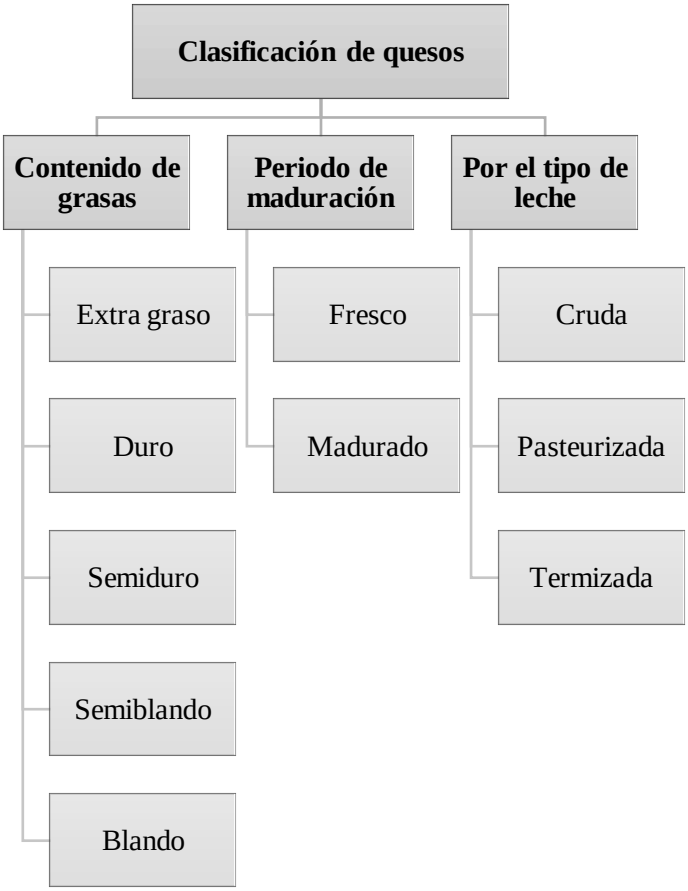
Graph 1: Fresh cheese making process

Source: (Prodar, 2018)

Classification of Cheeses

The diversity of cheeses produced in the world is impressive, there is a great variety of cheeses of different flavors, textures, shapes and aromas, in different catalogs and research works more than 2000 varieties and types of cheeses have been compiled (FIL-IDF, 2021). For this reason, several ways of classifying cheeses according to their significant family, physicochemical characteristics, manufacturing methods, type of milk,etc. have been sought. Graph 2 below presents the classification of cheeses under three criteria:

Figure 2: Classification of cheeses



Source: (Novoa, 1987)

Definition of Queso Fresco

According to the Ecuadorian Technical Standard NTE INEN 1528, it defines fresh cheese as "a cheese not matured, nor blanched, molded, with a firm texture, slightly granular, which can be prepared with whole milk, semi-skimmed, coagulated with enzymes and acids, generally without lactic cultures"

Fresh cheese is characterized by being a cheese that can be consumed after its manufacture and is not subject to any additional physical or chemical change. The organoleptic characteristics that define fresh cheese are: its regular edges, smooth faces, soft and non-fluffy texture, it has a color that can range from white to cream, they have a characteristic dairy flavor and smell. It has a humidity of 60%, fats 20% and another 20% is distributed between proteins, mineral salts, carbohydrates and vitamins (Nolivos, 2011).

The safety of fresh cheeses is related to the quality and hygienic conditions in the reception of milk, the handling of raw material by personnel, contamination of the product during processing by machinery, methods and conditions of storage and transport, etc., so hygiene

care is essential in the process of making these products, and should be based on good manufacturing practices and quality standards in the country (Sánchez, 2015).

Cheese-Borne Diseases

Milk and dairy products such as cheese can be contaminated with several pathogenic microorganisms such as: *Enterobacteria, Escherichia coli, Staphylococcus aureus, Listeria monocytogenes and Salmonella* that can cause illness to people who consume these products. The symptoms associated with these pathogens are diarrhea, stomach cramps or cramps, fever, headache and vomiting, these symptoms can typically last several hours, weeks or more (Carrión, 2016).

Pathogenic microorganisms

The quality of any type of cheese is directly related to the quality of the milk that is used as raw material for its elaboration. Cheeses have a very diverse microbiology consisting of beneficial microorganisms that help the formation and natural conservation of cheese, but at the same time pathogenic microorganisms can be found that are associated with the deterioration of cheese and produce diseases (Vargas, 2018).

Enterobacteriaceae

The *Enterobacteriaceae* family is a large and heterogeneous group of gram-negative bacilli. The main characteristics of this bacterium is that they are facultative anaerobes, produce catalase, do not grow in the presence of sodium chloride and most are mobile due to their peripheral flagella (Molleda, 2016). These microorganisms are distributed in plants, soils, water and interim of man and animals, are among the most common pathogens that are responsible for serious infections Some genera of this family are common colonizers of the gastrointestinal tract (Lan, 2013)

Enterobacteria are found naturally in cheeses, it has the ability to provide some specific characteristics to artisanal cheeses. But its presence causes controversy due to sanitary and technological aspects, since its presence can pose concern and danger due to the presence of pathogenic strains and species. These bacteria can indicate fecal contamination and are indicators of poor hygiene practices during food processing, in addition these microorganisms can produce proteolytic and lipolytic enzymes that modify the organoleptic propertiesof cheeses (León, 2018).

Escherichia coli

Escherichia coli is a Gram-negative bacillus, belonging to the family Enterobacteriaceae of the tribe Escherichia. The main characteristics of this bacterium is that it is facultative anaerobe, mobile, oxidase negative, nitrate reducer, non-sporulated, ferments glucose with gas and acid production and presents O, H and K antigens. *E. coli* colonize the gastrointestinal tract of humans and animals within hours of birth and are harmless. Some strains of *E. coli* can be pathogenic causing foodborne illness and produce different clinical picturessuch as diarrhea (Basavaraju & Gunashree, 2022).

Escherichia coli is known as an index microorganism as it indicates the presence of fecal contamination pathogens and is also an indicator of hygiene and food safety. This bacteria can contaminate foods such as pasteurized milk and other finished products, post-pasteurization. E. *coli* testing is performed on most products including raw vegetables, raw milk, cheeses, seafood, etc. (Vasquez, et al. , 2018)

Staphylococcus aureus

Staphylococcus aureus belongs to the Micrococcaceae family, they are shaped like coconuts are grouped forming clusters, they are immobile, gram-positive, aerobic and facultative anaerobes, they have coagulase, phosphatase and deoxyribonuclease enzymes that distinguish it from other staphylococci, it also produces hemolytic and enterotoxic exotoxins. This bacterium is present in the environment, air, water and food, especially in dairy products and sausages (Rodas, 2016). When this microorganism is present in food it produces toxins when stored at room temperature, these toxins can be in dangerous amounts and do not show signs of deterioration such as bad odors (Gajewska, 2022)

When *Staphylococcus aureus* is present in food, it indicates that contamination occurred after pasteurization. This contamination is produced by human sources through the skin, mouth and nose of the workers who handle the products during processing, in addition to inadequate cleaning or disinfection of equipment that is in contact with the raw material (Nagwa, et al. 2008)

Food Labeling

Food labels play an important role in the food marketing system through their impact on product design, consumer confidence in food quality, and consumer education about diet and health (Padberg, 2015).

The labelling of products is mandatory and is also a fundamental right of consumers. The information provided by the label is important in the choice of a product since here it is: the origin, mode of conservation, ingredients and nutrients of the product (Díaz, 2017).

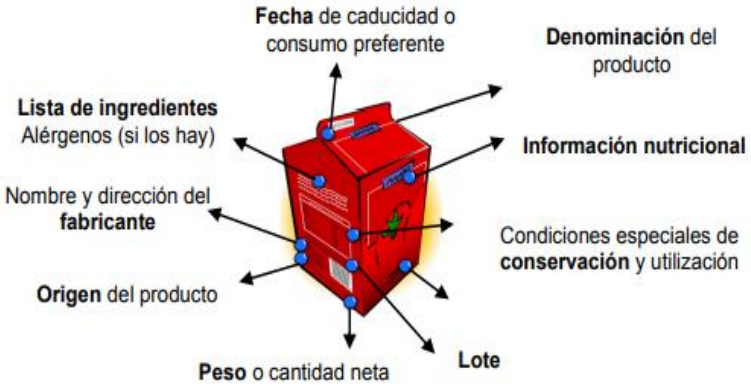


Figure 1: Food labeling

Source: (KontsumoBide)

According to the NTE INEN 1334 standard, the mandatory requirements in the labeling of packaged products are the following:

a. **Name of the food:** indicates the true nature of the product.
b. **List of ingredients:** The list of ingredients is declared, unless it has only one ingredient.
    - The list must be preceded by the title "Ingredient"
    - The ingredients must be in descending orderdepending on how it is used in the preparation
    - The added water must be indicated, except when the water is part of the brine, syrup or broth used in the food and is already declared as such in the list of ingredients.
    - When any food contains: crustaceans, eggs, fish, peanuts, soy, milk, dairy products, nuts or sulfites in concentrations of 10 mg / kg or more, they must be declared as they can cause hypertension.
    - Food additives must be included in the list of ingredients, because, when they are used in the raw material, they are transferred in significant quantities to perform a technological functioning. When additives and adjuvants are transferred in small quantities in food, they do not need to be declared
c. **Net content and drained mass (drained weight): declared in the** main part of the labelling in units of the International SI system.

- Liquid foods: volume
- Solid foods: dough
- Semi-solid or viscous foods: mass or volume

d. Identification of the manufacturer, packer, importer or distributor: the product indicates the name of the manufacturer, packer or owner of the brand and in case of being imported must indicate the name of the legal representative.

e. City and country of origin

f. Batch identification: packages must have a code engraved after the word "Lot" or letter "L" that allows the batch to be traced.

g. Date marking and storage instructions:

- The maximum date of consumption or expiration must be indicated, which must include at least the month and day for foods consumed in a period of three months and the year and month for foods that can be consumed on a date in a period greater than three months.
- The year, month and day must be identified in numerical or alphanumeric order
- If necessary, the label must indicate any condition that is required for the conservation of the food.

h. Instructions for use: the label must indicate the necessary instructions on how the food should be used**.**

i. Health registration: the health registration number issuedby the competent health authority must appear in a visible place on the label.

j. Notifiable nutrients:

- Table 1-2 shows the nutrients that must be declared on a mandatory basis and the daily value DV:

Table 1: Nutrients and Daily Value

Nutritious	Unit	Daily Value
Energy value (calories)	Medical history	8380
	Kcal	2000
Total fat	G	65
Saturated fatty acids	G	20

Cholesterol	Mg	300
Sodium	Mg	2400
Carbohydrates	G	300
Dietary fiber	G	25
Proteins	G	50

Source: NTE INEN 1334-2

Methods and Techniques

Sample collection and transport

The sample was taken and transported to the place of analysis as established in the NTE INEN 0004 standard for sampling of milk and dairy products, the samples have an approximate weight of 500g and being a product packaged in small quantities direct for consumption the sample is taken randomly, in this case as specified in the NTE INEN 1528 standard. For the transport of the samples, an aseptic and insulated container was used, to maintain the temperature of 0ºC and 5ºC specified by the standard, cold gel bags were used or to maintain said temperature.

Preparation of peptoned water

The peptoned water according to the requirements of the standard must have a concentration of 0.1% w / v, the preparationwas carried out under the standard NTE INEN 1529-1: 99 of microbiological control of food preparation of culture media and reagents, where it is mentioned that 1g of peptone must be weighed and dissolved in 1 liter of distilled water, then it is sterilized in autoclave at a temperature of 121ºC for 15 min., then it must be allowed to cool up to 45ºC to be able to use the peptoned water.

Stock solution preparation and dilutions

The preparation of the stock solution and dilutions is based on the NTE INEN 1529-2:99 standard for microbiological control of food, sending and preparation of samples for microbiological analysis.

Mother solution

Take the fresh cheese sample with a sterile spatula and weigh 10 g on an analytical balance and place in a 100 mL Erlenmeyer containing 90 mL of sterilized peptoned water and shake the Erlenmeyer until the sample, which corresponds to the stock solution (10^{-1} solution) is homogenized.

Dilutions

For microbiological analyses, a solution of 10^{-4} shall be used, obtained as follows:

- With a sterile pipette 1mL of the stock solution is taken and transferred to a test tube, which must previously contain 9 mL of peptone water, shake to homogen and raise the dilutionandlet stand, this dilution corresponds to 10^{-2}
- From the 10^{-2} dilution, 1mL is taken with another sterile pipette, transferred to another test tube containing 9 mL of peptoned water, stirred to homogenize and 10^{-3} dilution is obtained.
- With a new sterile pipette, 1 mL of dilution 10^{-3} is taken and transferred to another test tube with 9 mL of peptone water, homogenized and obtained the dilution 10^{-4} that will be used for microbiological analysis.

Microbiological analysis in petrifilm dishes

For the inoculation work protocol for the determination of *Enterobacteria*, *Escherichia coli* and *Staphylococcus aureus* in Petrifilm dishes, the enthusiasm for the Association of Analytical Communities (AOAC) 2003 was taken as a reference. 01, 991.14 and 2003.07

Inoculation

- In a laminar flow chamber, the petrifilm dishes for each test are placed and coded according to the samples to be sown.
- With a sterile pipette, 1 mL of the 10^{-4} dilution is taken.
- Lift the top film of the petrifilm carefully and without touching the growing area.
- Disperse the 1 mL of the dilution in the centre of the inner film of the petrifilm, with the pipette upright to the inoculation surface.
- Slowly lower the top film over the sample, preventing bubbles from forming.
- It is allowed to stand for 1 to 5 minutess so that the sample spreads evenly, and also so that the gel solidifies and can be taken to the incubator.

Incubation

Tabla 2: Temperature and Incubation Time

Microorganism	Temperature	Time
Enterobacteriaceae	36± 1°C	6 - 24 h
Escherichia coli	36± 1°C	24 ± 2 hrs
Staphylococcus aureus	36± 1°C	24 2 hrs

Recount

- The petrifilm dishes are removed from the incubator
- With the help of an illuminated magnifying glass count the colonies that were formed.

Table 3: Characteristics of the study microorganisms

Microorganism	Characteristics of the colonies
Enterobacteriaceae	Red and yellow color with yellow halos with or without bubbles
Escherichia coli	Blue color with bubbles
Staphylococcus aureu	Pink

CFU Calculations

For the calculation of colony forming units (CFU), Petrifilm dishes containing between 10 and 100 colonies for *Escherichia coli* and between 15 and 150 colonies for *Enterobacteriaceae* and *Staphylococcus aureu* shall be taken into account.

The UFC is calculated with the following formula:

$$UFC/g = \frac{\text{N de colonias por placa} \times \text{factor de dilución}^{*}}{ml\ de\ la\ muestra\ sembrada}$$

INTERPRETATION OF RESULTS

Sampling Results

Table 1-4 presents a checklist with the parameters required by the NTE INEN 0004 Standard: Sampling of milk and dairy products**,** to perform a correct sampling for the microbiological analysis of the fresh cheeses under study.

Table 4: Sampling checklist

Sampling	Date	Sample	Sample weight ≤ 1000g		Temperature 0 to 5 ºC	
			Meets	**Non-compliant**	**Meets**	**Non-compliant**
1	**07/12/2022**	**QF1**	X		x	
		QF2	X		x	
		QF3	X		x	
		QF4	X		x	
		QF5	X		x	
		QF6	X		x	
2	**12/12/2022**	**QF1**	X		x	
		QF2	X		x	
		QF3	X		x	
		QF4	X		x	

		QF5	X		x	
3	**20/12/2022**	**QF1**	X		x	
		QF2	X		x	
		QF3	X		x	
		QF4	X		x	
		QF5	X		x	

3 control processes are carried out around thevariable weight and temperature of the 5 brands of ques or randomly selected, 8 days after the first control, in table 4, it can be seen that all the brands under study comply with the parameter established for the weight of fresh cheese that are small quantities or packaged in quantities for direct sale to the consumer, For this reason, samples were taken directly and stored in Ziploc sleeves sterilized with 70% ethyl alcohol and subsequently coded. In addition, it can be seen that all samples meet the requirements of the temperature parameter, because it was controlled with the help of a digital thermometer and the transport time was maximum was 1 hour so this parameter did not present variation or incidence in the results obtained in the microbiological analysis, in this way youcan conclude that the 5 brands of cheese comply with the provisions of the NTE INEN 0004 standards.

In a similar investigation carried out by Plaza, 2013, it is indicated that the sampling was carried out under similar conditions taking the samples randomly and directly from the hangers of the different supermarkets under study, in addition, the samples were moved in a container with ice with a temperature of 6ºC so that the sample is kept to the laboratory where they were analyzed, In this way, it establishes that when complying with these parameters, the results of the microbiological analyzes carried out were reliable.

In a study carried out by Moreno (2021) in the city of Latacunga, he mentions howsampling was carried out for the evaluation of the microbiological quality of fresh cheeses of artisanal production. The research indicates that the samples were taken randomly for 4 weeks, applying aseptic techniques and the samples were stored in sterile plastic bags and coded,

they were also placed in a container with dry ice to maintain a temperature of 5ºC until the transfer of the sample to the laboratory and avoid any type of alteration.

Based on the above, the sampling carried out for the development of this research agrees with other investigations and complies with the requirements of the NTE INEN 0004 standard and, therefore, with all the measures taken, the integrity of the sampled products intended for the objective microbiological analysisor of this research is guaranteed.

Results of the microbiological analysis

Enterobacteriaceae count analysis

Table 5 shows the results obtained from the count of *Enterobacteriaceae* in Petrifilm dishes, which are characterized by red and yellow colonies with yellow halos with or without bubbles, and then make the comparison with the provisions of the NTE INEN 1528 standard.

Table 5: Results of *Enterobacteriaceae* count in Petrifilm dishes

Sample	***Enterobacteriaceae***			Media de x10^4UFC/g
	Repetition 1	**Repetition 2**	**Repetition 3**	
	x10^4UFC/g	**x10^4UFC/g**	**x10^4UFC/g**	
QF1	0	30	0	10
QF2	160	180	101	147
QF3	170	195	58	141
QF4	38	90	31	53
QF5	143	150	46	133

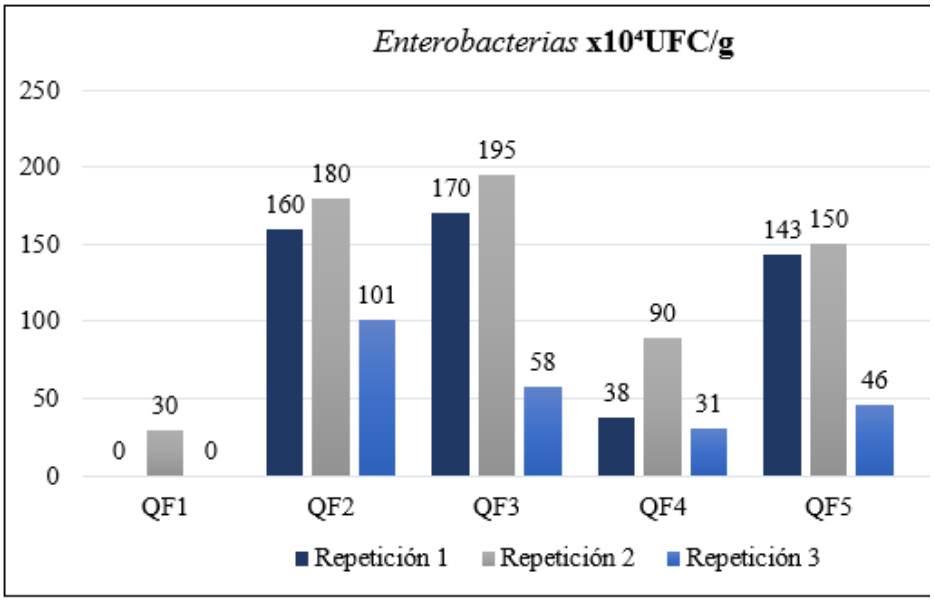


Figure3: Analysis of the count of *Enterobacteriaceae* in the different samplings

During the experimental process, it can be observed that there are variations of the results in the three repetitions, in the second repetition the 5 samples present a notable increase, this is because the sampling was made 5 days after the first taking and the conservation system of fresh cheeses is not the most appropriate, Since, it was observed that the cheeses are in containers with water and in refrigerators that do not have the right temperature for their conservation. In the third repetition it can be observed that the values of the CFU tend to fall and in the QF1 sample the value is null, because the samples were taken 8 days in order to have fresh cheesesthat will arrivethe same day at the distribution points where the sample was taken.

Table 6: Student's T test for a sample for *Enterobacteriaceae*

Single sample test						
	Test value = 200					
	t	**Gl**	**Sig. (bilateral)**	**Mean difference**	**95% confidence interval of difference**	
					Inferior	Superior
Enterobacteriaceae	5,242	14	0,000	927800,000	548197,280	1307402,719

Source: SPSS method

To compare the results obtained during the 3 repetitions to determine the presence of *Enterobacteria*, the Student's T Test was used for a sample that allows comparing the mean of the studied group, with the reference value taken from the NTE INEN 1528 standard that is equal to 200 CFU / g which is the maximum permissible index to identify acceptable levels of quality. According to the results obtained from the Test we have an average of 927800,000 which indicates an average outside the permissible value, however, it can be contrasted with the value of the significance calculated with a confidence level of 95%, where a value of 0.000 is obtained, which is less than 5% (0.05), which means that it is statistically significant, It is claimed that the cheeses presented pathogenic microorganisms. This means that none of the cheeses are suitable for human consumption, and may represent a risk to the health of the consumer.

Enterobacteriaceae cause most acute gastroenteritis, 30% of bacteremia and 70% of urinary tract infections. When this family of microorganisms occurs in food it is considered a public health problem, since it is an indicator of fecal contamination and on the contrary its absence is an indicator of good manufacturing practices (GMP), when there is a high count of said microorganism it indicates poor processing or subsequent contamination due to improper handling or by keeping food at room temperature for prolonged periods. The presence of *Enterobacteriaceae* is more prevalent in artisanal fresh cheeses (Guzmán, Rodríguez & Calderón, 2017).

In a similar investigation in the city of Babahoyo carried out by Espinoza, et al., (2020) mention that to analyze if the fresh cheeses marketed in 3 city markets comply with the quality standards of the NTE INEN 1528-2012 regulation, 35 samples of fresh cheeses of 250g each were taken and 3 repetitions were made, once, microbiological analysis was performed, it was obtained as a result that the *Enterobacteriaceae* presented an average of 754.57±292.84 CFU / g that is outside the permissible limits of indicates the standard, so they conclude that the fresh cheeses marketedin these 3 markets are not suitablefor human consumption and represents a threat to food safety.

Moreno in 2021, carried out an evaluation of the microbial quality in fresh cheeses of artisanal production marketed in the closed market Latacunga, for which he carried out 4 samplings in different weeks taking 5 samples of cheeses from 8 different marketing posts with a weight of between 300 and 500g. After carrying out the pertinent analyzes, it was obtained as a result that the count of *Enterobacteriaceae* is between the values of 10^4 to 10^5 CFU/g, which exceed

the permissible values of the INEN 1528: 2012 standard, therefore, they determined that the fresh cheeses marketed in this market present inadequate hygienic conditions that affect the product and consumers.

All the information provided, corroborates that the microbiological analysis carried out on the 5 samples to determine Enterobacteriaceae is correct, therefore, it can be mentioned that marketing these cheeses in the market is not adequate since they do not present an adequate handling and conservation system to prevent microorpathogenic organisms such as *Enterobacteriaceae* from proliferating in these foods.

Escherichia coli count analysis

Once the Escherichia coli colony count was carried out in Petrifilm dishes, the results obtained were tabulated and compared with the NTE INEN 1528 standard.

Table 7: Results of *Escherichia coli* count in Petrifilm dishes

Sample	***Escherichia coli***			Media de x10^4UFC/g
	Repetition 1	**Repetition 2**	**Repetition 3**	
	x10^4UFC/g	**x10^4UFC/g**	**x10^4UFC/g**	
QF1	0	0	0	0
QF2	13	55	10	26
QF3	10	65	12	29
QF4	0	69	0	23
QF5	18	59	22	33

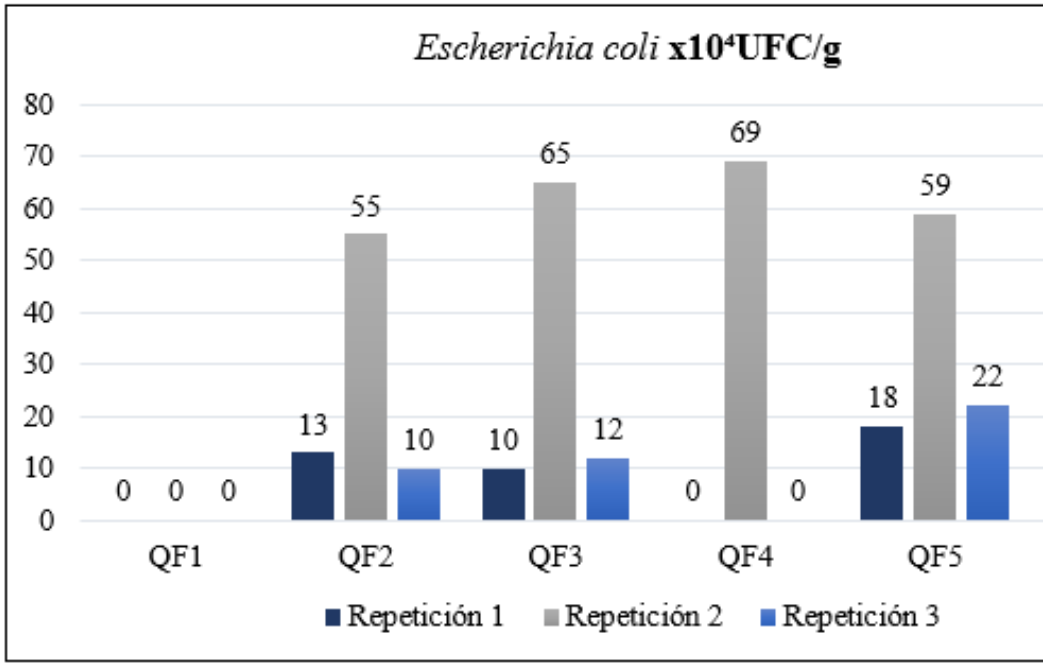


Figure3: Analysis of the *Escherichia coli* count in the different samples

In the experimental process for the analysis *Escherichia coli*, it can be observed that in the second repetition the 5 samples increase the measured value, except in QF1, while in the third repetition in the case of QF1 the value is null and the remaining 4 samples the experimental value studied tends to decrease. InQF1, the absence of *Escherichia coli* can be evidenced in the three repetitions performed.

Table 8: Student's T-test for a sample for *Escherichia coli*

Single sample test						
	Test value = 10					
	t	**Gl**	**Sig. (bilateral)**	**Mean difference**	**95% confidence interval of difference**	
					Inferior	Superior
Escherichia coli	3,314	14	0,005	221990,000	78338,842	365641,157

Source: SPSS method

Once the Student's T Test has been performed, to compare the results obtained from the *Escherichia coli* count of the 5 samples analyzed, with the reference value equal to 10 CFU / g established by the NTE INEN 1528 standard. An average of 221990,000 was obtained which indicates an average outside the permissible value, as well as, a significance value of 0.005

was obtained that is less than 0.05, for this reason, except for QF1 the 4 samples are not within the parameters established by the standard, therefore, the cheeses analyzed are not suitable for consumption.

Escherichia coli is a bacterium that is normally found in the intestine of humans and animals, the most frequent reservoir of this bacterium is cattle, for this reason, it is found in raw milk. It is transmitted to people by consuming contaminated food and its presence indicates fecal contamination, due to poor handling of food by the people who prepare them and the storage temperature is also important, since the development of this microorganism is an important factor (Castro, 2004).

In a similar investigation carried out by Trujillo in 2016, in the Santa Rosa market in thecity of Riobamba, he carried out a microbiological analysis of fresh cheeses made in an artisanal way, taking samples from 7 points of sale performing the analysis in triplicate. It obtained as results of *Escherichia coli* $7x10^5$ CFU / g and when comparing with the NTE INEN 1528 standard concluded that the fresh cheeses marketed in that market exceed those permissible in the standard and indicates a chain of deficient in the processing, transport and sale of fresh cheeses and are not suitable for human consumption.

The aforementioned information agrees with what was done in this investigation, and despite the fact that the fresh cheeses analyzed are from companies that have a health notification, with the exception of the QF1 samples, the remaining 4 samples present this microorganism which indicates a deficiency in hygiene since the collection ofstina milk for the preparation of cheeses and inadequate pasteurization, in addition to these factors also influences the handling and temperature at which the cheeses are preserved one is that they arrive at the points of sale within the market of the city of Riobamba.

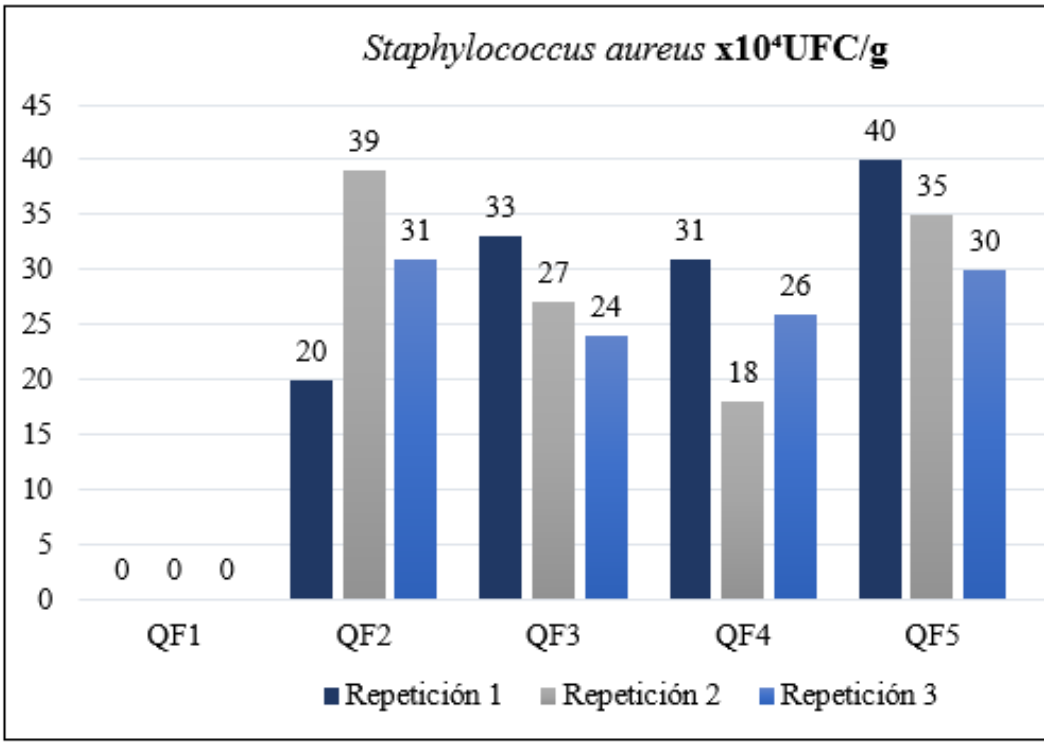
Staphylococcus aureus count test

Table9 tabulated the results obtained from the analysis and counting of colonies with a pink compositionthat is characteristicof the presence of *Staphylococcus aureus* and proceeded to comparative analysis.

Table 9 : Results of *Staphylococcus aureus* count in Petrifilm dishes

Sample	***Staphylococcus aureus***			Media de $x10^4$UFC/g
	Repetition 1	**Repetition 2**	**Repetition 3**	

	x10⁴UFC/g	**x10⁴UFC/g**	**x10⁴UFC/g**	
QF1	0	0	0	0
QF2	20	39	31	30
QF3	33	27	24	28
QF4	31	18	26	25
QF5	40	35	30	35



Graph 4: Analysis of the *Staphylococcus aureus* count in the different samplings

During the experimental process, se can be observed in the QF1 during the three repeats there is nullity of *Staphylococcus aureus*, while the remaining 4 samples in the three repeats show variations of growth and decrease of the microorganism under study.

Table 10: Student's T test for a sample for Staphylococcus aureus.

Single sample test						
	Test value = 1000					
	t	**Gl**	**Sig. (bilateral)**	**Mean difference**	**95% confidence interval of difference**	
					Inferior	Superior
Straphylococcus aureus	6,669	14	0,000	235000,000	159422,078	310577,921

Source: SPSS method

By means of the Student's T Test an average of 235000,000 of the 3 repetitions of the 5 samples was obtained, which indicates an average outside the permissible value, and this can be contrasted with the value obtained from the significance of 0.000 which is clearly less than 0.05, which indicates that the cheeses that are marketed in the wholesale market with the exception of QF1, has a value greater than 1000 CFU / g established by the standard, as cheeses present a high level of *Straphylococcus aureus,* these are not considered suitable to be distributed to consumers.

Humans and animals are the main hosts of *Staphylococcus aureus*., the presence of this microorganism in cheeses is due to a lack of hygiene of the personnel working during the selection, reception and storage of the raw material, deficient thermal processesto eliminate pathogens, use of contaminated utensils, transport and inadequate storage of the final product (Merchán, 2019).

Ferrín et, al. in 2020 carried out an investigationto determine the presence of *Staphylococcus aureus* in artisanal fresh cheeses in the municipal market of the Junín canton in the province of Manabí, 51 samples were collected randomly with a weight of 20g. After performing the analyzes, it was found that 100% of the samples presented a count higher than that established in the NTE INEN 1528 standard, so it represents a riskor for the health of consumers.

In an investigation carried outby Tenezaca (2019) in the city of Loja in the Gran Colombia market on the microbiological analysis of cheeses, 4 ounces were taken as a sample for each place of sale, obtaining a total of 35 samples. Once the analysis was carried out, it was obtained as a result that of the 35 samples 22 presented *Staphylococcus aureus* which represents 63% of the total, so itwas concluded that most of the cheeses do not comply with the INEN 1528 standard, therefore, those marketed in this market are not suitable for human consumption and the places of sale do not comply with adequate hygiene measures

Based on the aforementioned of the 5 cheeses analysis, 4 are outside the parameters of the standard, this due to the lack of hygiene of the dispensers during the handling of this product, in addition, that a deficient storage was observed which makes the product more susceptible to contamination with this microorganism and makes it proliferate faster and makes this product dangerous for consumers.

Tagging analysis

For the analysis of labeling of the 5 samples, a Check list was made, with the parameters established in the NTE INEN 1334 standard for labeling food products for human consumption, verifying compliance and non-compliance with the parameters.

Table 11: Labeling analysis checklist

Requirements	Samples									
	QF1		**QF2**		**QF3**		**QF4**		**QF5**	
	C	**NC**	**C**	**NC**	**C**	**NC**	**C**	**NC**	**C**	**NC**
Name of the food	x		x		x		x		x	
List of ingredients	x		x		x		x		x	
Net content and drained mass	x		x		x		x		x	
Identification ofthe manufacturer	x		x		x		x		x	
Cityand country of origin	x		x		x		x		x	
Batch identification		x		x		x		x		x

Date marking and storage instructions	x			x		x		x		x		
Instructions for use		x		x		x		x		x		
Sanitary registration	x		x		x		x		x		x	
Notifiable nutrients	x		x		x		x		x		x	
C: **Complies** NC: **Not compliant**												

Table 12: Percentages of compliance in the analysis of labeling

PARAMETER COMPLIANCE IN LABELING ANALYSIS		
BRAND QUESOS FRESCOS	**MEETS**	**NON-COMPLIANT**
QF1	80%	20%
QF2	70%	30%
QF3	70%	30%
QF4	70%	30%
QF5	70%	30%

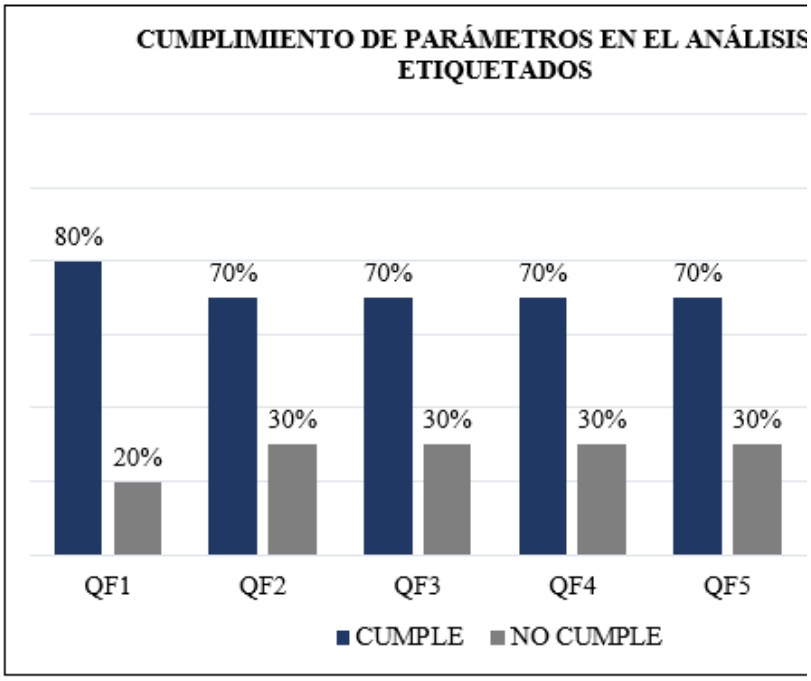


Figure 5: Compliance rates in labelling analysis

From thecheck list generated in Table 11, Table 12 of the percentage of compliance and non-compliance of each sample was made, obtaining that the QF1 sample complies with 80% of the parameters established by the NTE INEN 1334 Standard for labeling food products for human consumption and does not comply with 20% of the parameters. while the remaining 4 brands of fresh cheese comply with 70% of the parameters established in the labeling and do not meet the parameters by 30%.

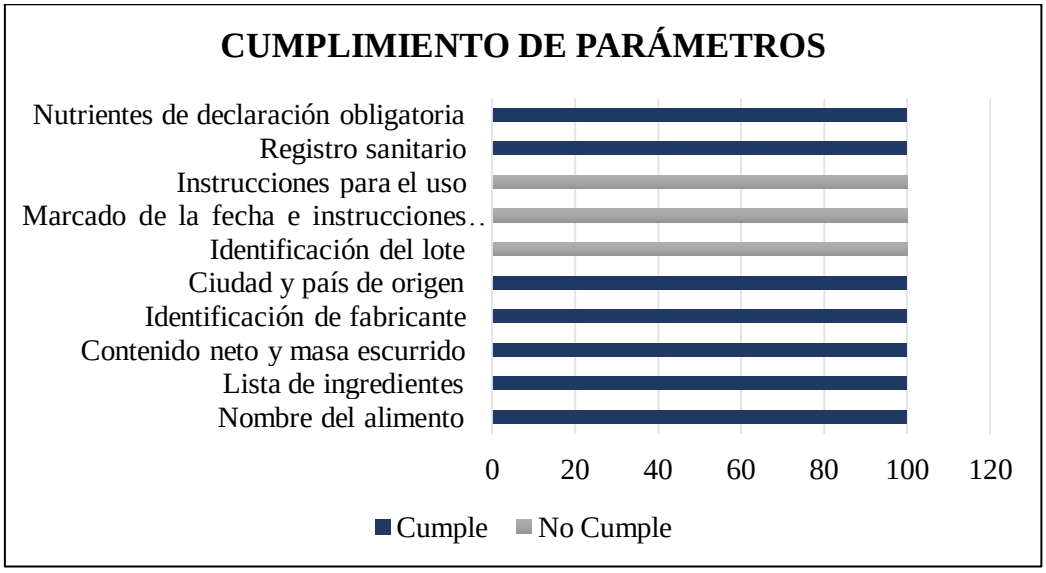


Figure 6: Compliance in labeling analysis

In Graph6 it can be seen that of the 10 parameters established in the NTE INEN 1334 Standard, there is non-compliance in three parameters that are: instructions for use, date marking and instructions for the conservation and identification of the lot, in the case of fresh cheeses QF2, QF3, QF4 and QF5, while, for QF1 the non-compliance is given in two parameters that are Instructions for use and identification of the lot.

Although the implementation of Good Manufacturing Practices is important in a company, it is also essential to take into account that the products are labeled according to the established Regulations, in order to provide information to consumers about the products they are going to consume and in this way a right of Ecuadorians is guaranteed, by having accurate and clear product information (Ango & Grijava, 2015)

Determining the shelf life of a product is very important, as it helps to establish the expiration date of a product. This information is useful for merchants to determine the time that the product can be offered to the consumer, while the consumer helps him to know the time frame in which he can consume a quality product. In recent years the legal regulations of the country require that within the labeling of the products must include the expiration or expiration date of the product, in addition, the identification of the lot must go, which is the set of products made under the same conditions and at the same time, it is identifiedon the label with a key number(López, 2016)

In an investigation carried out by the Government of Chile in 2015, three types of packaged cheeses were taken as a sample, for each brand 10 units were acquired for the determination of the nutritional composition and labeling of the cheeses. It was obtained as results that, based on the Food Health Regulations, of the Ministry of Health and Decree 297 of 1992, that the samples generally comply with the established, but at the same time there is controversy regarding the instructions for use.

Based on the information collected from the labeling of fresh cheese samples, these do not conform to the established, since they violate or do not comply with parameters such as the expiration date, so they do not offer the trader and the consumer to know in what time limit they can acquire and consume a quality freshproduct. The batch identification parameter is another that does not appear on the labels, so it can be mentioned that the companies that produce these foods do not identify or track each batch they produce. The instructions for use is another parameter in which all brands of fresh cheese frequently infringe, so companies do not give the necessary information for the use and conservation of their product.

Analysis of the sanitary registration parameter using the ARCSA

Next, the health notifications that each brand of fresh cheesecor ours on their labels are recorded in order to verify them on the official website of the National Agency for Regulation, Control and Sanitary Surveillance

Table 13: Verification of sanitary registration

Sample	Health notification	ARCSA	
		Meets	**Non-compliant**
QF1	06576 INHQAN 0406	x	
QF2	31748-ALN-0821		x
QF3	33066 ALN 1121		x
QF4	15346 INHQAN 0213		x
QF5	28692-ALN-0221	x	

Table 13 tabulated the results obtained from verified the sanitary records that presented each of the samples in their labels on the official website of the ARCSA, resulting in three of the samples analyzed have false sanitary registrations or other brands that make fresh cheeses, so, it can be mentioned that the samples marketed to consumers, at first glance they comply with this parameter, but in reality they present an erroneous or false labeling so it does not comply with what is established by the NTE INEN 1334 standard.

According to the National Agency for Regulation, Control and Sanitary Surveillance (ARCSA) the sanitary registration is used for medical devices, medicines, hygienic products, fertilizers, etc. and is granted in approximately 5 months to a year, if the applicant meets the requirements of quality, safety and efficacy to be able to consume or use such products, instead, The sanitary notification is granted to processed foods, cosmetic and hygiene products, in a period of 2 weeks if it meets the conditions of quality, safety and safety.

The certificate or code of Good Manufacturing Practices of the processing plant is granted by the National Agency for Regulation, Surveillance and Sanitary Control (Arcsa). Within 3 days, after receipt of favorable information from the company. This certificate will be valid for three

years and is granted by processing area where the variety of foods corresponds to the same type (Noboa, 2002),

Since December 21, 2015 through resolution ARCSA-DE-067-2015-GGG processed foods that have a health registrationonce it is not valid or valid must be replacedby a health notification or in case of having the certificate of Good Manufacturing Practices, it must appear on the labeling of food (ARCSA, 2017)

Based on the above and on what is observed on the labels of each sample analyzed, despite the fact that the ARCSA mentions that processed foods should no longer have the sanitary registration on their labels, the QF2 and QF4 sample infringe on this parameter. In addition, it can be mentioned that it is better to have a BPM code since it certifies the entire production line of a company, the control group has this code, for this reason, it has a varied productionunder the same code. On the other hand, the health notification onlycertifies it to a single product of the company.

CONCLUSIONS

- The microbiological analysis was carried out to verify the quality of the cheeses marketed in the city of Riobamba, and it was determined that the samples collected are not of good quality, because a high count of *Enterobacteria*, *Escherichia coli* and *Staphylococcus aureus* bacteria that produce different intestinal diseases in humans was found.

- A correct sampling is essential in an investigation, because this depends on having reliable results and following the standards framed in the NTE INEN 0004 edition of the year 1984, it was determined that the temperature is a very important factor when transporting the samples of dairy products because if they are not kept at an adequate temperature of between 0 and 5ºC, During transport the bacterial load can increase and when performing microbiological analyzes you do not have the correct results and you can give bad information about the samples analyzed.

- The microbiological analysis of the 5 samples of fresh cheese based on the standard NORMA NTE INEN 1528, determined once the count of the colonies of each microorganism under study, that the samples present $96.8x10^4$CFU / g of *Enterobacteria,* 22.2 $8x10^4$CFU / g of *Escherichia coli* and $23.6x10^4$CFU / g of

Staphylococcus aureus , which clearly indicates that the values are outside the ranges permissible by the standard, and therefore are not suitable for human consumption, in addition, this reflects a deficiency in the chain of production, transport and conservation of the product before being marketed and therefore these products are a source of disease transmission.

- Carried out the verification of the labels or labeling of the samples analyzed according to the parameters established in the NTE INEN 1334 standard, it was determined that the parameters are only met in 70 or 80% being the instructions of use, marking of the date and instructions for the conservation and identification of the lot the most frequent in not being complied with. Although the samples do comply with the health notification parameter, 60% present a notification from other companies, so it is concluded that the labeling of these samples does not comply with the provisions of the standard and also presents false and erroneous information to consumers.

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