Ministry of Higher Education

And Scientific Research

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Detection the Bacterial Contamination of local cheese(sweet &salt) from different areas in Diyala Province

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﴿ قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ﴾

صدق الله العظيم

سورة البقرة:الاية (٣٢)

الاهـــــداء . . .

الى الشفاه التي كلما اكثرت لنا الدعاء كلما نطقت ..

الى التي لولاها لنا مسكت اناملي قدما ...

وسهرت الليالي على راحتي ...

"والدتي العزيزة"

الى القلب الطيب ... ومعلمي الاول ...

وقدوتي في الحياة ..

" والدي العزيز"

الى ملامح طفولتنا وعنفوان شبابنا ...

الى من طال بهم شوط الانتظار ...

الى من اشد بهم ازري في الحياة ...

"اخوتي وأخواتي الاعزاء"

الى الشموع المحترقه ومناهل العلم ...

الى من علمونا حروفا من ذهب وكلمات من درر..

الى من صاغو لنا علمهم حروفا ومن فكرهم منارة ..

"اساتذتي الافاضل"

الى رفاق الدرب الطويل ...

الى الذين لم تلدهن امي من تحلو بالوفاء والعطاء ...

الى من عرفت كيف اجدهم وعلموني ان لا اضيعهم ..

"زملائي الاعزاء"

اقدم شكري وامتناني الى استاذي الفاضل الدكتور (هادي صالح مهدي) لما قدمه لي من ارشادات عمليه ومابذله من جهد كبير ومتواصل وكان له الفضل الكبير في ارساء اللمسات العلمية على هذا الجهد ...

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Abstract

Cheeses, even though characterized as safe for consumption, have been borne outbreaks associated with severe symptoms and implicated in food high fatality rate. The foodborne pathogens in raw milk originate from the farm environment and direct excretion from animals infected udder,. Important source of contamination during the handling and processing might be the workers as well. (The objective of this study was to review literature on the contamination of cheese in different area of Diyala city) .

70 samples of cheese collected (35 samples sweet cheese (without salt) &35 samples salt cheese)from different area of Diyala.
The results of this study were (54% positive) for pollution in sweet cheese and the area of Hebhb and Buhrz was the most polluted region of sweet cheese ,while (34%positive) for pollution in salt cheese and the area of Buhrz was the most polluted region of salt cheese .

INTRODUCTION

Cheese manufacture is one of the classical examples of food preservation, dating from 6000-7000 BC. Preservation of the most important constituents of milk (i.e. fat and protein) as cheese exploits two of the classical principles of food preservation, i.e. lactic acid fermentation and reduction of water activity through removal of water and addition of salt(NaCl). The establishment of a low redox potential as a result of bacterial growth contributes to the storage stability of cheese. (Fennema, 1996; Potter and Hotchkiss, 1995).

CHEESE VARIETIES

There are more than 400 varieties of cheese produced throughout the world, created by differences in milk source (geographic district or mammalian species), fermentation and ripening conditions as well as pressing, size and shape. Most of the cheese types that are produced today originated many centuries ago within smaller communities and are thus named, for example, Camembert and Brie from France, Gouda and Edam from the Netherlands, Cheddar and Cheshire from England, Emmentaler and Gruyere from Switzerland, Parmesan and Gorgonzola from Italy, and Colby from the USA; others are named for some aspect of their manufacture, e.g. Feta from Greece, processed cheese (best-known as the cheese slices that go on hamburgers) from the USA, and Mozzarella from Italy; (Gould et al., 2014) other names are more generic, e.g. cottage cheese from the USA and in Iraq there are two locally type of cheese (soft cheese or sweet and solid cheese or salt cheese ,which present in different forms.

General Cheese Processing Steps

- Standardize Milk
- Pasteurize/Heat Treat Milk
- Cool Milk
- Inoculate with Starter & Non-Starter Bacteria and Ripen
- Add Rennet and Form Curd
- Cut Curd and Heat
- Drain Whey
- Texture Curd
- Dry Salt or Brine
- Form Cheese into Blocks
- Store and Age
- Package

The times, temperatures, and target pH values used for cheddar cheese will depend on individual formulations and the intended end use of the cheese.

These conditions can be adjusted to optimize the properties of Cheddar cheese for shredding, melting, or for cheese that is meant to be aged for several years.

1. Standardize Milk

Milk is often standardized before cheese making to optimize the protein to fat ratio to make a good quality cheese with a high yield

2. Pasteurize/Heat Treat Milk

Depending on the desired cheese, the milk may be pasteurized or mildly heat-treated to reduce the number of spoilage organisms and improve the environment for the starter cultures to grow. Some varieties of milk are made from raw milk so they are not pasteurized or heat-treated. Raw milk cheeses must be aged for at least 60 days to reduce the possibility of exposure to disease causing microorganisms (pathogens) that may be present in the milk.

3. Cool Milk

Milk is cooled after pasteurization or heat treatment to 90°F (32°C) to bring it to the temperature needed for the starter bacteria to grow. If raw milk is used the milk must be heated to 90°F (32°C).

4. Inoculate with Starter & Non-Starter Bacteria and Ripen

The starter cultures and any non-starter adjunct bacteria are added to the milk and held at 90°F (32°C) for 30 minutes to ripen. The ripening step allows the bacteria to grow and begin fermentation, which lowers the pH and develops the flavor of the cheese.

5. Add Rennet and Form Curd

The rennet is the enzyme that acts on the milk proteins to form the curd. After the rennet is added, the curd is not disturbed for approximately 30 minutes so a firm coagulum forms.

6. Cut Curd and Heat

The curd is allowed to ferment until it reaches pH 6.4. The curd is then cut with cheese knives into small pieces and heated to 100°F (38°C). The heating step helps to separate the whey from the curd.

7. Drain whey

The whey is drained from the vat and the curd forms a mat.

8. Texture curd

The curd mats are cut into sections and piled on top of each other and flipped periodically. This step is called cheddaring. Cheddaring helps to expel more whey, allows the fermentation to continue until a pH of 5.1 to 5.5 is reached, and allows the mats to "knit" together and form a tighter matted structure. The curd mats are then milled (cut) into smaller pieces.

9. Dry Salt or Brine

For cheddar cheese, the smaller, milled curd pieces are put back in the vat and salted by sprinkling dry salt on the curd and mixing in the salt. In some cheese varieties, such as mozzarella, the curd is formed into loaves and then the loaves are placed in a brine (salt water solution).

10. Form Cheese into Blocks

The salted curd pieces are placed in cheese hoops and pressed into blocks to form the cheese.

11. Store and Age

The cheese is stored in coolers until the desired age is reached. Depending on the variety, cheese can be aged from several months to several years.

12. Package

Cheese may be cut and packaged into blocks or it may be waxed.

Cheese is popular in many countries (CDIC, 2014) because of the associated health benefits and flavor. The health benefits of cheese include natural probiotic and anti-tumor properties (Goldin and Gorbach, 1992). Additionally, cheese is a rich source of dietary calcium (IOM, 2011), phosphorus, and proteins (USDA, 2011), and has been shown to reduce the incidence of type II diabetes (Mozaffarian et al., 2010).

Although cheese is generally considered a safe food because of the physicochemical and antagonistic properties of lactic acid bacteria, 0.4% of all food borne outbreaks were related to contaminated cheese in 2006 in the European Union (Kousta et al., 2010). Many of these food borne outbreaks were a result of contamination with *Staphylococcus aureas*, and this was not surprising as *S. aureas* often causes mastitis in cows, leading to milk contamination (Rabello et al., 2007). Additionally, in the USA, *S. aureas* and *Listeria monocytogenes* were isolated from unpasteurized soft cheese on sale in California, which was smuggled into the USA from Mexico in 2010 (MDA, 2010).

. This implication may have resulted from the properties of soft cheese like the high water activity and low acidity as well as improper sanitation that is common route for pathogenic bacteria to contaminate the cheese during cheese-making process (Gould et al., 2014). Further, *L. monocytogenes* was often found in soft cheese, which has high moisture content: 67% of moisture on a fat-free basis or ≥50% of moisture content (Codex, 2000; FDA, 2012a).

This data suggest that cheese may pose a significant risk to consumers (Janštová et al., 2014). Given this picture, strict guidelines have been established to control foodborne pathogens in cheese, especially for *L. monocytogenes* (FDA, 2003; EFSA/ECDC, 2011). For instance, for cheese with an average pH greater than 5 and an average Aw above 0.94 along with a shelf life of more than 5 days, European Union law requires 0 colony-forming unit (CFU) of *L. monocytogenes* in five 25 g cheese samples at the time of production, and less than 100 CFU/g in five samples at the point of sale (European Commission, 2007).

FOOD SAFETY AND PRESERVATION

As mentioned previously, the first major addition of sodium to foods was as salt, which acted to prevent spoilage. Prior to refrigeration, salt was one of the best methods for inhibiting the growth and survival of undesirable microorganisms. Although modern-day advances in food storage and packaging techniques and the speed of transportation have largely diminished this role, salt does remain in widespread use for preventing rapid spoilage (and thus extending product shelf life), creating an inhospitable environment for pathogens, and promoting the growth of desirable micro-organisms in various fermented foods and other products. Other sodium-

containing compounds with preservative effects are also used in the food supply.

Salt's Role in the Prevention of Microbial Growth

Salt is effective as a preservative because it reduces the water activity of foods. The water activity of a food is the amount of unbound water available for microbial growth and chemical reactions. Salt's ability to decrease water activity is thought to be due to the ability of sodium and chloride ions to associate with water molecules (Fennema, 1996; Potter and Hotchkiss, 1995).

Adding salt to foods can also cause microbial cells to undergo osmotic shock, resulting in the loss of water from the cell and thereby causing cell death or retarded growth (Davidson, 2001). It has also been suggested that for some microorganisms, salt may limit oxygen solubility, interfere with cellular enzymes, or force cells to expend energy to exclude sodium ions from the cell, all of which can reduce the rate of growth (Shelef and Seiter, 2005).

Today, few foods are preserved solely by the addition of salt. However, salt remains a commonly used component for creating an environment resistant to spoilage and inhospitable for the survival of pathogenic organisms in foods. Products in the modern food supply are often preserved by *multiple hurdles* that control microbial growth (Leistner, 2000), increase food safety, and extend product shelf life. Salt, high- or low-temperature processing and storage, pH, redox potential, and other additives are examples of hurdles that can be used for preservation. As shown in Figure 4-1, no single preservation method alone would create a stable product; when combined, however, these methods result in a desirable, stable, and safe product. For example, a food might be protected by a combination of salt, refrigeration, pH, and a chemical preservative cheese,

Materials and methods:

1-Samples.

2-Nutrient agar.

3-Gram stain.

4-Microscope.

5-Septic container.

6-Burner.

7- Petri dish.

8-Autoclave.

9-Microbalance.

10-Loope.

11-Hote plate shaker.

12-Incubation.

13-Gloves and Mask

Collection of samples

50 samples of each type are collected from different area of Diyala province , the samples from markets. The samples are taken to the laboratory for bacteriological analysis.

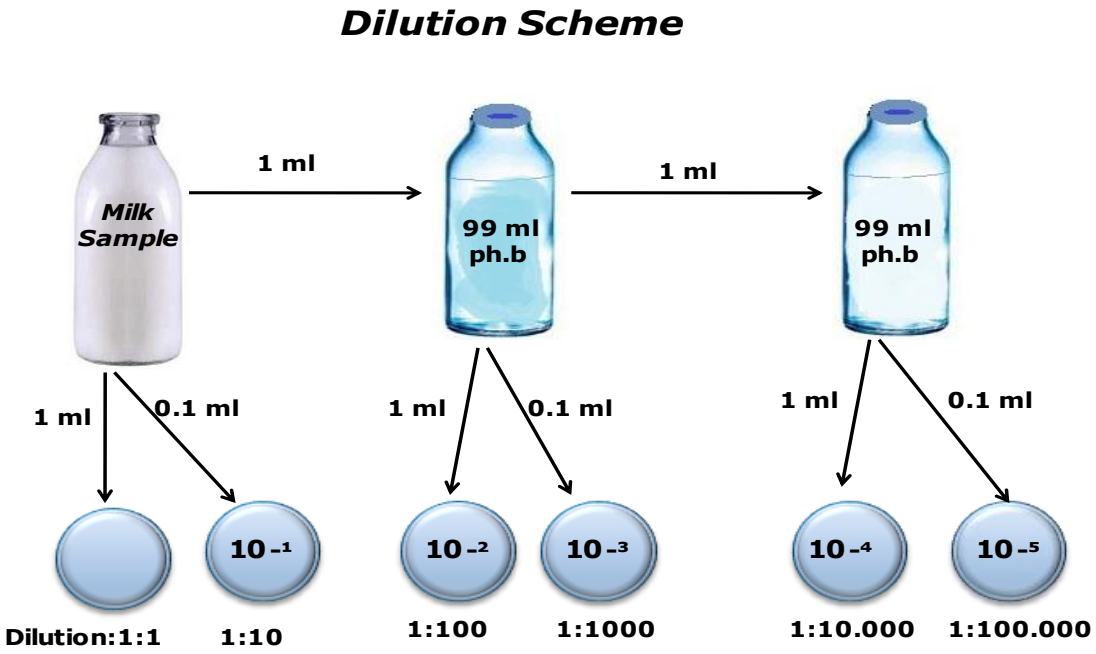
Study methodology:

11 grams of cheese were added to 99ml of sterile distilled water in a flask and shaken well to make 10^{-1} dilution. Further dilutions were prepared in sterile distilled water. Prepared samples were serially diluted (10^{-6}) in sterile water and used for the detection of the microbial contamination of the cheese. Total Plate count : Total plate count were enumerated by pour plate method using standard plate count .Diluted samples were cultured on nutrient Agar by using one ml of each dilution (10^{-6}), which added to petri-dish and incubated at 37C° for 24 hours, colonies were counted.

Dilution of cheese samples:

By using Standard Plate Count (SPC) Test

The chief cultural procedure for determining viable bacterial populations in dairy products has been an agar plate method. This method consists of growing the bacteria in a nutrient culture petridish or (petrifilm) and counting colonies which develop. It can be used for all types of dairy products and is generally used in the examination of Grade A raw and pasteurized milk. This method used to determine the general quality of the milk supply.(Laboratory Manual For Milk Testing, 2012).



Nutrient Agar

Nutrient Agar is used for the cultivation of less fastidious microorganisms, can be enriched with blood or other biological

fluids.

Composition

Ingredients Gms / Litre

Peptic digest of animal tissue 5.000

Sodium chloride 5.000

Beef extract 1.500

Yeast extract 1.500

Agar 15.000

Final pH (at 25°C) 7.4±0.2

Preparation of Nutrient Agar

Suspend 28.0 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense as desired and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring.

(HIMEDIA M001-500G).

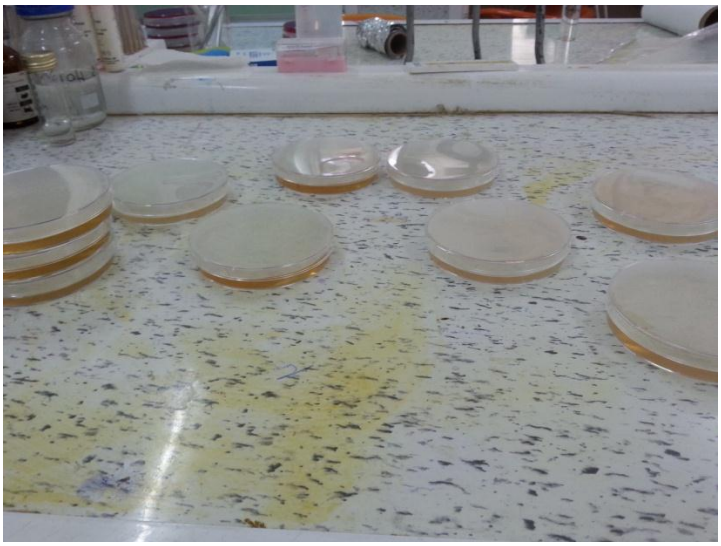


figure1: preparing the petri dishes

Figure 2 :Incubation the samples

Figure 3: culturing the petri dishes

Figure 4: incubation the samples

Figure 5: microscopically examination

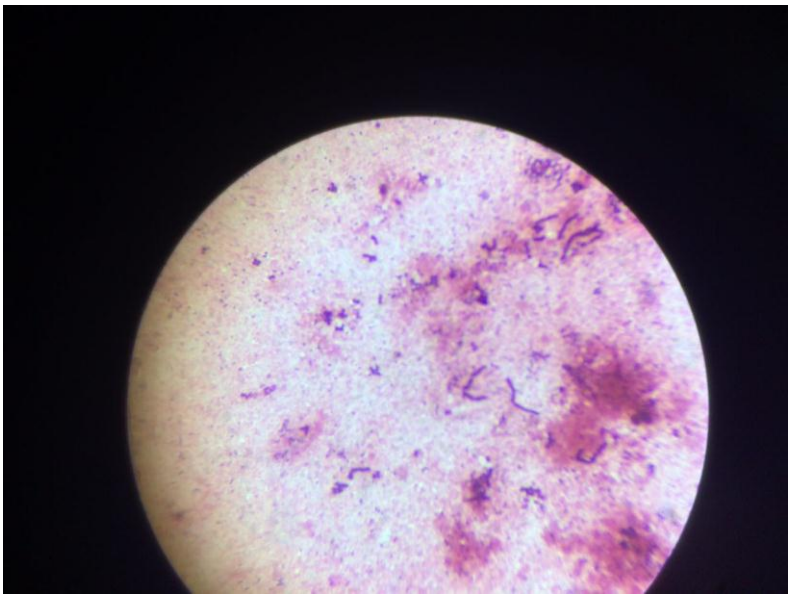


Figure 6 : samples of gram stain

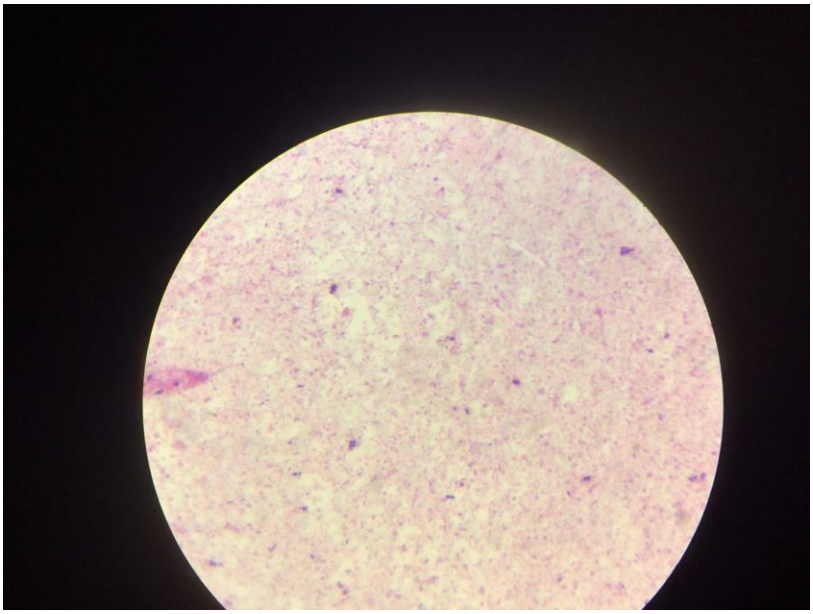


Figure 7 : samples of gram stain

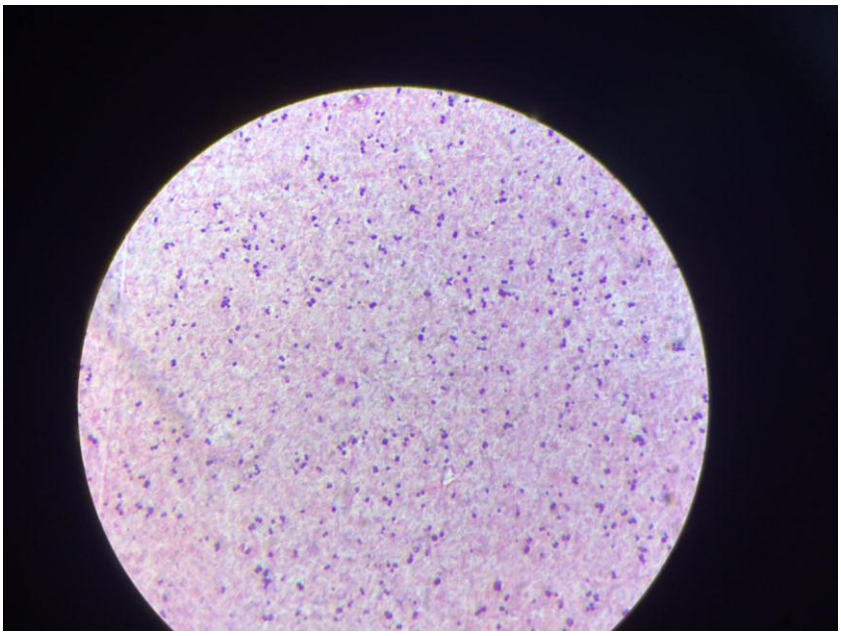


Figure 8 : samples of gram stain

Results:-

The samples are collected from different area of Diyala city.

list 1 : Sample of sweet cheese

Name of area	No. of sample	positive	negative
Khanaqin	5	2	3
Hibhib	5	4	1
Khan Bani Saad	5	3	2
Saadia	5	2	3
Jalawla	5	2	3
Bharz	5	4	1
Clare	5	2	3
Total	35	19	16

list 2 : Sample of salt cheese

Name of area	No. of sample	positive	negative
Khanaqin	5	2	3
Hibhib	5	٢	3
Khan Bani Saad	5	1	4
Saadia	5	2	3
Jalawla	5	0	5
Bharz	5	4	1
Clare	5	1	4
Total	35	12	23

Discussion :-

70 samples of cheese collected (35 samples sweet cheese (without salt) &35 samples salt cheese)from different area of Diyala.

The results of this study were (54% positive) for pollution in sweet cheese and the area of Hebhb and Buhrz was the most polluted region of sweet cheese ,while (34%positive) for pollution in salt cheese and the area of Buhrz was the most polluted region of salt cheese .

Through the results presented by this study is the investigation of bacterial contamination of sweet cheese, ie, salt-free and salt cheese

Sweet cheese is more likely to be contaminated than salty cheese because of the effect of salt on bacterial growth and the growth of microbial colonies , However, pollution in salty cheese can be attributed to salt content. the higher the salt content, the less pollution.

Salt was one of the best methods for inhibiting the growth and survival of undesirable microorganisms. Although modern-day advances in food storage and packaging techniques and the speed of transportation have largely diminished this role, salt does remain in widespread use for preventing rapid spoilage (and thus extending product shelf life), creating an inhospitable environment for pathogens, and promoting the growth of desirable micro-organisms in various fermented foods and other products. Other sodium-containing compounds with preservative effects are also used in the food

Salt is effective as a preservative because it reduces the water activity of foods. The water activity of a food is the amount of unbound water available for microbial growth and chemical reactions. Salt's ability to decrease water activity is thought to be due to the ability of sodium and chloride ions to associate with water molecules (Fennema, 1996; Potter and Hotchkiss, 1995).

Adding salt to foods can also cause microbial cells to undergo osmotic shock, resulting in the loss of water from the cell and thereby causing cell death or retarded growth (Davidson, 2001).

This implication may have resulted from the properties of soft cheese like the high water activity and low acidity as well as improper sanitation that is common route for pathogenic bacteria to contaminate the cheese during cheese-making process (Gould et al., 2014).

Recommendation:-

1-Milk used to make cheese must be free from contamination.

2.Cheese production should be done in healthy ways.

3.Transfer cheese from one area to another under good sanitary conditions for not polluting it .

4.The offer of cheeses for sale shall be in special displays and protected from any contamination .

5. Store the cheese in special coolers for not polluting and damaging them.

الخـــلاصـــة :-

تم جمع عينات الجبن من مناطق مختلفة من محافظة ديالى لغرض الكشف عن التلوث البكتيري في الجبن بنوعيهِ الحلو(خالي من الملح) والمالح ..

وكان عدد النماذج هو (٧٠) نموذج (٣٥ نموذج من الجبن الحلو اي الخالي من الملح من سبعة مناطق بواقع ٥ عينات من كل منطقة وكذلك ٣٥ نموذج من الجبن المالح من سبعة مناطق بواقع ٥ عينات من كل منطقة ..

وأظهرت النتائج الزرعية ان نسبة التلوث في الجبن الحلو هو (٥٤%) من مجموع العينات وكانت منطقة هبهب وبهرز من اكثر المناطق تلوثا للجبن الحلو .

وكذلك كانت نسبة التلوث للجبن المالح وحسب النتائج الزرعية هي (٣٤%) من مجموع العينات واظهرت النتائج ان منطقه البهرز من اكثر المناطق تلوثا بالجبن المالح ...

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