RESULTS AND DISCUSSION

4.1- Microbiological survey of some foods:

In general the numbers and types of microorganisms in a finished food product are influenced by the following : the general environment from which the food was originally obtained, the microbiological quality of the food in its raw or unprocessed state, the sanitary conditions under which the product is handled and processed and the adequacy of subsequent packaging, handled, and storage conditions of the product to maintain the flora at a low level **(James 1986)**.

Food containing vegetables, cereals and spices are naturally contaminated with a variety of microorganisms while other components such as milk and milk products contain both spoilage and pathogenic microorganisms **(Hobbs and Gilbert 1978)**.

The counts of aerobic plate count (APC), psychrotrophic bacteria (PTC), lactic acid bacteria (LAB), coliform bacteria (CF), yeasts and moulds (Y&M), halotolerant count (HTC) and occurrrence of *Bacillus spp.* recorded for all samples in Tables 7 to 12.

4.1.1- Pasteurized milk :

The initial microflora of freshly pasteurized milk consists primarily of thermoduric bacteria and spores. The types and numbers of the bacteria are dependent on the microbial population of the raw milk before pasteurization. The absence of psychrotrophic bacteria or if large numbers of thermoduric bacteria survive pasteurization, certain thermodurics, particularly psychrotrophic sporforming *Bacillus spp.* can grow and cause spoilage **(Bodyfelt 1980; Overcast and Atmaram 1974)**.

Data in Table (7) for 8 samples of different kinds of pasteurized milk indicates the range of 58 x 10^4 to 128 x 10^4 cfu/g., 35 x 10^4 to 97 x 10^4 cfu/g., 33 x 10^3 to 167 x 10^3 cfu/g., 15 to 35 cfu/g. and 32 x 10^2 to 95 x 10^2 cfu/g. corresponding to (APC), (STC),

(LAB), (CF) and (HTC), respectively while(Y&M) were not detected but *Bacillus spp.* were found in one sample only. The percentage occurrence of *Bacillus spp.* in these samples was 12.5%. On the other hand **Chen *et al.* (1988)** found in 25 pasteurized milk samples that the total bacterial count was 5×10^6 cfu/mL., and this is higher than hygienic allowance, coliform bacteria were detected in 23 samples with 0.3×10^3 to 2.4×10^3/mL., *Bacillus cereus* only was found in 32% of the samples while **Wagner *et al.* (1984)** tested 10 samples of milk heat treated, 2 had total bacterial count greater than 10^5/mL at the end of 7 days guaranteed shelf life and were contaminated with *B. cereus* var. *mycoides*. **Collins (1971)** isolated a number of psychrotrophic *Bacillus sp.* including *B. cereus* from pasteurized milk. **Reinheimer(1993)** found *Bacillus sp.* in pasteurized milk and the microbial count of 10^6 to 10^9 cfu/mL. after storing milk at 7 and 12°C. when off flavours were evident. **Vaisanen *et al.* (1991)** identified a total of 130 *bacillus* strains isolated from dairy products 98 of these strains were members of the *B. cereus* group. Comparing the results of present work with the U.S. public health service and American association of medical milk commissions Inc., the microbial count were higher than hygienic allowance.

Table (7): Microbiological Analyses Of Milk Samples.

Samples	APC	PTC	LAB	CF	Y&M	HTC	*Bacillus sp.*
full cream milk pasteurised	$75x10^4$	$47x10^4$	$33x10^3$	<30(21)	ND	$37x10^2$	+
full cream milk pasteurised	$82x10^4$	$61x10^4$	$45x10^3$	<30(18)	ND	$42x10^2$	ND
Modified milk pasteurised	$63x10^4$	$40x10^4$	$37x10^3$	<30(27)	ND	$95x10^2$	ND
modified milk pasteurised	$58x10^4$	$44x10^4$	$116x10^3$	35	ND	$40x10^2$	ND
fresh skim milk pasteurised	$124x10^4$	$97x10^4$	$43x10^3$	<30(15)	ND	$86x10^2$	ND
fresh skim milk pasteurised	$162x10^4$	$77x10^4$	$52x10^4$	<30(22)	ND	$50x10^2$	ND
fresh skim milk pasteurised with flavour	$88x10^4$	$35x10^4$	$167x10^3$	<30(17)	ND	$32x10^2$	ND
fresh skim milk pasteurised with flavour	$128x10^4$	$48x10^4$	$38x10^4$	<30(23)	ND	$44x10^2$	ND

Legend: APC, aerobic plate count; PTC, psychrotrophic count; LAB, lactic acid bacteria; CF, coliforms; Y&M, yeast and moulds; HTC, halotolerant count; ND, not detected.

4.1.2-Vegetables:

The incidence of microorganisms in vegetables may be expected to reflect the sanitary quality of stores (refrigerators) and the microbiological condition of the local market. Populations of microorganisms on vegetables vary widely and often depend on the type of vegetables **(Splittstoesser 197 0).**

Data in Table 8 for the six vegetables examined indicates the range of 70 x 10^5 to 184 x 10^6 cfu/ g., 66 x 10^4 to 150 x 10^5 cfu/g., 42 x 10^4 to 92 x 10^5 cfu/g., 89 x 10^2 to 66 x 10^3 cfu/g., 41 x10^2 to 53 x10^3 cfu/g. and 41 x 10^2 to 183 x 10^2 cfu/g. corresponding (APC), (PTC), (LAB), (CF), (Y&M) and (HTC), respectively while *Bacillus spp.* were found in two samples only, which are broccoli and cauliflower. The percentage of *Bacillus spp* occurrence in these samples was 33.3%. On the other hand **Leca and Esterase (1981)** found that total bacterial count ranged from 3 x 10^4 to 10^8/g in lettuce, *B. cereus* count 0 to 10^2/g and fungi count 5.4 x 10^3 to 5.64 x10^4/g while **Riser *et al.* (1984)** found APC can be as high as 10^7cfu/g in lettuce. **Geeson (1979)** and **Priepke *et al.* (1976)** found that cabbage have total count as few as 10^4 or as many as 10^9cfu/g. **Sato and Rodring (1988)** found the highest aerobic bacterial contamination in endive ranging between 7 x 10^5 and 18 x 10^8cfu/g, fungal count of many vegetable samples exceed 3 x 10^4 cfu/g, with the exception of carrots and spores of *B. cereus* were absent from all samples. **Kendo and Mieno (1989)** found the average cell counts/g. in 38 samples of packed cut vegetables were 2.2 x 10^6 for total count, 3.8 x 10^4 for coliform, 4 x 10^3 for *B. cereus* and 1.5 x 10^3 for fungi. **Hunter *et al.* (1994)** found in mayonnaise based delicatesen salads including vegetables the total viable count ranged from less than 200 to greater than 10^5 cfu/g. Yeast were isolated from 66 of the 87 salads (27 samples contained greater than 10^4 cfu/g) and fungi from 10samples while **Albrecht (1995)** analysed vegetables salad ingredients (lettuce, tomatoes, broccoli and cauliflower).

Table (8): Microbiological Analyses Of Vegetables.

Samples	APC	PTC	LAB	CF	Y&M	HTC	*Bacillus sp.*
Broccoli	$162x10^6$	$137x10^5$	$89x10^4$	$112x10^2$	$77x10^2$	$95x10^2$	+
Cauliflower	$96x10^6$	$75x10^5$	$57x10^4$	$89x10^2$	$53x10^3$	$61x10^2$	+
Cabbage	$82x10^5$	$73x10^5$	$53x10^4$	$95x10^3$	$46x10^2$	$131x10^2$	ND
Carrot	$70x10^5$	$66x10^4$	$42x10^4$	$220x10^2$	$38x10^2$	$60x10^2$	ND
Lettuce	$184x10^6$	$150x10^5$	$82x10^5$	$66x10^3$	$51x10^3$	$41x10^2$	ND
Spinach	$154x10^6$	$132x10^5$	$92x10^5$	$254x10^2$	$41x10^2$	$183x10^2$	ND

Legend: APC, aerobic plate count; PTC, psychrotrophic count; LAB, lactic acid bacteria; CF, coliform; Y&M, yeast and moulds; HTC, halotolerant count; ND, not detected.

The total bacterial count for the vegetables ranged from 5.51 to 6.63 log cfu/g. Yeast and moulds were found in all vegetables.

4.1.3- Pasta products:

Pasta products, usually manufactured from durum wheat flour, essentially fall into two categories: egg based pasta such as noodles and macaroni type pasta such as macaroni. Both products are manufactured in much the same fashion. The initial microbiological profile of the mixed dough is directly related to the quality of the ingredients.

Data presented in Table 9 for pasta products indicated the range of 121 x 10^4 to 55 x 10^6 cfu/g., 102 x10^4 to 37 x 10^6 cfu/g., 48 x 10^3 to 30 x 10^5 cfu/g., 128 x 10 to 32 x 10^3 cfu/g., 37 x 10^2 to 81 x 10^2 cfu/g., 38 x 10^2 to 87 x 10^2 cfu/g. corresponding to (APC), (SPC), (CF), (Y&M) and (HTC) while *Bacillus sp.* were not detected. On the **Mellino *et al.* (1989)** examined samples of simple and pasta filled. He found 87.4% of filled pasta and 69.4% of simple pasta had total plate counts greater than 10^6 cfu/g. High total coliform counts were frequent in filled pasta than simple pasta, 35.9% of filled pasta and 13.9% of simple pasta had total coliform counts greater than 10^3/g but **Aureli *et al.* (1986)** found in vaccum packed samples of pasta with meat or cheese and vegetables that the most total count ranged from 10^6 to 10^7 cfu/g for meat based, from 10^5 to 10^6 for cheese and vegetables based products, spores counts were ranged from 10^3 to 10^4 cfu/g. In all type moulds count were very low, no coliform. *Bacillus cereus* was found to be less than 10^3 cfu/g in only a few meat based products. While **Arai *et al.* (1981)** found the total count in two factories respectively were about 10^5 to 10^9 cell/g. They indicated *B. cereus* count in noodles in one factory to be about 10^6/g. **Simpson *et al.* (1994)** found in sous vide spaghetti that the total bacterial count, lactic acid bacteria and *Bacillus sp.* were increased after storage 14 to 21 days between 5 to 15°C.

Table 9: Microbiological Analyses Of Pasta Product.

Samples	APC	PTC	LAB	CF	Y&M	HTC	*Bacillus spp.*
Freshnoodles (rice flour)	$98x10^5$	$83x10^5$	$49x10^4$	$72x10^2$	$81x10^2$	$65x10^2$	ND
Freshnoodles (rice flour)	$181x10^4$	$126x10^4$	$86x10^3$	$41x10^2$	$43x10^2$	$71x10^2$	ND
Freshnoodles (wheat flour)	$196x10^5$	$127x10^5$	$78x10^3$	128x10	$61x10^2$	$53x10^2$	ND
Freshnoodles (wheat flour)	$170x10^5$	$153x10^5$	$136x10^4$	$42x10^2$	$55x10^2$	$47x10^2$	ND
Japanese noodle (wheat flour)	$71x10^5$	$50x10^5$	$67x10^3$	$45x10^2$	$41x10^2$	$38x10^2$	ND
Japanese noodle (wheat flour)	$121x10^4$	$102x10^4$	$48x10^3$	$63x10^2$	$37x10^2$	$41x10^2$	ND
Taingsnoodle (wheat flour)	$55x10^6$	$37x10^6$	$30x10^5$	$32x10^3$	$38x10^2$	$52x10^2$	ND
Taingsnoodle (weat flour)	$82x10^5$	$54x10^5$	$57x10^4$	$81x10^2$	$51x10^2$	$63x10^2$	ND
Fresh pasta	$38x10^6$	$42x10^5$	$36x10^4$	$76x10^2$	$65x10^2$	$71x10^2$	ND
Fresh pasta	$42x10^6$	$37x10^5$	$168x10^4$	$83x10^2$	$51x10^2$	$87x10^2$	ND

Legand: APC, aerobic plate count; PTC, psychrotrophic count; LAB, lactic acid bacteria; CF, coliform; Y&M, yeast and moulds; HTC, halotolerant count; ND, not detected.

4.1.4- Fried rice:

Keeping cooked rice in houses or restaurants under ambient conditions provides an ideal environment for bacterial growth. Boiled rice and fried rice that have been prepared in restaurants have been implicated as vehicles in outbreaks of *B. cereus* emetic type food poisoning in many countries.

Data in Table 10 for fried rice indicates the range of 92 x 10^5 to 97 x 10^6 cfu/g, 65 x 10^4 to 99 x 10^5 cfu/g, 41 x 10^3 to 122 x10^4 cfu/g, 113 x 10 to 107 x 10^2 cfu/g, 30 x 10^2 to 86 x 10^2 cfu/g and 50 x 10^2 to 133 x 10^2 cfu/g corresponding to (APC), (PTC), (LAB), (CF), (Y&M) and (HTC), respectively, while *Bacillus sp.* were detected in 4 samples. The percentage of *Bacillus spp.* on fried rice were 40%. **Ueda *et al.* (1980)** examined 77 samples of boiled rice which contained greater than 10^5 aerobic bacteria /g. They isolated small number of *B. cereus* meanwhile **Tzonetakes and ManoLkides (1986)** found the bacterial count on custard and budding rice samples to be between 11 to 5 x 10^3/g. Some samples had coliform, yeast and moulds. *B. cereus* did not exist in any samples.

4.1.5-Meat and meat products:

The microbiological quality of meat and meat products are dependent on a number of factors, such as quality of raw materials, efficiency of the cooking process, sanitation during processing and packaging, maintenance of adequate refrigeration from the processing to the retail level and to the consumer and finally sanitation during handling and or slicing at the retail store **(Oblinger and Kennedy 1980)**.

Meat and meat products have been reported to be one of the important vehicles of *B. cereus* food poisoning **(Bachhile and Jaiswal 1988)**. Both *Bacillus* and *Clostridium* may be found in meats of all type. **Steinkraus and Ayres (1964)** found these organisms to be occurred at very low levels, generally less than 1/g.

Table 10: Microbiological Analyses Of Fried Rice.

Samples	APC	PTC	LAB	CF	Y&M	HTC	*Bacillus spp.*
fried rice	$35x10^6$	$37x10^5$	$122x10^4$	114x10	$57x10^2$	$84x10^2$	+
fried rice	$40x10^6$	$99x10^5$	$80x10^4$	$38x10^2$	$86x10^2$	$50x10^2$	+
fried rice	$33x10^6$	$66x10^4$	$61x10^4$	$54x10^2$	$59x10^2$	$73x10^2$	+
fried rice	$45x10^6$	$92x10^5$	$32x10^4$	$31x10^2$	$70x10^2$	$123x10^2$	+
fried rice	$194x10^5$	$65x10^4$	$107x10^3$	$42x10^2$	$41x10^2$	$66x10^2$	ND
fried rice	$35x10^6$	$86x10^4$	$55x10^4$	$44x10^2$	$30x10^2$	$55x10^2$	ND
fried rice	$239x10^5$	$83x10^4$	$81x10^4$	$107x10^2$	$41x10^2$	$71x10^2$	ND
fried rice	$97x10^6$	$82x10^5$	$67x10^4$	113x10	$66x10^2$	$92x10^2$	ND
fried rice	$92x10^5$	$150x10^4$	$41x10^3$	$42x10^2$	$48x10^2$	$68x10^2$	ND
fried rice	$111x10^5$	$71x10^4$	$102x10^3$	$31x10^2$	$51x10^2$	$85x10^2$	ND

Legand: APC, aerobic plate count; PTC, psychrotrophic count; LAB, lactic acid bacteria; CF, coliform; Y&M, yeast and moulds; HTC, halotolerant count; ND, not detected.

Minced beef meat:

Data in Table 11 for minced beef meat indicates the range of 62 x 10^4 to 280 x 10^5 cfu/g, 33 x 10^4 to 92 x 10^4 cfu/g, 40 x10^2 to 91 x 10^3 cfu/g, less than 30 to 51 x10^2 cfu/g, and 36 x 10^2 to 98 x 10^2 cfu/g. corresponding to (APC), (PTC), (LAB), (CF) and (HTC) respectively while moulds and yeast was not detected and *Bacillus spp.* were detected in one sample only. On the other hand **Mates(1983)** found the APC in beef ranged from less than 10^6 to more than 5 x 10^7 cfu/g. **Janewyatt and Guy (1980)** found a total aerobic plate count in excess of 5 x 10^6cfu/g to be a violation and *E. coli* only is in excess of 50cfu/g. **Ray *et al.* (1984)** found in beef the aerobic plate count 4.1 x 10^3 cfu/g, psychrotrophs 3.2 x 10^3 cfu/g and coliform less than 1/2g. In the same time they found that mechanically separated beef had 3 x 10^3 coliform/g. **Elder and Simard (1985)** found in beef stored between 4 and 10°C the range of the population was 10^2 to 10^4 cfu/g for both aerobic and psychrothrophic counts. They also found coliforms in meat stored at 10°C only. Newsome (1984) found in beef loins coliform $\log_{10}$ 0.84/g, aerobic (35°C) $\log_{10}$ 2.91/g, aerobic (20°C) $\log_{10}$ 3.5 and lactobacillus $\log_{10}$ 0.36/g in the zero time before storage. Simard *et al.* (1984) found that $\log_{10}$ 7.15 ± 0.36/g, $\log_{10}$ 6.73 ± 0.33/g, $\log_{10}$ 4.39 ± 0.27/g, $\log_{10}$ 2.71± 0.55/g for psychrotrophic, aerobic plate count, *Lactobacilli*, moulds and yeast and coliform respectively. **Hirota *et al.* (1988)** found 6.6% from meat was contaminated by *B. cereus.*

Meat products:

The addition of soy protein at level of 10 to 30% to ground meat is fairly widespread in the fast food industry, at least in the United States, and the microbiology of these soy blends has been investigated. The earliest most detailed study is that of **Craven and**

Mercuri (1977) who found that when ground beef or chicken was extended with 10 to 30% soy, APC of these products increased over unextended control when both were stored at 4°C for 8 - 10 days. While coliforms were also higher in beef soy mixtures than in the control, this was not true for the chicken blends.

Fresh beef burger:

Data in Table (11) for fresh beef burger indicate the range of 43 x 10^5 to 120 x 10^6 cfu/g, 31 x 10^4 to 81 x 10^5 cfu/g, 133 x 10^2 to 71 x 10^2 cfu/g, less than 30 to 71 x 10^2 cfu/g, less than 15 to 47 x 10^2 cfu/g and 37 x 10^2 to 77 x 10^2 cfu/g corresponding to (APC), PTC), (LAB), (CF), (Y&Y) and (HTC), respectively while *Bacillus spp.* were not detected. On the other hand **El-khateib *et al.* (1988)** found in chicken burger total bacteria 10^7 cell/g, psychrophiles 10^5 cell/g and *B. cereus* 10^3 cell/g. while **El-Sherif *et al.* (1991)** found that 84% of examined (beeff burger) samples had aerobic plate count of 10^3 to 10^6/g. *Bacillus. cereus* was detected in 10% of samples.

Pastrami:

Data in Table (11) for pastrami indicated the range of 64 x 10^5 to 124 x 10^5 cfu/g, 275 x 10^3 to 243 x 10^4 cfu/g, 65 x 10^2 to 107x 10^2 cfu/g, less than 30 to 83 x10 cfu/g and 31 x 10^2 to 77 x 10^3 cfu/g. correspending to (APC), (PTC), (LAB), (CF), and (HTC), respectively. While moulds and yeast were not detected and *Bacillus spp.* was detected in one sample. The percentage of *Bacillus spp.* occurance in these samples was 25%. On the other hand Oplinger and Kennedy (1980) found in pastrami the range (as log count) for APC at 35°C 3.63 to 8.o4, APC at 20°C 3.82 to 8.18, yeast and moulds 1.6 to 5.23 and coliform 0.56 to 3.18.

Table (11): Microbiological Analyses Of Meat And Meat Products.

Samples	APC	PTC	LAB	CF	Y&M	HTC	*Bacillus spp.*
Minced beef meat	$62x10^4$	$81x10^4$	$40x10^2$	$51x10^2$	ND	$98x10^2$	+
Minced beef meat	$88x10^4$	$65x10^4$	$53x10^2$	$34x10^2$	ND	$96x10^2$	ND
Minced beef meat	$100x10^5$	$33x10^4$	$61x10^2$	<30	ND	$36x10^2$	ND
Minced beef meat	$280x10^5$	$92x10^4$	$91x10^3$	$41x10^2$	ND	$41x10^2$	ND
Fresh beef burger	$120x10^6$	$81x10^5$	$33x10^2$	$71x10^2$	$47x10^2$	$77x10^2$	+
Fresh beef burger	$41x10^6$	$72x10^5$	$33x10^2$	$32x10^3$	ND	$37x10^2$	ND
Fresh beef burger	$43x10^5$	$31x10^4$	$61x10^3$	$51x10^2$	$36x10^2$	$55x10^2$	ND
Fresh beef burger	$52x10^6$	$81x10^4$	$71x10^2$	<30	ND	$61x10^2$	ND
Pasterami	$124x10^5$	$74x10^4$	$82x10^3$	51x10	ND	$57x10^2$	+
Pastrami	$76x10^5$	$243x10^4$	$107x10^2$	83x10	ND	$31x10^2$	ND
Pastrami	$69x10^5$	$40x10^4$	$65x10^2$	<30	ND	$77x10^3$	ND
Pastrami	$64x10^5$	$275x10^3$	$80x10^2$	81x10	ND	$42x10^3$	ND

Legand: APC, aerobic plate count; PTC, psychrotrophic count; LAB, lactic acid bacteria; CF, coliform; Y&M; yeast and moulds; HTC, halotolerant count; ND, not detected.

Chicken sausage:

Data in Table (12) for chicken sausage indicate the range of 40 x 10^6 to 70 x 10^6 cfu/g, 70 x 10^5 to 213 x 10^5 cfu/g, 66 x 10^3 to 41 x10^4 cfu/g, 41 x 10 to 34 x 10^2 cfu/g, less than 15cfu/g and 32 x 10^2 to 73 x 10^2 cfu/g corresponding to (APC), (PTC), (LAB), (CF), and (HTC), respectively while yeast and moulds were not detected and *Bacillus spp.* were detected in one sample only. The percentage of *Bacillus spp.* occurrence in these samples was 25%. This results were agreement with **Von Holy *et al.* (1992)** who found *Bacillus spp.* in Vienna sausage about 26.2%, while **Dykes *et al.* (1996)** found the count for yeast below 100 cfu/g after 71 days storage, total aerobic plate count and lactic acid bacteria increased with storage time and the predominance of *Bacillus* decreased to 13.7% in acid treated samples, 41.5% in pasteurized samples and 46.7% in pasteurized acid treated. In the same time Franz and **Van Holy (1996)** reported the proportions of *Bacillus* increased from 3.9% of total isolates from non pasteurized sausages to 32.4% of total bacterial isolates from different treated sausages, but **Van Holy (1989)** reported that *Bacillus spp.* were previously shown to be unable to dominate the spoilage ecology of non pasteurized vacuum packaged Vienna sausage. **El-khateib *et al.* (1988)** found in dairy sausage total bacteria 10^7 cfu/g, psychrophiles 10^5 cell/g and *B. cereus* 10^3 cell/g. **Yurtyeri *et al.* (1993)** found the commercial smoked ripened dry sausage had total count 10^7 to 10^9 cfu/g, coliform count 10^2 to 10^6 cfu/g, *B. cereus* occurred in 15 samples at 10^4 cfu/g, count of yeasts and moulds varied widely.

Chicken frankurter:

Data presented in Table 12 for chicken frankfurter indicate the range of 55 x 10^5 to 40 x 10^6 cfu/g, 93 x 10^4 to 213 x 10^5 cfu/g, 31 x10^2 to 144 x 10^3 cfu/g, less than 30 to 66 x 10 cfu/g, and 82 x 10^2 to 111 x10^2 cfu/g coorespinding to (APC), (PTC), (LAB), (CF), and

(HTC), respectively. While yeast and moulds were not detected and *Bacillus spp.* was found in one sample only. The percentage of *Bacillus spp.* occurrence in these samples were 25 %. On the other hand **El-Khateib *et al.* (1988)** found that in frankfurter total bacterial was 10^6 cfu/g., psychrophiles 10^4 cfu/g and *B. cereus* 10^3 cell/g. **Hallerbach and Potter (1981)** found in frankfurter total aerobic ranged from 7×10^5 to 4×10^6 cfu/g, *Lactobacillus* and *Pediococcus* 4×10^2 to 5.5×10^4 cell/g and **Tiwari and Kadis (1981)** found in frankfurter APC from 10^3 to 10^8 cfu/g, SB from 10^3 to 10^8 cfu/g, yeasts from less than 10 to 10^4 cfu/g but coliform not detected in all samples.

Hamburger:

Data in Table 12 for hamburger indicate the range of 144×10^4 to 93×10^5 cfu/g., 66×10^3 to 81×10^5 cfu/g., 216×10^2 to 43×10^3 cfu/g., less than 30 to 53×10^2 cfu/g., less than 15 to 34×10^2 cfu/g. and 33×10^2 to 56×10^2 cfu/g. corresponding to (APC), (PTC), (LAB), (CF), (Y&M) and (HTC), respectively while *Bacillus spp.* were detected in two samples. The percentage of *Bacillus spp.* occurrence in these samples is 50%. On the other hand **Muller (1975)** analyzed hamburger and found the APC ranged from 9.7×10^3 to 3.1×10^5 cfu/g. before reheating and 8×10^3 to 4×10^4 cfu/g. following reheating. Coliform was originally present in small numbers of samples. **Duitschaever *et al.* (1977)** examined the bacteriological quality for fried hamburger. Seventy-six % of fried hamburger had psychrotrophic less than 1×10^3/g. Coliform was absent from all samples. Although no organisms known to cause foodborne illness were isolated.

Table (12): Microbial Analyses Of Meat Products

samples	APC	PTC	LAB	CF	Y&M	HTC	Bacillus sp.
chicken sausage	$58x10^6$	$132x10^5$	$40x10^4$	66x10	ND	$32x10^2$	+
chicken sausage	$43x10^6$	$188x10^5$	$66x10^3$	$30x10^2$	ND	$43x10^2$	ND
chicken sausage	$70x10^6$	$70x10^5$	$41x10^4$	34x102	ND	$51x10^2$	ND
chicken sausage	$40x10^6$	$213x10^5$	$144x10^3$	41x10	ND	73x102	ND
chicken frankfurter	$63x10^5$	$93x10^4$	$135x10^2$	35x10	ND	$91x10^2$	+
chicken frankfurter	$132x10^5$	$87x10^5$	$71x10^2$	66x10	ND	$101x10^2$	ND
chicken frankfurter	$55x10^5$	$41x10^5$	$83x10^3$	<30	ND	$82x10^2$	ND
chicken frankfurter	$49x10^5$	$61x10^4$	$31x10^2$	<3o	ND	$111x10^2$	ND
hamburger	$62x10^5$	$81x10^4$	$71x10^2$	$53x10^2$	$34x10^2$	$51x10^2$	+
hamburger	$93x10^5$	$53x10^4$	$43x10^3$	<30	ND	$56x10^2$	+
hamburger	$91x10^5$	$66x10^3$	$216x10^2$	91x10	ND	$54x10^2$	ND
hamburger	$144x10^4$	$51x10^4$	$230x10^2$	$51x10^2$	ND	$33x10^2$	ND

Legand: APC, aerobic plate count; PTC, psychrotrophic count; LAB, lactic acid bacteria; CF, coliform; Y&M, yeast and moulds; HTC, halotolerant count; ND, not detected.

The level for *Bacillus spp.* in all samples (13 samples) was less than 100/g. There was little correlation between the incidence of *Bacillus spp.* and other population. In starch based Asian foods (10 samples), failed to detect organisms in five samples immediately after production but found population ranging from 50 to 5.6 x 10^4 cfu/g. in retail samples, both of coloni appear to be *B. cereus.*

Data in Table 13 represent the average log cfu/g of aerobic plate count (APC), psychrotrophic bacteria(PTC), lactic acid bacteria(LAB) coliform bacteria (CF), yeasts and moulds(Y&M) and occurrence of *Bacillus spp.* in different food products.

Table 13:Microbial Count Log cfu/g And % Occurrence Of *Bacillus spp.* In Different Food Products*

Food items	Sample size	APC	PTC	LAB	CF	Y&M	HTC	*Bacillus spp.*
Pasteuriz-ed milk	8	5.96 ± 0.06	5.73 ± 0.05	5.12 ± 0.16	1.33 ± 0.04	ND	3.69 ± 0.06	12.5%
Raw vegetables	6	7.73 ± 0.27	6.63± ± 0.21	6.16 ± 0.25	4.42 ± 0.17	4.03 ± 0.22	3.92 ± ± 0.10	33.3%
Fresh noodle and pasta	10	7.09 ± 0.19	6.75 ± 0.15	5.52 ± 0.21	3.79 ± 0.11	3.71± 0.03	3.76 ± ± 0.04	ND
Fried rice	10	7.45 ± 0.09	6.30 ± 0.16	5.54 ± 0.15	3.73 ± 0.22	3.72 ± 0.04	3.87 ± 0.04	40%
Minced beef meat	4	6.55 ± 0.40	5.80 ± 0.40	4.02 ± 0.32	3.08 ± 0.54	ND	3.61 ± o.12	25%
Fresh beef burger	4	7.51 ± 0.31	6.29 ± 0.35	3.92 ± 0.30	3.39± 0.66	2.40± 0.70	3.79 ± o.o7	ND
Pastrami	4	6.90 ± 0.06	5.82 ± 0.20	4.16 ± 0.25	2.50 ± 0.35	ND	4.14 ± 0.34	25%
Chicken sausage	4	7.71 ± 0.06	7.41 ± 0.11	5.30 ± 0.14	3.11 ± 0.23	ND	3.68 ± 0.07	25%
Chicken frankfurter	4	6.84 ± 0.10	6.33 ± 0.27	4.10 ± 0.30	2.08 ± 0.35	ND	3.98 ± 0.03	25%
Hamburg-er	4	6.72 ± 0.19	5.54 ± 0.24	4.29 ± 0.16	2.97 ± 0.53	1.76 ± 0.59	3.43 ± 0.24	50%

Legand: N, number of samples; APC, aerobic plate count; PTC, psychrotrophic count; LAB, lactic acid bacteria; CF, coliform; Y&M, yeast and moulds; HTC, halotolarent count; ND, not detected.

*Mean ± S.E.

Identification of *Bacillus spp.*

4.2.1- Identification of *Bacillus spp.* using API system:

The genus *Bacillus* includes 60 validity described species and additional species of uncertain taxonomic status, aerobic or facultatively anaerobic endospore forming , gram positive or gram variable, some species being clearly positive only in young cultures.

The keys and the tables for different *Bacillus spp.* have been available for a long time. There are two ways to identificate *Bacillus spp.*. Firstly the diagnostic tests used , many of the classical tests for *Bacillus* described by **Smith *et al.* (1946, 1952)** and **Gordon *et al.* (1973)**. This way require special media, which are very time consuming and expensive to prepare. Several tests take 3 weeks or more from pure culture to final reading and this is too long for many diagnosticians to wait.

The second way leading to neglect of *Bacillus* identification is the character of the genus. *Bacillus* is an unusually wide taxon which contains most aerobic endospore forming rods. In term of DNA base ratios it is the equivalent of some bacterial families **(Priest 1981)**.

Some species are ill-defined, existing with closely related species as complexes or spectra in which the boundary of a particular species is difficult or impossible to identify, at the end classical test schemes do not permit identification of a typical strains. So the demand for more rapid, simpler systems for *Bacillus* identification has been created. Commercially available diagnostic kits API system **(Logan and Berkeley 1984)** have been made in recent years but their use is intended for *Bacillus spp.* of medical importance **(Kotzekidou 1996)**.

The results from the API test for 40 strains of *Bacillus spp.* are recorded in Table 14. All of these strains were determined to be *B. cereus* by using confirmation tests but API

test data in Table 14 show that 22 strains only (55%) are *B. cereus*. One strain only(No30) was accepted (86.7%) as *B. cereus* by API kit. The other strains required addition tests for confirmation as *B. cereus,* which was either rhizoid growth test or anaerobic growth test.

The rhizoid growth test was done for strains No's 1 - 12, 22 and 25 to differentiate between *B. cereus* and *B. cereus* var. *mycoides* (*B. cereus* var. *mycoides* has characteristic to be positive in this test). The positions of *B. mycoides* and *B. thuringiensis* as species distinct from *B. cereus* are not clear. **Logan (1980)** and **Logan and Berkeley (1981)** were unable to separate these three species. **Logan and Berkeley (1984)** suggested that *B. mycoides* and *B. thuringiensis* should be considered as varieties of *B. cereus*.

Anaerobic growth was done for strains no. 21, 23, 24, 26, 27, 28, 29 to differentiation between *B. cereus, B. firmus* and *B. lentus* (*B. cereus* only can grow anaerobically).

All *B. cereus* strains were isolated from 11 samples but the level for *Bacillus spp.* in all samples was less than 100cfu/g.or mL. Te-Giffel *et al.* (1997) found the count for *B. cereus* in pasteurized milk was low, of less than 5/mL. in 77% of all samples. **Wong *et al.*(1988)** found 52% of ice cream, 35% of milk powder, 17% of fermented milk and 2% of pasteurized milk were contaminated with *B. cereus*. **Angeles *et al.* (1989)** found *Bacillus* spores in 82.4% of different food samples (14.7% contained *B. cereus* spores). **VanNetten *et al.*(1990)** reported that 8% of 483 pasteurized milk samples were positive for *B. cereus*. **Logan *et al.* (1979)** reported that a distinction between diarrheal and emetic types could be made with an API system and numerical methods, while, **Logan and Berkeley (1981)** and **Shinagawa (1993)** found it is possible to discriminate between *B. cereus* strains causing the diarrhoeal and emetic types of food poisoning on the basis of failure to ferment salicin,

dextrin, starch and glycogen. In contrast, some workers have found no consistent between diarrhoeal and emetic strains (**Major *et al.* 1979**).

The results of carbohydrate metabolism indicated that there are many different types of *B. cereus* strains. These data are in agreement with the results of **Logan and Berkely (1984), Rangasamy et al (1993) and Te-Giffel (1995)**

Data in Table14 indicated that nine strains were determined to be *B. lentus* (22.5%), three strains were determined to be *B. pumilus* (7.5%), two strains were determined to be *B. brevies* (5%), two strains were *B. licheniformis*(5%) after checking the growth under anaerobic to differentiate between *B. megaterium*, *B. licheniformis* and *B. subtilis*. Two strains were determined to be *B. firmus* and one strain was unknown (5%). **Goep *et al.* (1977)** and **Johnson(1984)** reported that several *Bacillus spp.* have often been found to be responsible for outbreaks of food poisoning. **Schiefer *et al.*(1976)** reported that some strains of *B. cereus* cause bovine mastitis, often with necrotic mastitis.

Table 14: Identification And Extracellular Activity (haemolysis and starch hydrolysis) Of *Bacillus spp.*

No.	Source of food	API test	Starch hydrolysis	Haemolytic activity
1	Milk	*B. cereus*	+	+
2	Milk	*B. cereus*	+	+
3	Cauliflower	*B. cereus*	+	+
4	Cauliflower	*B. cereus*	+	+
5	Fried rice no. 1	*B. cereus*	+	+
6	Fried rice no. 1	*B. cereus*	+	+
7	Pasterami	*B. cereus*	+	+
8	Minced meat	*B. cereus*	+	+
9	Pasterami	*B. cereus*	+	+
10	Frankfurter	*B. cereus*	+	+
11	Hamburger no. 1	*B. cereus*	+	+
12	Hamburger no. 1	*B. cereus*	+	+
13	Sausage	*B. pumilus*	-	+
14	Sausage	*B. pumilus*	-	+
15	Minced meat	*B. pumilus*	-	+
16	Fried rice no. 2	*B. brevis*	+	+
17	Milk	*B. brevis*	+	+
18	Broccoli	*B. lentus*	+	-
19	Broccoli	*B. lentus*	+	-
20	Frankfurter	*B. lentus*	+	-

Table14: Continued

No.	source of food	API test	Starch hydrolysis	Haemolytic activity
21	fried rice no. 3	*B. cereus*	-	+
22	fried rice no. 2	*B. cereus*	+	+
23	Sausage	*B. cereus*	-	+
24	Milk	*B. cereus*	+	+
25	fried rice no. 4	*B. cereus*	-	+
26	Milk	*B. cereus*	-	+
27	fried rice no. 3	*B. cereus*	-	+
28	fried rice no. 4	*B. cereus*	-	+
29	Pasterami	*B. cereus*	-	+
30	Frankfurter	*B. cereus*	-	+
31	Cauliflower	*B. lentus*	-	+
32	Broccoli	*B. lentus*	-	+
33	fried rice no. 1	*B. lentus*	-	+
34	Hamburger no. 1	*B. lentus*	-	+
35	Pastermia	*B. lentus*	-	+
36	Broccoli	*B. lentus*	-	+
37	Milk	*B. firmus*	-	+
38	Milk	*B. licheniformis*	+	-
39	Sausage	*B. licheniformis*	+	+
40	Broccoli	Unknown	+	+

4.2.2- Bacillus biotyping :

The isolates from outbreaks of diarrhoeal disease have been found to hydrolyze starch. Whereas those from the emetic type of outbreaks have been found to be starch negative.

Data in Table 14 indicated that 14 strains (63.6%) of *B. cereus* were positive to starch hydrolysis while eight strains were negative. **Nishikawa *et al.* (1996)** found 38 (88%) of 43 outbreak strains which produced vacuolation responses in HEP-2 cell were all negative for starch hydrolysis while an other 76 strains associated with outbreaks gave all negative results except four strains and 56 (74%) of these strains were starch hydrolysis positive. **Christiansson *et al.* (1989)** found only seven strains (5.1%) from 136 dairy isolates of *B. cereus* did not hydrolyze starch. **Shinagawa *et al.* (1979)** reported that the *B. cereus* strains isolated from diarrhoeal type food poisoning and those isolated from uncooked rice were positive for starch hydrolysis, the isolates from the emetic type of food poisoning and those isolates from cooked rice were negativefor starch hydrolysis. Data in Table 14 indicated that three of nine *B. lentus* from, two strains of *B. brevies,* two strains *B. licheniformis* and one unidetifined strain were positive for starch hydrolysis while all *B. pumilus* strains were negative for starch hydrolysis.

4.2.3- Haemolytic activity :

Ottolenghi (1965) suggested that food poisoning caused by *B. cereus* might be due to α-haemolysin analogous to that produced by *Clostridium perfringens*. However, **Johnson and Benventre (1967)** observed that the toxin in *B. cereus*B-48 was not haemolytic.

Data in Table 14 indicated that all strains of *B. cereus, B. pumilus, B. brevies, B. lentus, B. firmus, B. licheniformis* and the unknown strain produced hemolysin on blood

agar except three from *B. lentus* strains and one *B. licheniformis* strain. These results are in near agreement with those of **Kamat *et al.* (1987)** who found all strains of *Bacillus spp.* produced haemolysin on blood agar, while **Christiansson *et al.* (1989)** found 2 to 28 % of *B. cereus* dairy isolates produced haemolysin.

4.3- Toxin production by *B. cereus* and other *Bacillus spp.*

4.3.1- Diarrhoeal toxin production by *B. cereus* and other *Bacillus spp.*:

Food poisoning strains from the geneus *Bacillus* produce the following toxins and extracellular products: lecithinase, proteases, hemolysin, β-lactamase, mouse lethal toxin, cereolysin, emetic enterotoxin and the diarrheagenic toxin. The enterotoxins are responsible for the two food poisoning syndromes caused by this organism. The diarrheagenic toxin induces vascular permeability in the skin of rabbits, elicits fluid accumulation in the rabbit ileal loop and causes diarrhea in rhesus monkeys. It has been shown to be a protein of molecular weight of about 50000 with an isoelectric point of 4.9 **(Turnbull *et al.* 1979)**. Its production is favoured by low dissolved O_2 and most is produced during the logarithmic phase of growth **(Spira and Silverman 1979)**. It is heat labile and sensitive to trypsin and pronase. Production is favoured in the pH range 6 - 8.5 with the optimum between 7 - 7.5 and is produced over the temperature range 18 - 43°C **(Johnson 1984)**.

The diarrhoeal enterotoxin production of the different strains of *B. cereus, B. pumilus*, *B. brevis*, and *B. lentus* are shown in Table 15. All *B. cereus* strains tested produced diarrhoeal enterotoxin aerobically at 30°C, under 10°C and anaerobically at 37°C except isolates No. 11, which did not produce the toxin at low temperature (10°C). Data in Table 14 indicated that most of these strains were positive for starch hydrolysis and hemolytic activity. **Griffith (1990)** tested 38 strains of *B. cereus* which produced diarrhoeal enterotoxin. He found all of them produced toxin when grown in BHI broth at 25°C. **Chopra *et al.* (1980)** reported that 6 out of 25 (24%) milk isolates of *B. cereus* produced diaarrhoegenic toxin. **Wong *et al.* (1988)** found the majority of *B. cereus* strains isolated from a variety of dairy products were capable of producing toxin when grown in

BHI broth at 35°C with aeration. **Per *et al.* (1993)** found 59% of 85 strains of *B. cereus* isolated in Norway from dairy products were enterotoxigenic. **Christiansson *et al.* (1993)** found 7 dairy isolates of *B. cereus* produced enterotoxin after growth at 8°C for 4 days using two kits. **Day *et al.* (1994)** examined 14 strains of *B. cereus* isolated from different sources for production of diarrhoeal enterotoxin by two kits. Six strains were positive with the RPLA kit while 13 strains were positive with an ELISA kit. **Serve and Sita (1993)** found several *B. cereus* strains isolated from different sources gave positive results with ELISA kit but 66.6% only gave positive results with RPLA. One strain gave a positive results with ELISA kit when it was grown at 10°C for 72 h. and 15°C for 24 h. **Van Netten *et al.* (1990)** examined 1700 samples for the presence of *B. cereus*, 9.8% of those samples have *B. cereus* (those foods implicated in diarrhoeal syndrome food poisoning). Growth and enterotoxin producing by psychrotrophic *B. cereus* could be prevented by temperatures below 4°C and pH values not exceeding 5. **Per *et al.* (1993)** reported that *B. cereus* grow quite well under anaerobic conditions, though slower than under aerobic conditions, so that enterotoxin may be produced in the small intestine.

Data in Table 15 indicated that two strains of *B. pumilus* produced enterotoxin aerobically at 30°C, and one strain gave a positive result when grown under aerobic conditions at low temperature(10°C), but both gave negative results after anaerobic. incubation at 37°C. .Two strains of *B. brevis* produced enterotoxin under all conditions while only one strain of *B. lentus* produced enterotoxin aerobically at 30°C but did not produce this toxin under anaerobically at low temperature(10°C.).

The production of diarrhoeagenic enterotoxin was not confined to *B. cereus* and *B. cereus* related strains. There are other species capable of producing enterotoxin such as

Table 15: Enterotoxin Production By *Bacillus spp.*

No. of strain	Source of food	Strain	30°	37°C/An. O_2	10°C
1	Milk	*B. cereus*	+	+	+
2	Milk	*B. cereus*	+		
3	Cauliflower	*B. cereus*	+	+	+
4	Cauliflower	*B. cereus*	+		
5	Fried rice	*B. cereus*	+	+	+
6	Fried rice	*B. cereus*	+		
7	Pastrami	*B. cereus*	+	+	+
8	Minced meat	*B. cereus*	+	+	+
9	Pastrami	*B. cereus*	+		
10	Frankfurter	*B. cereus*	+	+	+
11	Hamburger	*B. cereus*	+	+	-
12	Hamburger	*B. cereus*	+		
13	Sausage	*B. pumilus*	-		
14	Sausage	*B. pumilus*	+	-	-
15	minced meat	*B. pumilus*	+	-	+
16	fried rice	*B. brevis*	+	+	+
17	Milk	*B. brevis*	+	+	+
18	Broccoli	*B. lentus*	-		
19	Broccoli	*B. lentus*	+	-	-
20	Frankfurter	*B. lentus*	-		

B. lentus, B. pumilus, B. brevis, B. polymyxa, B. carotrarum and B. circulans. The production of diarrhoegenic toxin by strains of *Bacillus spp.* other than *B. cereus* has been described **(Marth 1978)**.

Obtained results in Table 15 were nearly in agreement with those of **Griffith (1990)** who examined four strains of *B. lentus*, of which three produced enterotoxin, and two strains of *B. pumilus* one of them produced enterotoxin . **Gilbert *et al.* (1981) and kramer *et al.* (1982)** reported that *B. brevis* may be potential food poisoning agent. **Goepfert *et al.* (1972)** and **Johnson (1984)** reported that several *Bacillus sp.* have often been found to be responsible for outbreaks of food poisoning . **Johnson(1984), Clause and Berkeley (1986)** and **Kramer and Gilbert (1989)** reported that *B. brevis* has been implicated in food poisoning.

Data in Table (15) continued indicated that five *B. cereus* from ten produced enterotoxin at 30° C. and this strains also produced enterotoxin under anarobic conditions at 37° C. but one strain No. 29 produced enterotoxin 10° C. for 7 days.

Tow strains of *B. lentus* from 6 produced enterotoxin at 30° C. under aerobic conditions, at the same time they produced enterotoxin under anaerobic condition at 37° C for 48 hrs but they did not produce enterotoxin at 10° C. *Bacillus firmus* produced enterotoxin at 30, 37°C. aerobic and anaerobic respectively but did not produce enterotoxin at 10°C. *Bacillus licheniformis* (two strains) and *B. cereus* diarrhoeal refrence produced enterotoxin under all condition.*Bacillus cereus* emetic toxin did not produce enterotoxin under aerobic condition at 30°C. with agitation. The results in Table 15 continued for *B. licheniformis* were agrrement with **Gilbert *et al.* (1981)** who found many species of genus *bacillus* produeced enterotoxin include *B. licheniformis.*

Table 15: Enterotoxin Production By *Bacillus spp.*

No. of strain	Source of food	Strain	30°	37°C/An. O_2	10°C
21	fried rice no. 3	*B. cereus*	-		
22	fried rice no. 2	*B. cereus*	-		
23	Sausage	*B. cereus*	+	+	-
24	Milk	*B.cereus*	+	+	-
25	fried rice no. 4	*B. cereus*	-		
26	Milk	*B. cereus*	+	+	-
27	fried rice no. 3	*B. cereus*	-		
28	fried rice no. 4	*B. cereus*	-		
29	Pasterami	*B. cereus*	+	+	+
30	Frankfurter	*B. cereus*	+	+	-
31	Cauliflower	*B. lentus*	-		
32	Broccoli	*B. lentus*	-		
33	fried rice no. 1	*B. lentus*	-		
34	Hamburger no. 1	*B. lentus*	+	+	-
35	Pastermia	*B. lentus*	+	+	-
36	Broccoli	*B. lentus*	-		
37	Milk	*B. firmus*	+	+	-
38	Milk	*B. licheniformis*	+	+	+
39	Sausage	*B. licheniformis*	+	+	+
40	Broccoli	Unknwon	-		
41	Diarrheagenic reference	*B. cereus*	+	+	+
42	Emeitc reference	*B. cereus*	-		

4-3-2 Emetic toxin production by *B. cereus*:

A number of methods have been discovered for detection of the *B. cereus* emetic toxin. These methods include cell culture method Hep-2 (human carcinoma of the larynx) cells **Huges *et al.* (1988)**, CHO (Chinese hamster ovary) cells **Szabo *et al.* (1991)** and old method (Monkey feeding).

Agata *et al.* (1994) purified emetic toxin pure from *B. cereus* strain NC7401A by chemical method and the toxin was named as cerulide. Agata method was adapted to isolate emetic toxin from *B cereus* emetic reference strain and other *B. cereus* strains isolated from rice products but the method could not be succeeded. The area is not possible here to discuss what have been done. Is one strain only is enough to the Agata results to be valid. Agata published his paper in FEMS microbiology letters 1994 and the method is not clear.

Further more from 1994 until 1998 we did not found any published data in magazine or conference or any one follow Agata method. If this method is good why all biologists especially in Japan (Agata country) can not use this method. I found a lot of new published paper from Japan and thy did not use this method including new published data for **agata (1996)**.

4.4- Enzymatic activity for *B. cereus* and *Bacillus spp.*:

Bacillus sp. are important as food spoilage organisms and can be isolated from fruits, vegetable products, nuts, cereals, milk and dairy products, meat, dried foods and spices **(Goepfert *et al.* 1972, Johnson 1984)**.

Bacillus cereus is the predominant organism that determines the keeping quality of pasteurized milk and its products **(Stewart 1975)**.*Bacillus cereus and B. cereus* var. *mycoides* are associated with defects such as off -flavours, sweet curdling in fluid milk and bitty cream which are caused by the action of their protease, lipase and phospholipase enzymes **(Meer *et al.* 1991)**.

Member of the genus *Bacillus* are able to secrete a wide variety of enzymes into the culture medium **(Priest 1977 and Mezes and Lampen 1985)**.

In Indonesia many species of the genus *Bacillus* are used to produce traditional foods such as terasi, which is salty fermented product **(Ingrid and Akiyoshi1994)**. A simple new technique which has been used for the identification of bacteria is the API ZYM system. This is a semiquantitative micromethod which was originally designed to detect enzymatic activity in a varitety of specimens such as tissues, cells, biological fluids and bacteria. It allows the systematic and rapid study of enzymatic reactions from very small samples.

The enzymatic activity of *Bacillus* isolates determined with API ZYM kit are recorded in Tables 16, 17, 18 and 19. The data indicated that all isolates produced a wide variety of enzymes including : phosphatase alcaline, easterase (C4) easterase lipase (C8), lipase(C14), leucine arylamidase, valine arylamidase, chymotrypsin phosphatase, naphtol-AS-BI-phosphohydrolase , and α- glucosidase.

Nearly all of the isolates showed high leucine arylamidease and phosphatase acid activity. Similarly nearly all isolates showed moderate phosphatase alcaline, esterase(C 4), esterase lipase (C8) and naphthol-AS-BIphosphohydrolase activity. On the other hand the enzyme activities of the isolates were weak with respect to lipase(C 14) , valine arylamidase, chymotrypsine and α -glucosidase, except one strain only (No. 24 *B. cereus*) which showed high α-glucosidase activity (≥ 40 nanomole).

Data indicated that *B. pumilus* (three strains) gave high β glucosidase activity and two strains were weak for ∝-mannosidase. Three *B. lentus* strains did not produce chymotrypsin, phosphatase acid or naphthol-AS-BI-phosphohydrolase. One *B. brevis* strain (No. 16) had weak trypsin activity and one *B. lentus* strain (No. 18) was weakly produced N-acetyl-βglucosaminidase.

These results closely agreed with those of many workers, such as **Amoa-Awua and Jakobsen (1995)** and **Okafor and Ejiofor (1986)** who reported that *Bacillus* isolates (seven species including *B. pumilus*, *B. cereus* and *B. licheniformis*) produced a wide spectrum of enzymes and that they also exhibited a similar pattern of enzymatic activity. All isolates showed high lipase, esterase and alkaline phosphatase activity. Only *B. pumilus* and *B. amyloliquefaciens* showed linamarase determined as β glucosidase activity. At the same time, Ingrid and Akiyoshi(1994) found most of the isolates from terasi starter, including *B. pumilus* and *B. brevis* exhibited high esterase (C 4) activity and esterase lipase activity (C 8) in the presence or absence of 10 % NaCl. They also found enzyme from the isolates to be weakly proteolytic.

Data in Table 19 showed that enzymatic activity for *B. cereus* strains No. 1 and No. 9 increased after aerobic incubation for 8 hrs with no further increase after incubation for 12 hrs. On the other hand, heat shock at 65°C for 30 mins led to a decrease in the

enzymatic activity for two *B. cereus* strains. The data showed that the enzymatic activity after aerobic incubation for 72 hrs at 10°C was nearly equal to the enzymatic activity after aerobically incubation for 4 hrs at 37°C. Anaerobic condition led to more or less some enzymatic activity.

Table 16: Enzymatic Activity For *B. cereus* And *B. pumilus*

↓→	2	3	4	5	6	7	8	10	11	12	13	14	15
1													
2	30+	30	30	≥40	10	20	10	10	≥40	≥40	20	≥40	≥40
3	20	10	30	20	10	10	10	10	5	10	10	5	5
4	20	20	30	20	10	20	20	10	10	20	30	5	20
5	5	5	5	5	5	0	5	0	0	0	0	0	0
6	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	20	≥40	5	0	5
7	5	5	5	5	5	0	5	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0	0
10	5	5	10	10	10	05	5	10	5	5	20	20	10
11	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	5	5	5
12	10	10	10	10	10	5	5	0	≥40	≥40	10	5	0
13	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0
16	10	10	10	10	10	5	5	10	5	5	0	0	0
17	0	0	0	0	0	0	0	0	0	0	30	20	20
18	0	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	10	5	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0

↓No. of substrate (Table 4). →No. of strains (Table 12).

+ Quantity of hydrolysed substrate (nanomole).

Table 17: Enzymatic Activity For *B. brevies*, *B. lentius*, And *B. cereus*.

↓→	16	17	18	19	20	21	22	23	24	25	26	27	28
1													
2	≥40+	≥40	20	10	10	20	20	20	20	20	20	20	≥40
3	5	10	10	5	10	20	20	20	5	20	20	20	10
4	10	20	20	10	10	20	30	20	20	20	30	30	20
5	0	0	0	0	0	0	0	0	0	0	0	0	5
6	20	30	5	0	0	≥40	30	≥40	20	≥40	≥40	30	≥40
7	0	0	0	0	0	10	0	5	5	10	5	5	10
8	0	0	0	0	0	0	0	0	0	0	0	0	0
9	5	0	0	0	0	0	0	0	0	0	0	0	0
10	5	20	0	0	0	10	30	5	10	5	5	5	5
11	≥40	≥40	0	0	0	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40
12	≥40	≥40	0	0	0	20	20	20	20	20	30	30	20
13	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0	0
16	10	5	0	0	0	5	20	5	≥40	5	5	5	20
17	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	5	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0

↓No. of substrate (see table 4). →No. of strains (see table 12).

+ Quantity of hydrolysed substrate (nanomole).

Table 18: Enzymatic Activity For *Bacillus sp.*

↓→	29	30	31	32	33	34	35	36	37	38	39	40
1												
2	20+	10	10	10	20	≥40	20	10	10	20	20	10
3	20	10	10	10	20	20	20	10	10	20	20	10
4	20	20	10	10	20	≥40	20	20	10	20	30	20
5	0	0	0	0	0	0	0	0	0	0	0	0
6	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	20
7	5	10	10	20	5	10	10	20	10	5	5	0
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	10	5	5	5	10	5	10	10	20	5	≥40	5
11	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40	≥40
12	20	20	20	20	20	20	20	10	10	20	20	10
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	5	5	5	5	5	5	5	5	5	5	10	5
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0

↓No. of substrate (see table 4). →No. of strains (12).

+ Quantity of hydrolysed substrate (nanomole).

Table 19: Enzymatic Activity For *B. cereus* Strains No.1 And 9

↓→	1-1	1-2	1-3	1-4	1-5	1-6	9-1	9-2	9-3	9-4	9-5	9-6
1												
2	30+	≥40	≥40	0	30	≥40	10	20	20	5	≥40	10
3	30	30	30	5	20	10	5	10	10	5	10	5
4	30	≥40	≥40	5	30	20	10	20	20	10	20	10
5	5	0	0	0	0	0	5	5	5	5	5	5
6	≥40	≥40	≥40	10	20	5	≥40	≥40	≥40	≥40	≥40	≥40
7	5	5	5	0	5	0	5	10	10	5	20	5
8	0	0	0	0	0	0	0	0	0	0	0	0
9	0	0	0	0	0	0	0	0	0	0	0	0
10	10	5	30	0	10	10	5	10	10	0	20	5
11	≥40	≥40	≥40	10	≥40	≥40	≥40	≥40	≥40	5	≥40	≥40
12	20	20	20	5	20	20	5	10	10	0	5	5
13	0	0	0	0	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0	0	0
16	10	10	10	0	20	5	5	10	10	0	20	5
17	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0

↓No. of substrate (see table 4). → [(-1, 2, 3 after incubation 4, 8, 12 hr respectively)(-4 after heat shock at 65°C. for 30 min.)(-5 under 10°C for 72 hr.) (-6 under anaerobic)]. + Quantity of hydrolysed substrate (nanomole).

4.5- Antagonistic activity for *Bacillus spp.:*

4.5.1- Antagonistic activity between *Bacillus spp.:*

A total of 39 strains from five species of genus *Bacillus* and one unknown strain were tested for antagonistic activity against each other. The results are recorded in Table 20 and 21.

Data indicated that a total of 21 strains produced inhibition zones against other *Bacillus* strains. Strains No. 13, 14, 28, 30, 37, 39 and 40 are the most frequently inhibited strains. Three strains of *B. lentus* No. 18, 19 and 20 are the bigest inhibitor strains.

The culture supernatants from inhibitor strains were tested against inhibited strains. Some of this supernatants inhibited some strains but with small zone.

4.5.2- Antagonistic activity between *Bacillus spp.* with other organismes:

Antagonistic activity of *Bacillus* strains was tested against 11 strains from other genera *Bacillus* by the agar well diffusion method and the results recorded in Table 22.

This results showed that no inhibitory activity from *Bacillus spp.* against *E. durans* and *P. aeruginosa.* One strain only (*B. brevies*) inhibited *E. faecalis.* Two strains inhibited *L. monocytogenes* (*B. lentus* No. 18 and 19). *Bacillus lentus* strains 18, 19 and 20 are the best inhibitor strains from all *bacillus* strains against other organismis. *Bacillus firmus* and *B. licheniformis* did not inhibit any strains. *Bacillus cereus* inhibit *S. chromogenes* only.

Table 20: Antagonistic Activity of *B. cereus* .

Test strains	Inhibitor strains									
↓ →	1	2	3	4	5	6	7	8	9	10
1		-	-	-	-	-	-	-	-	-
2	-		-	-	-	-	-	-	-	-
3	-	-		-	-	-	-	-	-	-
4	-	-	-		-	-	-	-	-	-
5	-	-	-	-		-	-	-	-	-
6	-	-	-	-	-		-	-	-	-
7	-	-	-	-	-	-		-	-	-
8	-	-	-	-	-	-	-		-	-
9	-	-	-	-	-	-	-	-		-
10	-	-	-	-	-	-	-	-	-	
11	-	-	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-	-	-
13	2/1*	2/1	2/1	2/1	2/1	2/1	2/2	2/2	-	2/2
14	3/2	3/2	3/2	2/2	2/2	2/2	2/2	2/2	-	2/2
15	-	-	-	-	-	-	-	-	-	-
16	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	-	-	-	-	-
18	-	-	-	-	-	-	-	-	-	-
19	3/-	3/-	-	-	3/-	-	3/-	-	-	-
20	-	-	-	-	-	-	-	-	-	-
21	-	-	-	-	-	-	-	-	-	-
22	-	-	-	-	-	-	-	-	-	-
23	-	-	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-	-	-
25	-	-	-	-	-	-	-	-	-	-
26	-	-	-	-	-	-	-	-	-	-
27	-	-	-	-	-	-	-	-	-	-
28	3/1	3/-	3/-	-	2/1	2/1	2/1	2/	-	2/-
29	-	-	-	-	-	-	-	-	-	-
30	2/1	2/1	2/1	2/1	2/1	2/1	2/1	2/1	-	2/1
31	-	-	-	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-	-	-	-
34	-	-	-	-	-	-	-	-	-	-
35	-	-	-	-	-	-	-	-	-	-
36	-	-	-	-	-	-	-	-	-	-
37	2/-	2	-	-	2/-	2/-	2/-	-	2/-	-
38	-	-	2/-	-	2/-	-	-	-	-	-
39	2/-	2/-	2/-	2/-	2/-	2	2/-	2/-	-	2/-
40	1/1	1/1	1/1	-	2/1	2/1	2/1	2/1	-	2/-

↓→ no. of strains . *culture supernatants. the zone mesure by mm.

Table 21: Antagonistic Activity Of *Bacillus spp.*

Test strains	Inhibitor strains										
↓ →	11	12	13	14	15	16	17	18	19	20	21-40
1	-	-	-	-	-	-	-	3/3*	3/1	3/1	-
2	-	-	-	-	-	-	-	3/3	3/2	3/2	-
3	-	-	-	-	-	-	-	4/3	4/2	4/2	-
4	-	-	-	-	-	-	-	3/3	3/2	3/2	-
5	-	-	-	-	-	-	-	-	2/2	2/2	-
6	-	-	-	-	-	-	-	3/2	3/2	3/3	-
7	-	-	-	-	-	-	-	3/3	3/1	3/1	-
8	-	-	-	-	-	-	-	5/3	5/2	5/2	-
9	-	-	-	-	-	-	-	3/3	3/1	3/1	-
10	-	-	-	-	-	-	-	3/2	2/2	2/2	-
11		-	-	-	-	-	-	3/3	3/2	3/2	-
12	-		-	-	-	-	-	4/2	4/2	4/2	-
13	-	-		-	-	-	2/2	3/2	3/-	3/-	-
14	2/2	2/-	-		-	2/2	2/2	3/2	3/-	3/-	-
15	-	-	-	-		-	-	5/2	5/2	5/-	-
16	-	-	-	-	-		-	4/3	4/2	4/2	-
17	-	-	-	-	-	-		3/3	3/2	3/2	-
18	-	-	-	-	-	-	-		-	-	-
19	-	-	-	-	-	-	-	-		-	-
20	-	-	-	-	-	-	-	-	-		-
21	-	-	-	-	-	-	-	-	2/-	2/-	-
22	-	-	-	-	-	-	-	3/3	3/-	-	-
23	-	-	-	-	-	-	-	3/3	3/1	1/1	-
24	-	-	-	-	-	-	-	-	-	3/1	-
25	-	-	-	-	-	-	-	-	2/1	2/1	-
26	-	-	-	-	-	-	-	2/1	2/1	1/1	-
27	-	-	-	-	-	-	-	-	2/1	2/1	-
28	2/-	2/1	-	-	-	2/1	2/1	1/1	1/1	-	-
29	-	-	-	-	-	-	-	2/2	2/1	2/1	-
30	2/1	2/1	-	-	-	2/-	2/1	3/3	3/1	3/1	2/0(22)
31	-	-	-	-	-	-	-	2/2	2/1	-	-
32	-	-	-	-	-	-	-	1/1	3/1	3/1	-
33	-	-	-	-	-	-	-	2/-	2/-	-	-
34	-	-	-	-	-	-	-	-	2/-	2/-	-
35	-	-	-	-	-	-	-	3/2	3/1	3/1	-
36	-	-	-	-	-	-	-	3/2	2/-	-	-
37	-	-	-	2/-	-	-	-	-	4/1	-	-
38	-	-	-	-	-	-	-	3/2	3/2	3/2	-
39	2/-	2/-	2/-	2/-	-	2/-	2/-	-	4/1	2/1	2/-(30)
40	2/1	2/1	2/-	2/-	-	2/-	2/1	-	3/1	3/1	-

↓→ no. of strains . *culture supernatants. the zone mesure by mm

Table 22 : Antagonistic Activity Between *Bacillus spp.* And Other Genera.

Inhibitor strains ↓→	Test strains 1	2	3	4	5	6	7	8	9	10	11
1	-	-	-	-	2/-	3/2	-	2/-	-	-	3/-
2	-	-	-	-	1/-	3/-	-	3/-	-	-	3/-
3	-	-	-	-	1/-	4/-	-	2/-	4/-	-	3/-
4	-	2/1	-	-	1/-	4/-	-	2/-	-	-	3/-
5	-	-	-	-	-	3/-	-	-	3/2	-	3/-
6	-	-	-	-	-	3/-	-	2/-	-	-	1/-
7	-	-	-	-	-	3/2	-	2/-	4/2	-	3/-
8	-	1/1	-	-	2/-	3/-	3/-	3/-	-	-	3/-
9	-	-	-	-	2/-	3/-	-	2/-	3/-	-	3/-
10	-	1/1	-	-	2/-	4/-	-	3/2	3/-	-	2/-
11	-	-	-	-	-	-	-	2/2	3/-	-	3/-
12	-	-	-	-	-	3/-	-	-	3/-	-	2/-
13	-	-	-	-	5/-	-	5/2	8/-	-	-	8/-
14	-	-	-	-	5/-	-	5/3	8/-	-	-	8/-
15	-	-	-	-	3/-	3/-	3/3	8/-	-	-	8/-
16	-	-	-	-	2/-	3/-	-	-	3/-	-	2/-
17	2/-	1/-	-	-	2/-	-	-	2/-	3/2	-	3/-
18	-	-	-	4/3	5/-	8/3	5/2	2/2	4/3	-	5/2
19	-	-	-	4/3	-	8/5	5/4	-	4/4	-	4/4
20	-	-	-	-	5/-	6/-	3/2	5/2	4/2	-	3/3
21	-	-	-	-	1/-	2/-	-	2/-	-	-	1/-
22	-	-	-	-	1/-	3/-	-	2/-	-	-	1/-
23	-	-	-	-	-	2/-	-	1/-	-	-	-
24	-	1/-	-	-	2/-	3/-	-	3/-	-	-	3/-
25	-	-	-	-	1/-	1/-	-	1/-	-	-	1/-
26	-	-	-	-	-	2/-	-	2/-	2/-	-	2/-
27	-	-	-	-	-	2/-	2/-	-	4/-	-	1/-
28	-	-	-	-	-	-	-	1/-	-	-	-
29	-	-	-	-	-	2/-	-	4/-	-	-	-
30	-	-	-	-	5/-	4/-	-	1/-	-	-	-
31	-	-	-	-	1/-	2/-	-	-	-	-	2/-
32	-	-	-	-	1/-	2/-	-	2/-	-	-	1/-
33	-	-	-	-	1/-	2/-	-	2/-	-	-	1/-
34	-	1/-	-	-	-	2/-	-	2/-	-	-	1/-
35	-	-	-	-	-	2/-	-	2/-	-	-	1/-
36	-	-	-	-	-	2/-	-	1/-	2/-	-	2/-
37	-	-	-	-	-	-	-	-	-	-	-
38	-	-	-	-	-	-	-	-	-	-	-
39	-	-	-	-	-	-	-	-	-	-	-
40	-	1/1	-	-	2/-	2/-	-	-	-	-	-

→ 1-*E. faecalis.* 2-*E. faecium.* 3-*E. durancs.* 4-*L. monocytogenes* 5-*S. warner.* 6-*S. saprophyticus.* 7-*S. aureus.* 8-*S. chromogenes* 9-*E. coli.* 10-*Ps. aerogenos.* 11-*Sa. Typhimurium* *culture supernatants

4.6- Susceptibility of *B. cereus* and other *Bacillus spp.* to antibiotic:

Antibiotics effective against *B. cereus* are aureomycin, dihydrostreptomycin, terramycin, baciteracin **(Brown and Scherer 1957)**, oxytetracycline, chloramphenicol and gentamicin **(Chmielowski 1979)**. Slight inhibition was observed with neomycin, cloxacillin, ampicillin and penicillin **(Chmielowski 1979)**.

Brown and Scherer (1957) found no inhibition of *B. cereus* by penicillin or polymyxin. **Reva *et al.* (1995)** studied antibiotic susceptibility for some species belonging to the genus *Bacillus* to develop a method for identification based on a ststistical evaluation of species differences in antibiotic susceptibility.

The antibiotics susceptibilities of 40 strains, including five species and one unknwon strain are shown in Table 23. Data indicated that all isolates were susceptible to all antibiotics. A comparison of the diameters of the zones of the growth inhibiton data indicates that the susceptibities of all of the strains to the antibiotic bacitracin and ampicillin were nearly the same except strains 13, 20 and 35 (*B. pumilus, B. lentus* and *B. lentus* respectively . There was no relationship between the zone of the inhibition within genus or within species.

Chloramphenicol, erythromycin, oxytetracycline, novobiocin and tetracycline are highly effective antibiotics against *B. cereus* and other species, followed by gentamycin, kanamycin and neomycin, while bacitracin and ampicillin were weakly effective.

These results agree with those of many workers **(King-Thom 1986, Bernhard *et al.* 1978 and Kamat *et al.* 1989)** except for ampicillin, while **Reva *et al.* 1995** found strains sensitive to this antibiotic.

Table 23: Antibiotic Susceptibility For *Bacillus* Strains.

↓→	1	2	3	4	5	6	7	8	9	10
1	2*	3	3Δ	1°	1	2	3	3	1	1
2	2	3	3	2	2	2	3	3	1	1
3	2	3	3	2	1	2	3	3	1	1
4	2	3	3	1	1	2	3	3	1	1
5	2	3	3	1	2	2	3	2	1	1
6	2	3	3	2	2	2	2	3	1	1
7	2	3	3	1	1	1	3	3	1	1
8	2	3	2	2	1	2	3	3	1	1
9	2	3	3	1	1	1	2	3	1	1
10	2	3	2	1	2	2	3	3	1	1
11	2	2	2	1	1	1	3	3	1	1
12	2	2	2	1	1	1	2	3	1	1
13	3	3	2	2	1	2	3	2	1	3
14	3	3	1	2	2	2	3	1	1	1
15	2	2	2	1	1	2	3	2	1	1
16	2	3	3	2	2	2	3	3	1	1
17	2	3	3	1	1	1	3	2	1	1
18	2	3	2	2	1	2	3	3	1	1
19	2	2	2	1	1	1	2	3	1	1
20	3	3	3	2	2	3	3	3	1	3

↓ No. of strains →Disks containing antibiotic

°1≤ 3 *3 ⟨ 2 ≤ 6 Δ6 ⟨ 3 ≤ 9. The zone by mm.

4.7- Growth and survival of *Bacillus cereus*

4.7.1- Growth temperatures for *Bacillus spp.*:

Bacillus species are common contaminated of many food and can cause spoilage of variety of processed food **(Johnson 1984, Kramer and Gilbert 1989 and McGuiggan *et al.* 1994)**.

Bacillus cereus is generally classed as a mesophile but psychrotrophic strains are not uncommon particulary in raw and pasteurised milk **(Griffiths 1990)**. Psychrotrophic strains can grow down to 4-5° C.**(Van Netten *et al.* 1990, Dufrenne *et al.* 1993 and Davis and Walker 1994)**.

Data in Table 23 showed that all *Bacillus spp.* (42 strains) able to grow at 10, 15, 20, 25, 30, 37 and 42° C. All strains were able to grow very fast at 30 and 37° C. after 12-18 hr. following at 25° C. after 24 hrs Two strains of *B. pumilus* (No.13 and 15), 3 strains of *B. lentus* (No. 18, 19 and 20) and 4 strains of *B. cereus* (No. 25, 26, 27 and 28) were able to grow at 50° C. after 24 hrs.

These results for *B. pumilus* were nearly in agreement with Bergy's manual but not agree for *B. lentus*. While the results for *B. cereus* were agreed with many worker such as **Johnson *et al.* (1983)** who found that *B. cereus* can grow from 5 to 50 ° C. and was faster at 30°C.. and **Kathleen and Emil (1987)** reported that some strains of *B. cereus* can grow at 50° C. in nutrient broth . While **Te-Giffel *et al.* (1997)** studied the growth for *B. cereus* at different temperatures including 50° C. but he did not mention any thing about this degree.

Bacillus cereus No. 25, 27 and 28 were isolated from fried rice **and Moreland 1976, Bryan *et al.* 1981, Gilbert *et al.* 1974 and Sly and Ross 1982** advised we should keep prepared rice hot (55-63° C. or 131-146° F.) or cool cooked rice quickly and reheat cooked rice thorougly befor serving.

Three strains of *B. cereus* (No. 29 which produced diorrhoeal and emetic toxin refrences strains), 2 strains of *B. lentus* (No. 35 and 38), 1 strain of *B. licheniformis* and unknwon strains were able to grow after 7 days at 7° C. while all strains were able to grow at 10° C.On the other hand **Kathleen and Emil (1987)** found that *B. cereus* can grow between 7 to 50° C.. **Robert *et al.* (1993)** found 3 strains of *B. cereus* did not grow at 5° C. and had a minmum growth temperature of 8° C..**Te-Giffel *et al.* (1997)** found 56 of the 106 strains were able to be grow at 7°C. and were consider to be psychrotrophic.Normally psychrotrophs are isolated by incubation at low temperatures (5-7°C.) for 7-10 days(**Cousin1982**). The International Dairy Federation defined psychrotrophs as those microorganisms that can growths at 7°C. or less, irrespective of their optimal growth temperature (**Collins 1981**).

Table 24 : Growth Temperatures For *Bacillus spp.*

↓ →	7	10	15	20	25	30	37	42	50
1	0*	2	2	3	4	5	5	3	0
2	0	2	2	3	4	5	5	3	0
3	0	2	2	3	4	5	5	3	0
4	0	2	2	3	4	5	5	3	0
5	0	2	2	3	4	5	5	3	0
6	0	2	2	3	4	5	5	3	0
7	0	2	2	3	4	5	5	3	0
8	0	2	2	3	4	5	5	3	0
9	0	2	2	3	4	5	5	3	0
10	0	2	2	3	4	5	5	3	0
11	0	2	2	3	4	5	5	3	0
12	0	2	2	3	4	5	5	3	0
13	0	2	2	3	4	5	5	3	0
14	0	2	2	3	4	5	5	3	1
15	0	2	2	3	4	5	5	3	0
16	0	2	2	3	4	5	5	3	1
17	0	2	2	3	4	5	5	3	0
18	0	1	2	3	4	5	5	3	0
19	0	2	2	3	4	5	5	3	1
20	0	1	2	3	4	5	5	3	1
21	0	2	2	3	4	5	5	3	1
22	0	2	2	3	4	5	5	3	0
23	0	2	2	3	4	5	5	3	0
24	0	2	2	3	4	5	5	3	0
25	0	2	2	3	4	5	5	3	0
26	0	2	2	3	4	5	5	3	1
27	0	2	2	3	4	5	5	3	1
28	0	2	2	3	4	5	5	3	1
29	1	2	2	3	4	5	5	3	1
30	0	1	2	3	4	5	5	3	0
31	0	1	2	3	4	5	5	3	0
32	0	1	2	3	4	5	5	3	0
33	1	1	2	3	4	5	5	3	0
34	0	1	2	3	4	5	5	3	0
35	1	1	2	3	4	5	5	3	0
36	0	1	2	3	4	5	5	3	0
37	0	2	2	3	4	5	5	3	0
38	2	2	2	3	4	5	5	3	0
39	0	2	2	3	4	5	5	3	0
40	1	2	2	3	4	5	5	3	0
d	1	1	2	3	4	5	5	3	0
e	1	1	2	3	4	5	5	3	0

↓No. of strains → temperature d and e, diarrhoeal and emetic toxin references strains.
*Faster of growth.

4.7.2- Growth Of *B. cereus* in Asian Rice Based Confectionery Product :

Bacillus cereus foodborne illness occurs in two distinct syndromes. The first description of the diarrooal syndrome was provided by Hauge **(1955)** and the first reported outbreak of emetic type *B. cereus* foodborne illness occured in England in 1971 **(Public Health Laboratory Service 1972)**.In addition to causing foodborne illness, *B. cereus* is also capable of causing mastitis, systemic infection, gangrene and other clinical problems. Foodborne illness outbreak have been reported in the United Kingdom, the United States, Canada, the Netherland, Scandinavia, Japan and elsewhere **(Johnson 1984)**.

Rice of some form seems to be almost exclusively associated with emetic outbreaks. Other starchy foods, such as macroni and cheese **(Holmes *et al.* 1981)** and vanilla slices **(Pinegar and Buxton 1977)** have been implicated in emetic outbreaks. Unconfirmed cases due to feta cheese and skim milk powder may have also involved *B. cereus* **(Schmitt *et al.* 1976)**.

Data in Table 26 indicated the growth of *B. cereus* strains isolated from milk, pastrami and diarrhoeal and emetic reference strains in nutrient broth at 30°C.. Nearly the growth are the same, but we have to remember there are some differences between the growth of *B. cereus* especially the germination of spores (fast or slow). This differences is depending on the source of this bacteria, air, soil, and **food (Johnson *et al.* 1982, Wong et al 1988 and Te-Giffel *et al.* 1995)**.

Table 26 :Growth Of *B. cereus* Strains In Nutrient Broth (Log cfu/mL.)

Time/h	Strain No.1	Strain No. 2	strain No.9	Diarrhoeal reference strain	emetic reference strain
0	2.38	2.23	2.41	2.54	2.53
1	3.47	3.59	3.23	2.95	3.06
2	4.07	4.15	3.62	3.54	3.42
3	4.69	4.59	4.02	4.16	3.74
4	5.22	5.46	5.12	4.69	4.69
5	6.02	6.13	6.13	5.67	5.27
6	6.65	6.73	6.86	6.45	6.04
7	7.2	7.35	7.24	6.94	6.77
8	7.37	7.39	7.45	7.08	6.92
9	7.46	7.41	7.51	7.35	7.22
10	7.82	7.67	7.71	7.09	7.19
11	7.65	7.85	7.79	7.17	7.33
12	7.83	7.54	7.72	7.25	7.62

Previous work in our laboratory and some departments of Australian health service in Sydney found that more than 60% of Asian rice products contained *B. cereus with* high numbers, storage temperature for all this products in the supermarket between 15-28.1° C However, *B. cereus* frequently contaminates cooked food, which often serve as vehicles for food poisoning **(Gilbert et al 1974, Melling *et al.* 1976 and Schiemann 1978).**

I visited some places, which produced these products. The equipments are too basic, nearly same as equipment's in any house kitchen. So the aim of this part: study the growth of *B. cereus* in one product of Asian rice products (Asian rice based confectionery product). I cooked this product using steam producing by autoclave (temperature transfer through 4

sides), and this is the first difference between my cooking method and the producer method (they used steam producing by boiling water and the temperature transfer by one side).

The cooked temperature was measured after opening the autoclave direct. It is between 94-96°C. Second difference I put this product after cooking directly in cold place (the temperature was nearly 20°C.), until the sample become cold and sliced, then stored directly at different temperatures. In the manufacture's place I believed they keep the product until become cold on the same cooking place, and the temperature is too high because they used big flame for producing steam by boiling water (the temperature is more than 30°C.).

Data in Table 27 indicated the growth of the rifampicin *B. cereus* emetic reference strain in rice layers under different storage temperatures. The inoculation numbers between Log. 5.33 to 5.96. The samples were inoculated by submerged into the broth for one min. (all samples are the same weight and volume). The samples were visually spoiled with increasing storage temperature. The numbers of *B. cereus* were decreased but not too much after storage by 6 days at 7°C. This may be happened because the vegetative cell was sensitive to low temperature and *B. cereus* is not strong competitor with psycrophilic bacteria. Any way the ingredient for rice product can not have the same inoculation numbers but from this experiment data indicated that we can control the growth of *B. cereus* in rice product by controlling the temperature, but the problem if this product store at 7°C. the texture become hard because the product have high percentage of starch.

Table 27: Growth Of *B. cereus* In Rice Layer Under Different Temperatures (°C.) (Log cfu/g) *.

Temp. ▸ / Days ▾	7	10	15	20	25	30	37
0	5.66	5.53	5.96	5.92	5.55	5.62	5.55
1	5.74	5.86	6.43	7.27	7.51	7.82	7.92
2	5.61	5.96	6.93	7.38	7.86	8.06	7.81
3	5.61	5.73	7.05	7.69			
4	5.58	6.37	7.28				
5	5.61	6.45					
6	4.69						
7	4.47						
8	4.3						

*Mean from duplicate plates of duplicate samples

Data in Table 28 indicated the effect of cooking and storage temperature of rice layers and on the growth of *B. cereus*. The number of *B. cereus* was decreased by 2.08 Log. after cooking. I cooked about 5 layers, each layer cooked for 5 min. except last layer which cooked for 10 min. same as the producer told me, but we found in the supermarket some rice layers products about 8-10 layers and this is another problem because if this product have 10 layers this mean the time for cooking is 55 min. plus 5-10 min. added each layers equal 65 min.. So if the ingredient have *B. cereus* vegetative cells or spores especially if the strain is fast growth the number for *B. cereus* can multiply in the ingredient during cooking time. However the organism can multiply in these ingredient during storage time.

No published data *about* this product, especially D and Z values for *B.* cereus spores in this product or any product have same composition and the main problem is the percentage of starch in this product is too high. This mean that the D and Z values for *B. cereus* in this product become long.

I tried many times to inoculate the rice layers by number of *B. cereus* until I destroy the spores by cooking temperature.

Table 28: Effect Of Cooking And Storage Temperature Of rice Layers On The Growth Of *B. cereus* (Log cfu/g.) *.

Temp. ▸ / Days ▾	7	10	15	20	25	30	37
A				5.73			
B				3.67			
1	3.82	4.9	5.29	5.96	7.14	7.23	7.26
2	3.87	5.56	6.5	7.27	7.64	7.69	7.6
3	3.7	6.18	6.72	7.08			
4	3.65	6.05	6.83				
5	3.72	5.55					
6	3.52						
7	3.66						
8	3.65						

A- before cooking.

B – after cooking.

*Mean from duplicate plates of duplicate samples.

Data in Table 29 indicated the effect of cooking temperature on the low inoculation of *B. cereus* and the growth of *B. cereus* under different storage temperatures, but we have to remember that the sensitivity of spread method may be can not detect small number of *B. cereus* and also the sensitivity of BEMPA medium and TSA (I changed BEMBA medium with TSA medium after I did fast comparison between PEMBA and TSA medium to reduce cost).

Bacillus cereus was not detected directly after cooking and after storage at 7°C. for 8 day until the samples visually spoiled. *Bacillus cereus* was found after storage by 3 day at 10°C.

Table 29: Effect Of Cooking And Storage Temperature Of Rice Layers On The Growth Of *B. cereus* (Log cfu/g.) *.

Temp. ▸	7	10	15	20	25	30	37
Days ▾							
A				3.41			
B				ND			
1	ND	ND	3.21	3.46	4.59	6.66	6.72
2	ND	ND	3.31	3.53	4.73	6.8	6.84
3	ND	3	3.38	3.64			
4	ND	3.04	3.51				
5	ND	3.14					
6	ND						
7	ND						
8	ND						

A- before cooking.

B – after cooking.

*mean from duplicate plates of duplicate samples.

During doing this part, I continued watching the analysis of rice products for the occurrence of *B. cereus* with undergraduate students. We found some PEMBA plates free from any bacteria after spreading the samples and incubation for 48hr at 30 °C. I stored these samples on fridge, but these samples can not be spoiled. This opens my eyes for trying some preservatives in this product. The use of chemical antimicrobial agents to prevent the growth of spoilage and pathogenic microorganism in food is will establish. Sorbic acid and its salts are among the most widely used food preservatives in the world, the most commonly used forms include sorbic acid and the potassium salt. Major groups of foods that may be preserved with sorbates include dairy products, fruit and vegetable products, certain meat and meat products and sugar and confectionery items **(Sofos and Busta 1981)**.

It is save if added to food at the recommended levels from 0.02 to 1.6% **(Banwart 1979)**. Generally, usage levels of sorbate are at 0.5% or less.

Linda *et al.* (1993) studied the effect of three preservatives (sodium benzoate, sodium nitrite and potassium sorbate) on the growth of *B. cereus, E. coli* and *Staphylococcus aureus.* Potassium sorbate was the most effective preservative tested against *B. cereus.*

Lashen *et al.* (1992) tested the antimicrobial activity of 11 fatty acids and their salt on the growth of *clostridium botulinum, clostridium sporogenes* and *B. cereus.* Linolenic acid was the most inhibitory of saturated fatty acids.

Data in Table 30 indicated the effect of adding 0.1 % potassium sorbate with cooking and storage temperature. *Bacillus cereus* was not detected after adding potassium sorbate and cooking and not detected after storage at 7, 10, 15 °C. for 8, 5, 4 days

respectively. *Bacillus cereus* was detected after storage at 20, 25, 30 and 37 °C. by one day.

Table 30: Effect Of Adding Potassium Sorbate, Cooking, And Storage Temperature Of Rice Layers On The Growth *B. cereus* (Log cfu/g) *.

Temp. ▸	7	10	15	20	25	30	37
Days ▾							
A				3.51			
B				ND			
1	ND	ND	ND	3.27	4.53	6.53	6.72
2	ND	ND	ND	3.39	4.77	6.79	6.81
3	ND	ND	ND	3.47			
4	ND	ND	ND				
5	ND	ND					
6	ND						
7	ND						
8	ND						

A- before cooking.

B – after cooking.

*Mean from duplicate plates of duplicate samples.

Data in Table 31 indicated the effect of adding 0.1 luric acid, cooking and storage temperature on the growth of *B. cereus*. *Bacillus cereus was* not detected after cooking and after stored at 7, 10°C. for 8, 4 day respectively but was detected after stored by 5 days at 10°C.

Table 31: Effect Of Adding Luric Acid, Cooking And Storage Temperature Of Rice Layers Temperature And Storage On The Growth Of *B. cereus* (Log cfu/g) *.

Temp. ▸ / Days ▾	7	10	15	20	25	30	37
A				3.43			
B				ND			
1	ND	ND	3.2	3.43	3.61	6.71	6.74
2	ND	ND	3.43	3.5	3.66	6.76	6.80
3	ND	ND	3.43	3.54			
4	ND	ND	3.48				
5	ND	3.21					
6	ND						
7	ND						
8	ND						

A- before cooking.

B – after cooking.

*mean from duplicate plates of duplicate samples.

No published data for this product was available especially the growth of pathogenic or sporforming bacteria in this products, but there are some work before about germination, heat resistance or effect of cooking temperature and growth of *B. cereus* in a broth medium and in rice (fried rice, boiled rice and rice mixed with broth) most of data indcated that the time temperature exposure during cooking destroyed heat sensitive spores but not heat resistant spores and the survivor will germinate and multiply in rice stored at room temperature (**King Thom and Hone Ling 1986, Gilbert *et al.* 1974, Kim**

and Goepfert 1971, Johnson *et al.* 1983, Johnson et al 1984 and Frank *et al.* 1981). So if the ingredient of rice layer product contaminate especially by high number of *B. cereus,* the cooking temperature can not destroy all *B. cereus* spores.

Sensory evaluation was done for rice layers product by triangle test. Sixty five % from the panelists can know the sample, which has potassium sorbate. This may be due to the taste is weak (coconut milk, rice flour, starch, sugar). Some rice product in the supermarket was found containing artificial aroma. So we can add artificial aroma or increase the percentage of sugar to fix the taste.

From this study the producer must follow this recommendation to produce rice layer free from *B. cereus*:

1- The ingredients for rice product must be free from *B. cereus* (at least the number of *B. cereus* must be less than hygienic allow).

2- The numbers of layers must be less than 5 layers to avoid germination of *B. cereus* in the ingredients during cooking time.

3- It is better if the producer change cooking pan by steamer box or pressure cooker to increase cooking temperature and consequently be more effective (temperature will transfer through 4 side) and also change the percentage of starch by water if possible.

4- Put or keep the product very fast after cooking direct between 15-20°C. to be cooled until slice this product and each tray must be alone on one shelf (if cooling temperature less than 15°C., the surface for this product will dray).

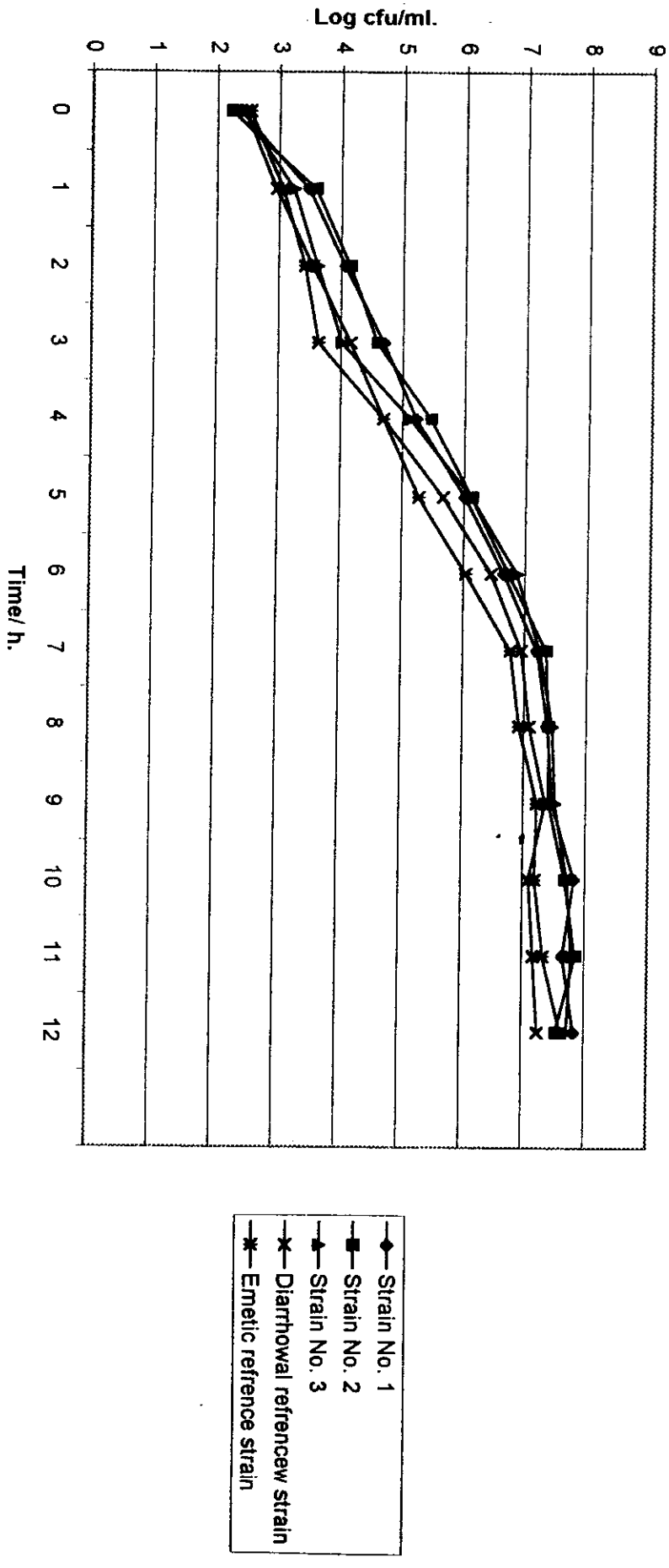
5- Cooking small quantities of this product, as the supermarket need.

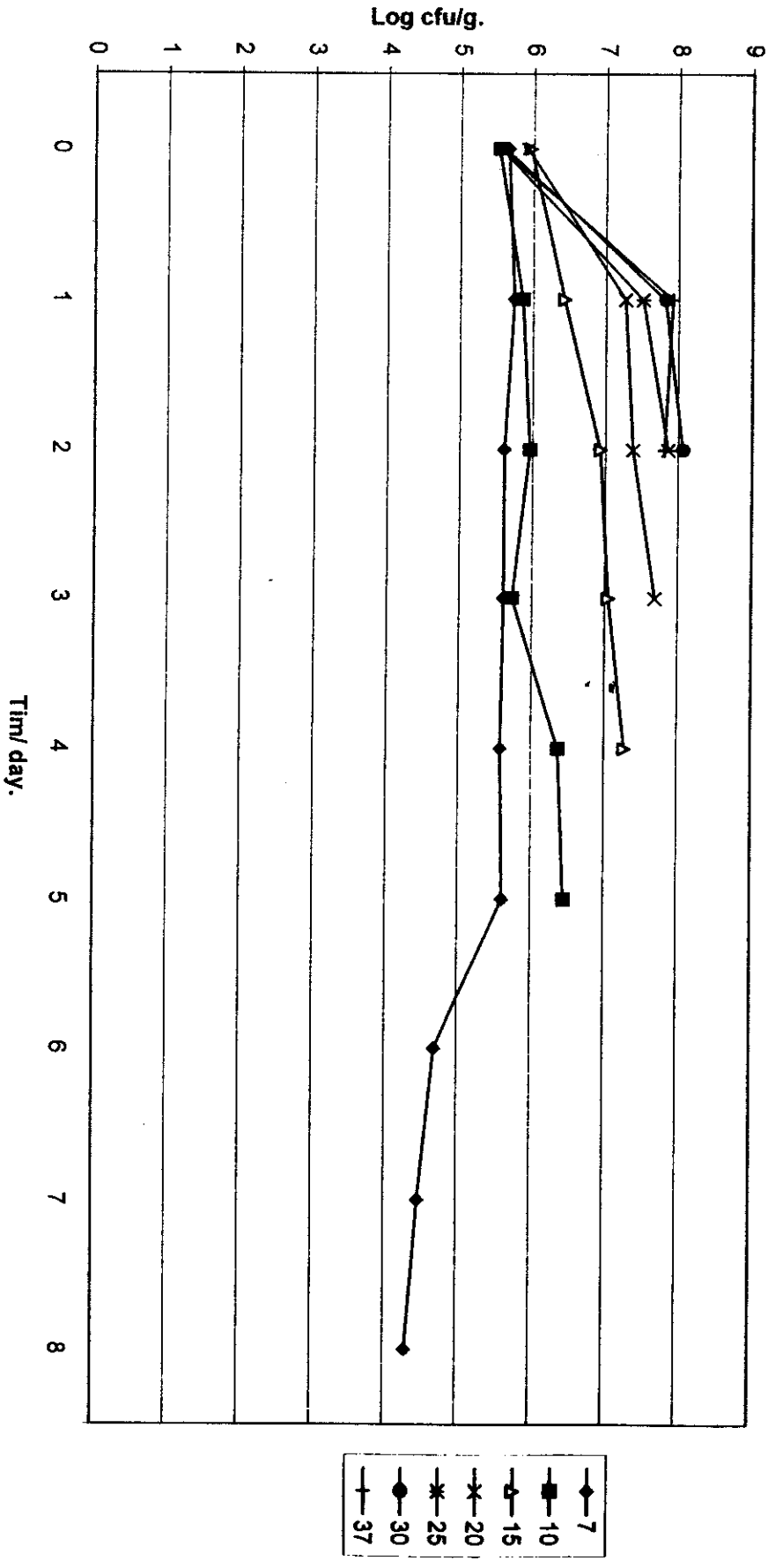
6- Keep the product on refrigerator until delivery, transfer by refrigerator, store on supermarket at low temperature (less than 10°C.).

7- Disinfect the equipment by good antiseptic before and after cooking any batch to kill any spore of *B. cereus.*

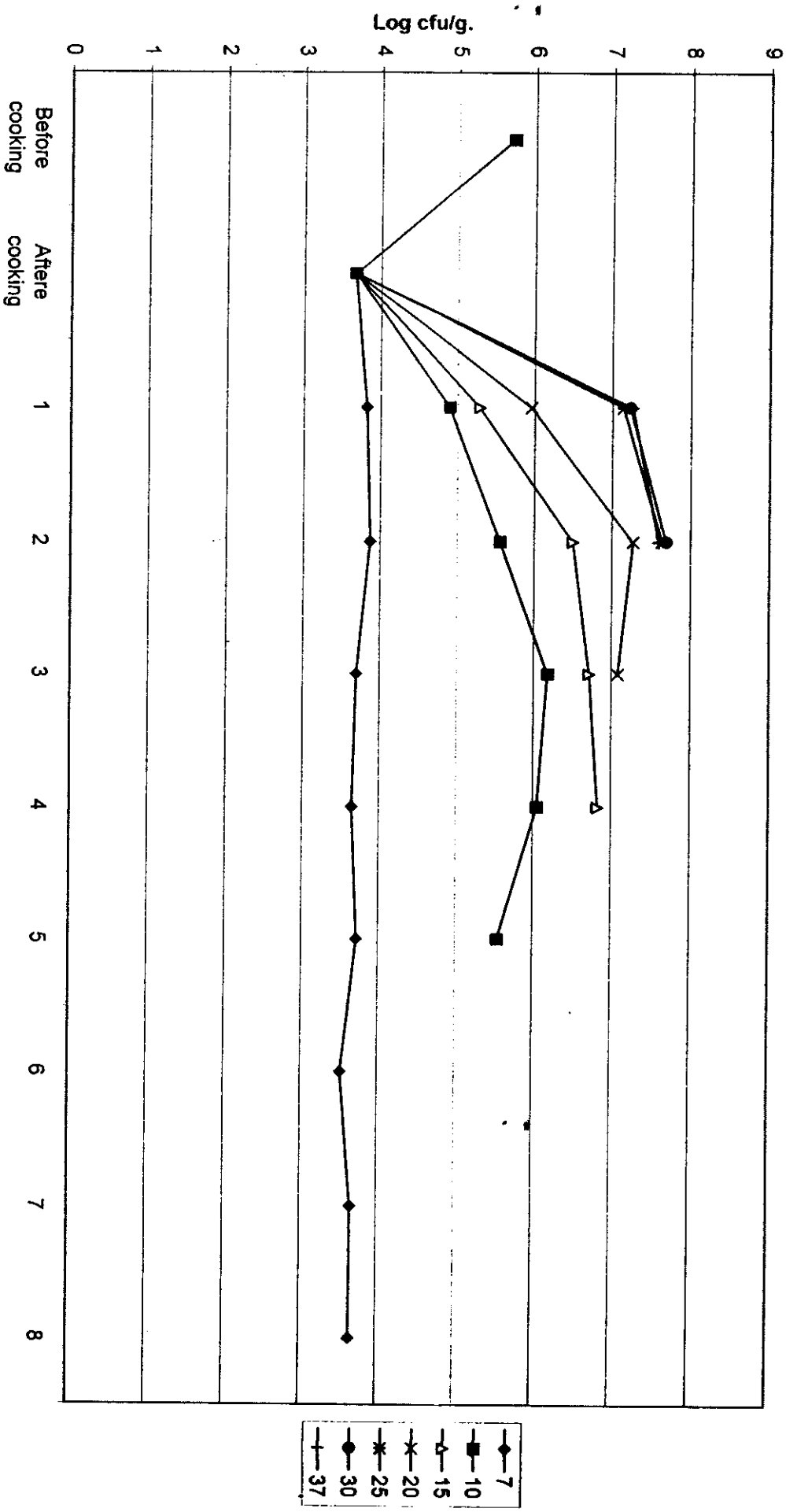
8- Many kind of rice product in the supermarket now and all manufactures for all this products is too basic and the people who working are not educated so they need knowledge about this product more than training. This open new area for research especially the growth of sporforming bacteria in this products, methods for cooking this products, nutrition for this product or HACCP for this products.

Growth Of *B. cereus* In Nutrient Broth.

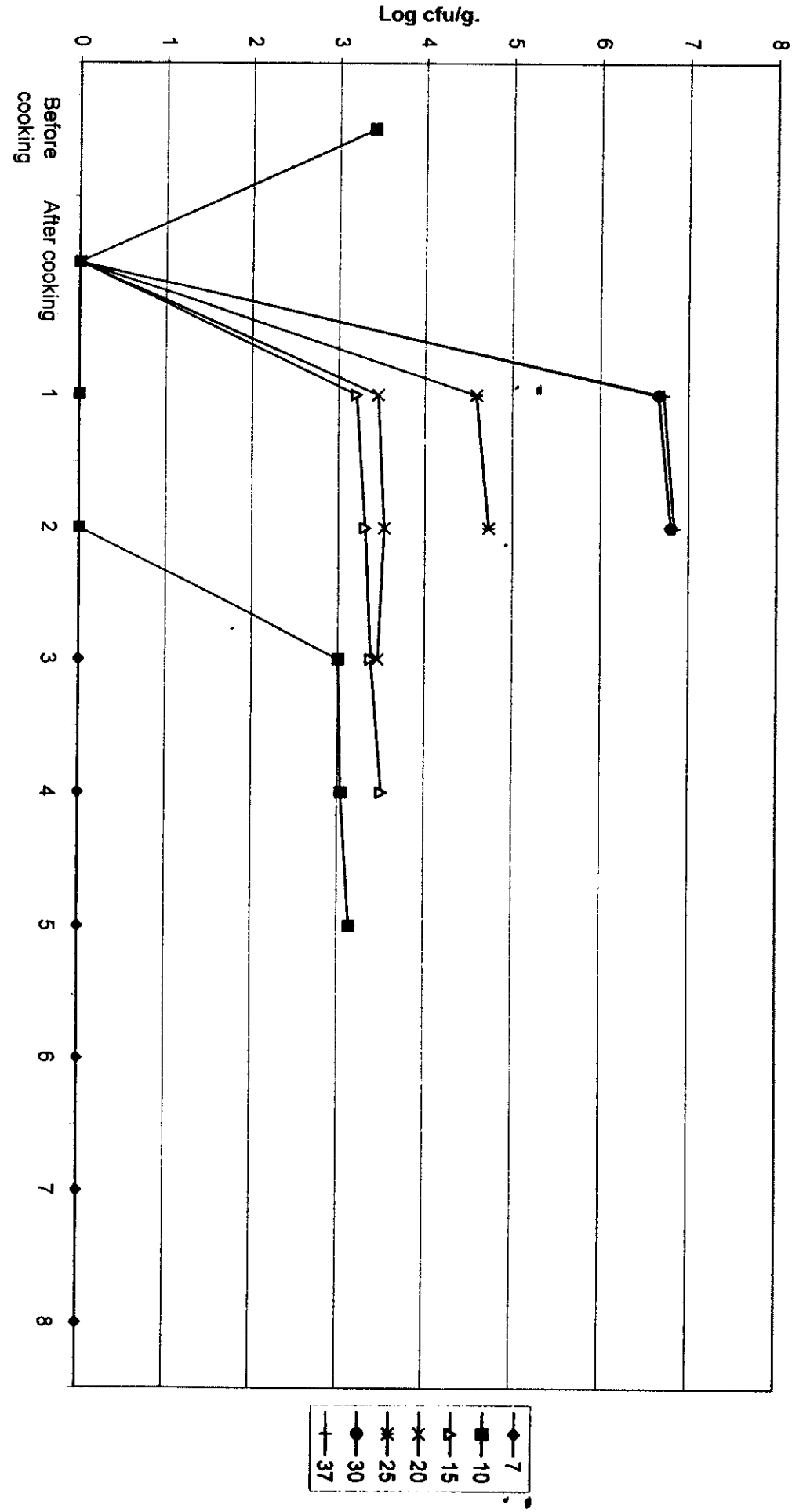




Growth Of *B. cereus* Under Different Temperature

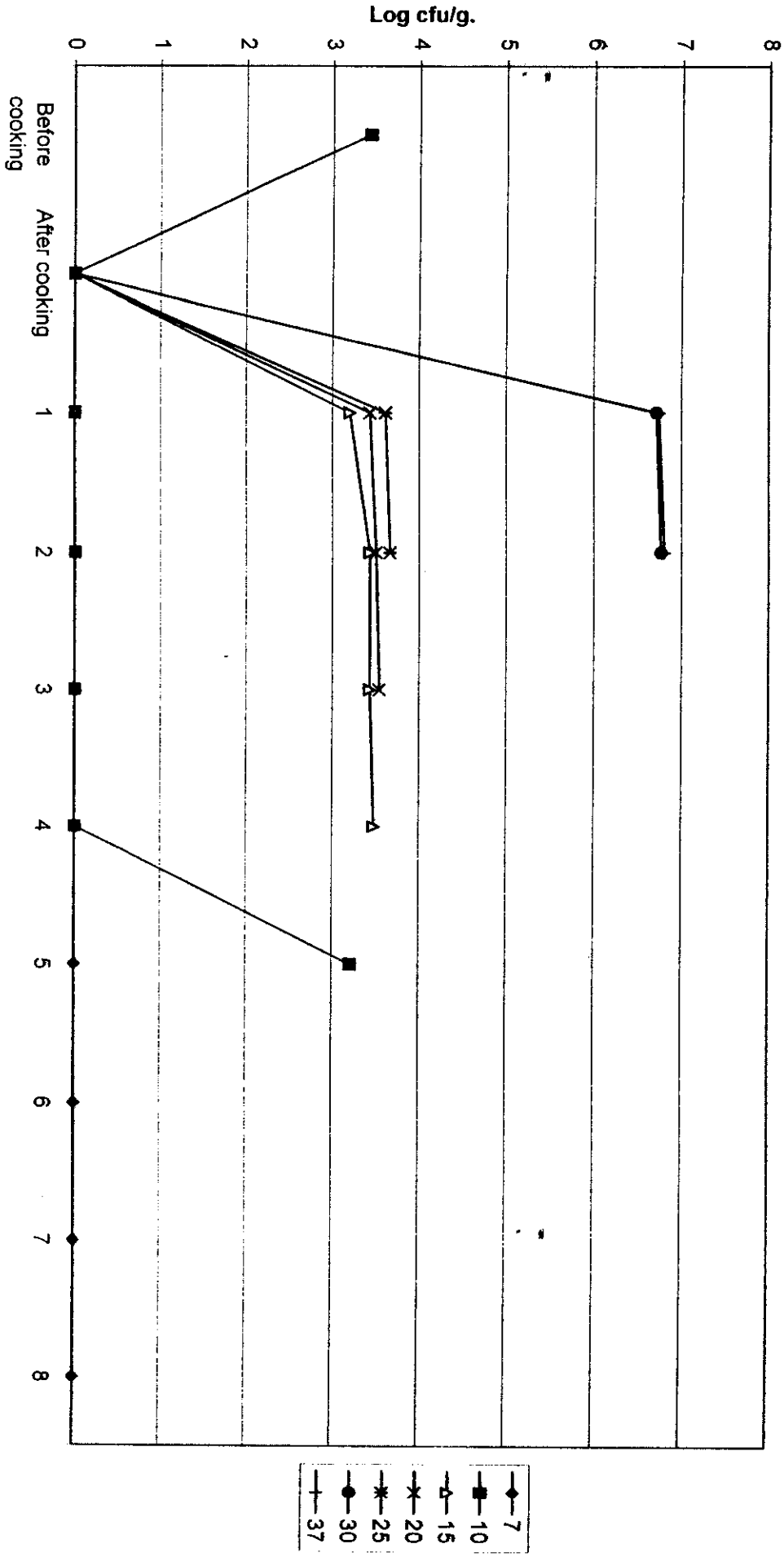


Effect of cooking and storage temperature of rice layers on rhe growth of B. cereus.



Effect Of Cooking And Storage Temperature Of Rice Layrs On The Growth Of *B. cereus*

Effect Of Adding Luric Acid, Cooking And Storage Temperature Of Rice Layer On The Growth Of *B. cereus*



4-7-3- Effect of combination of sodium chloride and pH on the growth of *B. cereus*.

Increased acidity or change in pH of food by adding acids is one of the ways for limiting microbial growth to control spoilage **(Ziauddin 1993)**.

Information concerns the effect of NaCl. concentration and pH on the growth of *B. cereus* is limited **(Goepfert *et al.* 1972, Troller 1973, Marrku and Constantin 1975, Lalita *et al.* 1985, Ziauddin *et al.* 1993 and Bonestroo *et al.* 1993)**. Some of them studied the effect of pH and NaCl in media and other in some food products such as salad .The aim of this part is to know additional information of the effect of combination NaCl and pH of the growth of *B. cereus* diarrhoeal and emetic toxin in laboratory media.

Results for the growth of *B.* cereus diarrheal and emetic toxin are presented in Table (31) and (32) respectively and following by figures for this growth at each pH. The temperature in this time (10 days) was around 20-25°C.. The inoculation numbers are between 2.91- 3.96 log cfu/mL., except one sample is 2.6 log cfu/mL.. At pH 7.5 the number of *B. cereus* diarrheal and emetic toxin was increased especially when the concentration of salt is low. This growth is very high. While the number of *B. cereus* diarrheal toxin was increased at pH 7.5 with 8 % NaCl the number of *B. cereus* emetic toxin nearly is not increased and not decreased. The increasing of number of *B. cereus* diarrheal toxin at 8 % NaCl is not too much especially if we make visually comparison with the same number of growth at 7 % salt.

Generally the growth of *B. cereus* diarrheal toxin was more than the growth of *B. cereus* emetic toxin at all pH values and salt concentrations. The growth of *B.* cereus diarrheal and emetic toxin was going down with decreasing of pH values and increasing of salt concentration. The growth of *B. cereus* diarrheal toxin was decreased at pH 4.5 with all concentrations of NaCl. On the other hand *B. cereus emetic* toxin could not be

detected after 4 days with same condition, but we have to remember the sensitivity of medium and method.

As I mentioned before not too much data about the effect of combination of NaCl and pH on the growth of *B. cereus* especially the range of NaCl 3-8 % because most of workers before such as **Nielson and Zeuthen 1986, Thomas *et al.* 1993** and other studied the effect of low concentration of salt and pH but with some preservatives.

The inoculation number of *B. cereus* was slightly high because I do not know what the way for *B. cerus* growth (increase or decrease). The number for *B. cereus* was accounted by spread method because optical density method can not give reading when the number is too low and can give wrong data if *B. cereus* produce any product can effect the turbidity. I chosen room temperature because all products, which have same pH values and salt concentration such as, pickle normally was stored at room temperature. I did not use MPN method because this method take long time, the samples should be analysis every 2 days and the number of samples were 48.

I could not find any relationship between the obtained results and another results because a lot of differences between the growth conditions such as pH, NaCl concentration, temperature, media and the differences between the strains, especially the sources for this strains (isolating from food, soil and air). Nearly or generally if we forget some directions, these results are in agreement with some results before. **Tomase *et al.* (1993)** reported that the maximum salt concentration for growth of *B. cereus* is 7 % at pH 6-7 and 30-35° C. **Kim and Goepfert (1971)** mentioned that *B. cereus* is able to grow in 7 % NaCl but not in 10 %. *Bacillus cereus* can grow at pH 4.3 to 9.3 **(kramer and Gilbert 1989, Raevuori and Genigorgis 1975 and Kim and Goepfert 1971). Lalit *et al.* (1985)** who is the only one found that *B. cereus* can tolerate to 10 % of salt and 4 % of

acidity at 26 ± 2° C. for 48 hr. on nutrient broth. On the other **hand Vijayalakshmi *et al.* 1981** found that *B. cereus* can grow in tamarind rice natural and artificial contamination ($2x10^2$-$4x10^3$) and the pH values for this rice were 4.1and 4.2 respectively.

From the data of this and other studies, it is indicated that decrease of pH alone does not enough to control the growth of *B. cereus* and the acid food should be tested for occurrence of *B. cereus* especially sweet pickle.

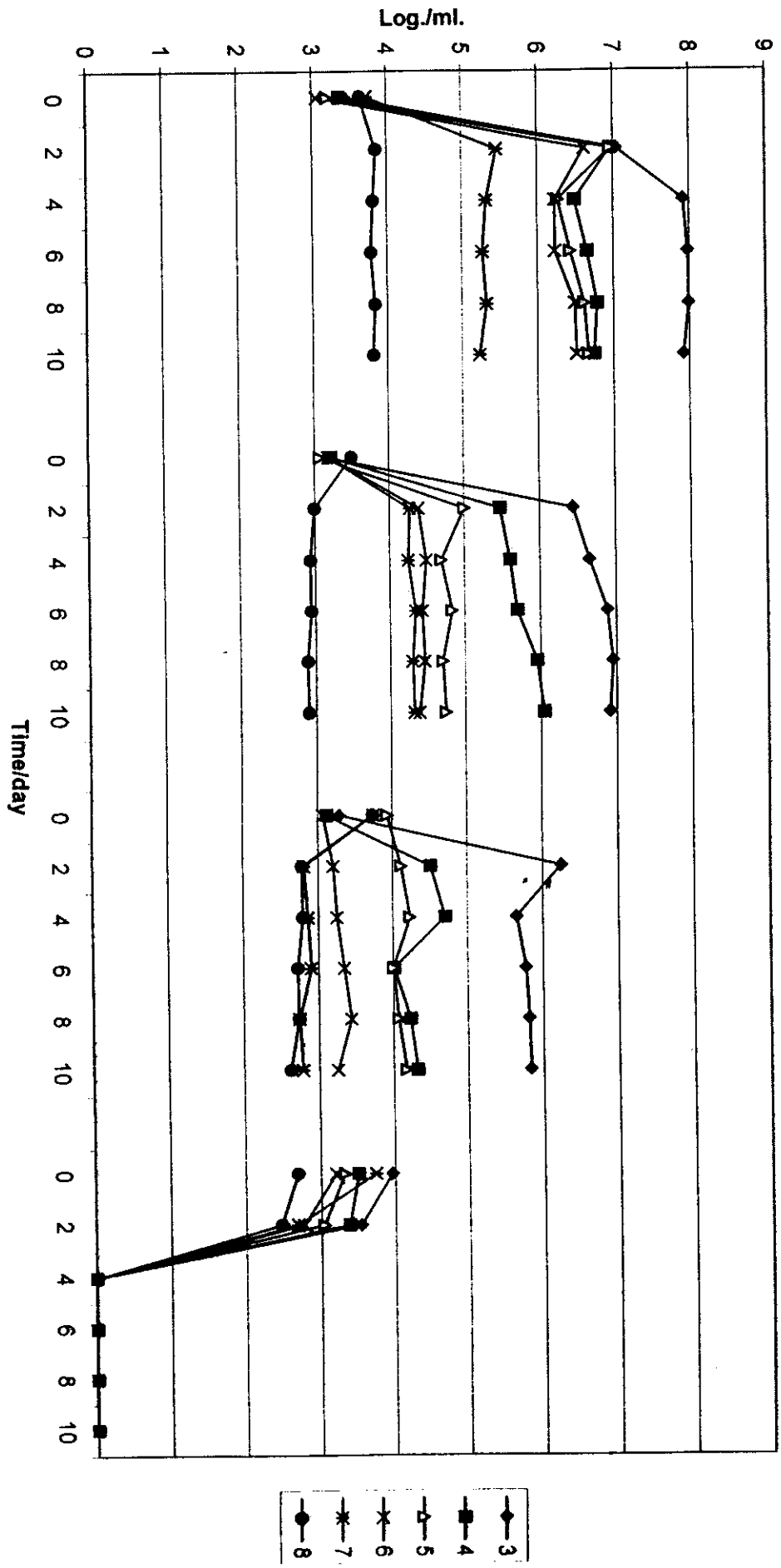
Table 32: Growth Of *B. cereus* Diarrheal Toxin At Different pH Values And Salt Concentration (Log cfu/mL.).

Salt % ▸ / Days ▾	pH value	3	4	5	6	7	8
0	7.5	3.35	3.23	3.26	3.05	2.6	3
2		8.44	7.59	7.16	6.73	6.06	4
4		8.44	7.59	7.34	7.36	7.06	5.38
6		8.94	7.24	6.86	6.97	6.74	5.7
8		8.87	7.32	6.81	6.84	6.65	5.69
10		7.82	7.27	6.81	6.87	6.74	5.6
0	6.5	3.34	3.36	3.35	3.2	3.08	3.18
2		8.09	7.37	7.65	6.89	5.91	3.37
4		6.65	6.65	6.94	6.78	5.82	4.81
6		6.38	6.65	6.14	5.6	5.84	2.87
8		6.43	6.35	6.14	5,69	5.80	2.91
10		6.39	6.36	6.07	5.84	5.77	282
0	5.5	3.34	3.29	3.71	3.18	3.2	3.17
2		6.71	4.25	4.09	3.77	3.54	2.67
4		6.91	6.31	5.54	3.72	3.43	2.66
6		6.16	5.65	5.42	3.69	3.08	2.67
8		6.09	5.40	5.27	3.65	3.02	2.60
10		6.06	5.37	5.13	3.60	2.98	2.51
0	4,5	3.45	3.56	2.97	2.91	3.96	2.94
2		3.40	2.74	3.25	2.65	2.64	2.66
4		2.76	2.58	2.53	2.74	2.67	2.56
6		2.68	2.72	2.61	2.61	2.60	2.57
8		2.65	2.69	2.57	2.57	2.56	2.55
10		2.65	2.67	2.56	2.56	2.55	2.54

Table 33: Growth Of *B. cereus* Emetic Toxin At Different pH Values And Salt Concentration (Log cfu/mL.).

Salt % ▸ / Days ▾	ph value	3	4	5	6	7	8
0	7.5	3.44	3.36	3.2	3.06	3.73	3.64
2		7.04	6.96	6.69	6.62	5.46	3.85
4		7.92	6.49	6.27	6.23	5.32	3.81
6		7.98	6.65	6.43	6.23	5.27	3.78
8		7.99	6.78	6.61	6.49	5.32	3.83
10		7.92	6.74	6.67	6.51	5,23	3.81
0	6.5	3.18	3.22	3.07	3.21	3.18	3.49
2		6.44	5.47	5	4.39	4.27	2.99
4		6.65	5.6	4.69	4.49	4.25	2.93
6		6.88	5.69	4.83	4.43	4.34	2.94
8		6.95	5.95	4.7	4.46	4.3	2.89
10		6.91	6,04	4.74	4.39	4.32	2.9
0	5.5	3.29	3.12	3.93	3.07	3.75	3.73
2		6.25	4.51	4.11	3.2	2.81	2.78
4		5.65	4.70	4.23	3.25	2.86	2.79
6		5.77	4.01	4	3.34	2.9	2.72
8		5.81	4.23	4.07	3.43	2.74	2.73
10		5.83	4.32	4.17	3.25	2.78	2.62
0	4.5	3.96	3.5	3.32	3.2	3.74	2.96
2		3.54	3.38	3.04	2.77	2.69	2.47
4		ND	ND	ND	ND	ND	ND
6		ND	ND	ND	ND	ND	ND
8		ND	ND	ND	ND	ND	ND
10		ND	ND	ND	ND	ND	ND

ND: not detected



Growth Of *B. cereus* Emetic Toxin at Different pH And Salt Concentration.