



Department of Food Hygiene

Incidence of Cronobacter species in milk and some dairy products

Thesis Presented by

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Summary

Milk as well as milk products under the influence of their high nutritive value are considered excellent media for pathogenic as well as non pathogenic bacteria. This can lead to milk borne outbreaks caused by different types of bacteria such as *Cronobacter* which can cause outbreaks of meningitis, sepsis and necrotizing enterocolitis in infants, elderly and immunocompromised patients.

Two hundred and fifty random samples were collected from different localities including raw milk samples and some dairy products as ice cream, domiati, kareish and ras cheese (50 samples each). These samples were examined for the incidence of *Cronobacter* species using Chromogenic *Cronobacter* Isolation agar medium.

The obtained data revealed that 8 (16%), 13 (26%), 1 (2%), 2 (4%), 5 (10%) of the examined samples of raw milk, ice cream, domiati cheese, kareish cheese and ras cheese, respectively were contaminated with *Cronobacter* species.

Biochemical tests used for differentiation between different *Cronobacter* species resulting in the identification of *Cronobacter* isolates as, 75% of *Cronobacter sakazakii*, 12.5% of *Cronobacter malonaticus* and *Cronobacter turicensis* in raw milk samples. On other hand, from the incidence percentage of *Cronobacter* species isolated from ice cream samples 61.5% were *Cronobacter sakazakii*, 15.4% were *Cronobacter malonaticus* and *Cronobacter turicensis* each, and 7.7% were *Cronobacter muytjensii*.

In addition, *Cronobacter* species were isolated from 1 of 50 examined domiati cheese samples which was identified as *Cronobacter sakazakii*. In kareish cheese, *Cronobacter sakazakii* was detected in two samples, while the other *Cronobacter* species could not be detected. On the other hands, *Cronobacter sakazakii* was isolated from 3 samples of ras cheese followed by *Cronobacter malonaticus* and *Cronobacter dublinensis* (1 sample).

Polymerase Chain Reaction (PCR) was performed on 20 isolates of *Cronobacter sakazakii* previously identified by biochemical tests, 15 of them were confirmed to be *Cronobacter sakazakii*.

Minimum inhibitory concentration of propolis and chitosan was determined using agar well diffusion method which indicated propolis as 6.25mg/ml and chitosan 1%. Application of propolis in a concentration 12.5mg/ml in the laboratory prepared ice cream stored at different temperature (-5 and -21°C) significantly affected *Cronobacter sakazakii* count as it decreased the count from 2×10^6 at the beginning of the experiment to reach 4.5×10^3 cfu/ml at the 12th week while, using propolis in a concentration 6.25 mg/ml, the count of *Cronobacter sakazakii* decreased slowly from 2.2×10^6 at zero time until reached 8.5×10^3 cfu/ml at the 12th week when the ice cream was stored at -5 °C. There was no big difference in the reduction of the count of *Cronobacter sakazakii* upon storage of ice cream at -21°C.

The antibacterial effect of chitosan on the count of *Cronobacter sakazakii* was less than propolis. The results indicated that the count decreased from 2.2×10^6 at zero time to 9×10^3 cfu/ml at 12th week when 1.5 % chitosan was used and decreased from 2.2×10^6 to 1×10^4 cfu/ml on concentration 1 % of chitosan at -5 °C, also there was no significant difference in the reduction of the count of *Cronobacter sakazakii* when the

ice cream was stored at -21°C . However, after the 12th weeks the organism still present and the count not decrease below the infectious dose. Addition of propolis in different concentrations on ice cream affected the color, odor, taste and texture but addition of chitosan did not have noticeable effect on the color, odor, taste and texture.

The public health importance of the organism and the steps, which should be taken to control this organism in the dairy industry as well as the recommended sanitary measures, were also discussed.