

سەڵاحەدین زانکۆی

Salaheddin University - Erbil

Isolation and Identification of Pathogenic Bacteria with Potential for Spoilage from Some Refrigerated Foods after Heating

Research Project Submitted to the department of (Biology) in partial

fulfillment of the requirements for the degree of B.A or BSc.in biology

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**Supervisor Certification**

We certify that this research project was prepared under our supervision in the Department of Biology, College of Education, Salahaddin University-Erbil and hereby recommend it to be accepted in partial fulfilment of the requirements for the degree of BSc.

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Data;

**Chair Certification**

In view of the available recommendation, I forward this research project

for debate by the examining committee.

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**Abstract**

Food is a chemically complex matrix, and predicting whether, or how fast, microorganisms will grow in any given food is difficult. Most foods contain sufficient nutrients to support microbial growth. Several factors encourage, prevent, or limit the growth of microorganisms in foods; the most important are water availability, pH, and temperature .

Low-temperature storage is a common way to preserve foods that contain heat-stable nutrients. Improper handling with improper use and abuse of temperature while storing these foods may lead to the growth of bacteria initially present in the raw materials. The production of extracellular enzymes by semi-organisms at different temperatures indicates the possibility of their exocytosis.

In this study, thirty samples were collected including rice, red meat, shishkin meat, kofta, doksawa, parma, rice ,Rice with tamato, dagua, green beans, brian, potatoes in water, lentils, grilled chicken breast, chickpeas, grits, yogurt,Foul, dolma, falafel, squash, fried eggplant, limo,Fish meat, mushroom soup, kibbeh, Kurdish pilaf, Eggs in Tamato, Bulgur and Sarobi samples were collected during the period from the 15th of the October 2022 to the November of the 2022 from the refrigerator after reheating. The samples were cultured on selective and differential media. Several common pathogenic bacterial species were screened by analyzing their morphological and cultural characteristics. *Klebsiella spp* (57.4) was prevalent in most of the samples, while *Pseudomonas.spp* (9.4) was less prevalent. A large number of samples showed adequate microbial loads at infection or sepsis levels.

**Introduction**

Refrigeration is the most widely used method to prevent foods for some time. Mostly the perishable foods are kept for refrigeration to control the microbial contamination at 4-50C. (1, 2) Refrigeration not only minimizes the contamination of foods but also the chemical and enzymatic spoilage of food by retarding the growth of microbes (3). There are certain studies which proved that the perishable foods even preserved at refrigeration temperature also undergo spoilage (4). The food spoilage depends on the type of storage container or wrapping material. Several other factors related to the food stored also influence the rate of spoilage (5).

When there is contamination with microbes, this can cause food spoilage (change in characteristics of food rendering it unacceptable or unsafe for consumption) and food borne diseases (dened by WHO), as any disease of an infectious or toxin nature caused by or thought to be caused by the consumption of food or water,Temperature is one of the main factors in controlling food quality and safety due to its influence on microbial growth rates. Despite the fact that the low temperature can reduce the growth rate of many species of microorganisms, it has been reported that psychrotrophic microorganisms can thrive under normal refrige-ration temperatures(Odewade,etal.,2018).

The main microbiological objective in low-temperature preservation of food is to prevent or reduce growth of microorganisms. Low temperature also reduces or prevents catalytic activity of microbial enzymes, especially heat-stable proteinases and lipases. Germination of spores is also reduced, but spores are not killed at low temperature . Low-temperature storage, especially freezing, is also lethal to microbial cells, and under specific conditions, 90% or more of the population can die during low-temperature preservation. The metabolic activities, enzymatic reactions, and growth rates of microorganisms are maximum at the optimum growth temperature. As the temperature is lowered, microbial activities associated with growth slow down. Normally, the generation time, is doubled for every 10C reduction in temperature. Thus, a species dividing every 60 min in a food at 22 C will take 120 min to divide if the temperature is reduced to 12C. The lag and exponential(Log) phases and the germination time (of spores) for some psychrotrophs (mesophilic types) become longer as the temperature is reduced to 0C or even to –1C. The term psychrophile is applied to organisms that grow over the range of subzero to 20°C, with an optimum range of 10-15°C. The term psychrotroph is an organism that can grow at temperatures between 0°C and 7°C and produce visible colonies (or turbidity) within 7-10 days. Some psychrotrophs in fact, mesophiles(Akpogumaetal.,2015).

 The microorganisms that cause the spoilage of meats, poultry, and vegetables in the 0-5°C range would be expected to be psychrotrophs. Many types of microorganisms can cause food problems. The microorganisms that can cause food-borne illness are called pathogenic microorganisms. These microorganisms grow best at room temperatures (60-90°F), but most do not grow well at refrigerator or freezer temperatures. Pathogenic microorganisms may grow in foods without any noticeable change in odor, appearance or taste. Spoilage microorganisms, including some kinds of bacteria, yeasts and molds, can grow well at temperatures as low as 40°F. When spoilage microorganisms are present, the food usually looks and/or smells awful(Nebraska ExtensionUNL FOOD,2023) https://food.unl.edu/how-food-spoils

**2. MATERIALS AND METHODS**

Thirty different food samples and juices were obtained after storing them in the refrigerator for two to three days after heating, and then storing them in the refrigerator. The swabs were first dissolved in sterile peptone water and then inoculated on different culture media: Mannitol Salt Agar, Salmonella-Shigella Agar and Blood Agar. The samples were incubated and observed for the growth of colonies. The colonies were identified by the colony characters and Microscopy (Gram staining). For all samples, after adding one gram or one milliliter of sample (according to the type of sample, 1 gram of solid sample with 90ml sterile physiology saline or 1 milliliter of watery sample) to 4 milliliters of Agar broth, the mixture was homogenized by using a shaking. The ' inoculated broth samples' were then incubated at 37 °C for 24 hours (Jose et al., 2015).

**Result**

Thirty different samples were collected from various foods that were cooled after heating and kept for three days after isolation and identification. The result showed that Klebsiella spp was the most prevalent in refrigerated food samples with (57.4%) recorded, and Enterobactar spp (4.7%) as shown in Table (1-3) and Figure (1-3) shows that the percentage of bacterial contamination reached 70% , including 21 samples, and the percentage of non-contamination reached 30%, including 9 samples.

**Table(1-3) The percentage of bacterial contamination and non-contamination of refrigerated food after heating it**

|  |  |  |  |
| --- | --- | --- | --- |
| **Bacteria** | **Gram stain** | **Frequency of occurrence** | **%**  |
| *E. coli* | **Negative** | 3 | 14.2 |
| *Klebsiella spp* | **Negative** | 12 | 57.4 |
| *Proteus.spp* | **Negative** | 3 | 14.3 |
| *Pseudomonas.spp* | Negaitve | 2 | 9.4 |
| Total | Negative | 21 | 100 |

**Figure (1-3) represents bacterial growth and non-growth of refrigerated food after heating**

|  |  |  |
| --- | --- | --- |
| Sample | %Growth | % No Growth |
| Rice | + |  |
| Read meat | + |  |
| Chichken meat | + |  |
| Kfta | + |  |
| Doxawa |  | + |
| Brma | + |  |
| Rice with Mungbean | + |  |
| Rice with Tamato |  | + |
| Doghawa | + |  |
| Green bean |  | + |
| Biryane | + |  |
| Patato in water |  | + |
| Lentil | + |  |
| Roasted chicken chest | + |  |
| Chickpea |  | + |
| Grit |  | + |
| Milk | + |  |
| Bean | + |  |
| Dolma | + |  |
| Falafel  | + |  |
| Squash | + |  |
| Fried eggplant |  | + |
| Lemo |  |  |
| Fish meat | + |  |
| Mushroom Soup | + |  |
| Kibbeh |  | + |
| Kurdish Pilau | + |  |
| Egg in Tamato | + |  |
| Bulgur | + |  |
| Sarupe |  | + |
| Total | 21(70%) | 9(30%) |

**Disscution**

Klebsiella was the most frequently isolated bacteria in our study [%57,4],Klebsiella bacteria are normally found in the human intestine.Klebsiella pathogenic strain responsible for multiple nosocomial infections, including pneumonia, urinary tract, and soft tissue infections,

proteus spp. second most frequently in our study were recovered from[%14,3] of the refrigerators examined

E. coli was isolated from %14,2 of refrigerators in this study widely. It is accepted indicator of fecal contamination suggesting that the refrigerator internal surfaces are frequently contaminated by the import of contaminated raw foods or by poor personal hygiene.

Pseudomonas spp isolated from %9,4 in the refrigerators in our study, are found widely in the environment, such as in soil, water, and plants. They usually do not cause infections in healthy. people. Infectious species include P. aeruginosa, P. oryzihabitans, and P. plecoglossicida. P. aeruginosa flourishes in hospital environments. It is the second-most common infection in hospitalized patients (nosocomial infections).

Enterobacter spp recovered from %4,7 from refrigerators

 The results showed that 70%(21/30) of respondents did not clean vegetables, fruit and meats before keeping in refrigerators may because of the common belief that store them without washing makes them resistant to rot for longer time. That action leads to spread of bacteria in refrigerators. The use of chromagarmedia give rapid detection to differentiate between the bacterial colonies by color and morphology characteristics.

It is impossible to completely exclude food pathogens from the kitchens; however their spread, growth and survival can be controlled with correct food storage and preparation of practices and regular cleaning and

disinfection of food contact cite

**Conclution**

This study showed that pathogenic bacteria can live in refrigerated food samples after heating them, and due to the high number of bacteria observed in food samples, they can thus pose a risk to the health of the consumer. Refrigerated foods can become vectors for foodborne pathogens through abuse of temperature and inadequate cleaning of household refrigerators. It is critical for the public to understand that the refrigerator can be an important habitat for foodborne pathogens to persist and spread due to its reliance on refrigeration as a method of food preservation. Care must be taken when eating refrigerated foods, especially after a long period of time.

To prevent contamination in the refrigerator, there are rules and best practices, includingThoroughly wash your hands before handling and preparing food stored in the refrigerator.Keep ready-to-eat and cooked food above raw food in the refrigerator.Place [raw poultry](https://www.foodsafety.ca/blog/how-buy-store-and-prepare-poultry-safely), meat and seafood on the bottom shelf of the refrigerator in food-grade, covered containers or sealed plastic bags to prevent juices from dripping onto other foods.Ensure the fridge is fully emptied prior to cleaning and sanitizing, and that spills are thoroughly cleaned when they occur.Store foods in properly labelled, sanitized containers with airtight lids or seals,Ensure you and your staff are trained in food safety policies and procedures, including contamination prevention.

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