SUMMARY AND CONCLUSION

Milk quality is an important factor in the production of high quality dairy products. High load of spore-forming bacteria in raw milk is an indication of persistently neglected cleancing of dairy farm or dairy plant.

Spore-forming bacteria capable to form heat-resistant endo-spores which cause a major technological problems in dairy industry.

Storing raw bulk milk at low temperature before processing has increased the probability that psychrotrophic bacteria will grow and produce heat-resistant enzymes, which can affect UHT-milk products and cause its spoilage.

The main object of this study stressed on spoilage of UHT-milk by microorganisms or by factors other than viable microorganisms i.e. microbial enzymes, therefore, this work was undertaken in five parts and the results can be summarized in the following aspects:

Part I. Spore-forming Bacteria in Raw milk:

1- The count of aerobic spore-forming bacteria in raw milk ranged form 66.8 x 10^2 to 507.5 x 10^2 c.f.u/ml with an average of 196.5 x 10^2 c.f.u/ml.

2- The anaerobic saccharolytic spore-forming bacteria ranged from 0.43 x 10^2 to 11x 10^2 /ml with an average of 4.8 x 10^2 /ml in raw milk samples while the anaerobic proteolytic spore-forming bacteria average of 0.88 x 10^2/ml.

Part II. **Bacteriological Quality of UHT-milk:**

1- The UHT-milk samples did not show any count of anaerobic mesophilic and thermophilic bacteria either saccharolytic or proteolytic.

2- The aerobic thermophilic bacteria not detected in all tested UHT-milk samples.

3- From all UHT-milk samples, three samples only contained 1.1 $x10^2$, 5 x 10^2, $6x10^2$ c.f.u/ml for aerobic mesophilic bacteria.

4- Incubation of UHT-milk samples showed no detectable anaerobic mesophilic or thermophilic bacteria in the tested UHT-milk samples incubated for 14 days at 30°C and 7 days at 55°C either saccharolytic or proteolytic. While, in respect of aerobic mesophilic and thermophilic bacterial counts the average was 1.15 x 10^2 c.f.u/ml and 0.4x 10^2 c.f.u/ml, respectively.

Part III. **Effect of sub-lethal Heat-shock and some Germinating Activators on the Bacterial count of UHT-milk:**

1- Using heat-shock as an activator increased the spore germination, the mesophilic aerobic count increased from 2 $x10^2$ c.f.u/ml (In control) to 16 $x10^2$ c.f.u/ml, the count of thermophilic aerobic was 5.4 x 10^2 c.f.u/ml, while there was no growth in control. The count of aerobic mesophilic bacteria was 15.6 x 102 c.f.u/ml, in UHT-milk samples incubated for 14 days at 30°C while, the count of aerobic thermophilic organisms was increased to 14.9 x 10^2 c.f.u/ml in UHT-milk stored for 7 days at 55°C.

2- Addition of some germinating activators to the nutrient agar medium such as starch, L-alanine, L-cysteine, tyrosine and adenosine increased the count of bacteria.

- Using L-alanine as an activator gave the highest aerobic thermophilic count as it was 54.6 $x10^2$ c.f.u/ml while, it was not detected in control

followed by adenosine ($43.7x10^2$ c.f.u/ml), tyrosine ($41.3 x10^2$ c.f.u/ml) and then starch ($14x 10^2$ c.f.u/ml).

- Using of adenosine increased the aerobic mesophilic bacterial count of UHT-milk samples, the aerobic mesophilic bacterial count of incubated UHT-milk for 14 days at 30°C and the aerobic thermophilic bacterial count of incubated UHT-milk for 7 days at 55°C as they $53x10^2$ c.f.u/ml while they were $2x10^2$, $1.7 x 10^2$ and $0.6x 10^2$ in control, respectively.

4) Using heat-shock or the germination activators didn't appear any bacterial count of anaerobic saccharolytic or proteolytic spore-formers bacteria in all tested UHT-milk samples.

Part IV: **Isolation and identification of aerobic spore-formers from UHT-milk.**

1) One hundred and nine pure cultures were isolated from UHT-milk after using germinating activators.
2) All cultures were spore-forming rods which morphologically characterized as genus *Bacillus.*
3) The Isolates were identified to species level by some biochemical tests.
4) The growth profile of the isolated showed that all isolated grow at 30°C 99% of total isolates grow at 40°C, 62.4% grow at 50°C, 43% grow at 55°C, 39.4% grow at 10°C 13.8% at 5°C and their was no growth at 65°C.
5) The isolates were identified as *B. licheniformis* (28.16%), *B. coagulans* (17.48%), *B. subtilis* (15.53%), *B. megaterium* (8.74%), *B. firmus (6.8%), B. cereus, B. pumilus* and *B. brevis* (3.88%) for each, *B. pantothenticus* (2.91%), *B. circulans, B. alvei, B. polymyxa* and *B. lentus* (1.94%) for each and *B. sphaericus* (0.97%).

Part V: **Evaluation of UHT-milk during storage:**

In this part the samples were divided into two portions. First portion was stilled under normal packaging (under package vacuum), but the second portion was out of normal packaging as it was transferred aseptically into a sterilized container.

1- The differences between total bacterial count of UHT-milk samples during 90 days of storage at 30°C with and without using spore activators under and out of normal packaging were almost insignificant except in the case of using starch or tyrosine as an activators the differences were significant.

2- The storage of UHT-milk at 30°C has a significant effect on its quality.

3- The proteolytic activity of UHT-milk was 0.94 u/ml/min at the beginning. With storage development the proteolytic activity in the UHT-milk stored under normal packaging was gradually increased to be 5.28 U/ml/min after 90 days. While in the samples stored out of normal packaging the increased was with a higher as it reached to 7.36 u/ml/min after 90 days of storage at 30°C.

4- The pH value of UHT-milk was slightly decreased during storage.

5- Milk appearance of UHT-milk was normal during storage for 90 days at 30°C under normal packaging but it spoiled (gelled) after 30 days of storage out of normal packaging.

6- Addition of high concentration of the germinant (1.0 % L-alanine) to the milk was capable of fast and great germination and out growth of survival spores exists in UHT-milk with a concentration of 1.0% increased greatly the proteolytic activity from 0.6 U/ml/min to 3.86 and 4.7 U/ml/min for milk under and out of normal packaging, respectively.

According to these findings, it could be recommended that:

1- Heat treatment of processing milk immediately on delivery at dairy plant and minimizing the cold storage period of raw milk.

2- Addition of some germinating activators to raw milk before heat treatment.

Application of these recommendations in addition to efficient hygiene procedure as an integrated system procedure can greatly overcome the problems usually caused by the spore-forming bacteria and heat-stable enzymes.