Berk Can AYDIN
001129-0080

BIOLOGY

EXTENDED ESSAY

THE EFFECT OF TEMPERATURE ON REPRODUCTION OF *Bacillus Cereus* IN BABY FOODS

TEACHER NAME: ÜMİT YAŞATÜRK MİDİLLİ

STUDENT NAME: BERK CAN AYDIN

CLASS:12/H

CANDIDATE NUMBER:001129-0080

WORD COUNT: 3500

Berk Can AYDIN
001129-0080

Abstract

The reason that I was choosing this topic is that my little cousin had a posining because of a baby food. I thought the baby food's expiration date passed but it wasn't so I researched what was in the baby food and what can make puke my cousin and I found a microorganism called *Bacillus cereus.* It can reproduce while heating the meal or baby food so while my cousins's mom was preparing his meal she heated the baby food so maybe *Bacillus cereus* poisoned so I wonder when the baby food heated, the amount of microorganism may increase and I wanted to explore this topic in my extended essay.

Berk Can AYDIN
001129-0080

Content Page

Berk Can AYDIN
001129-0080

Introduction

The first time I confronted in 7 months ago, I have a cousin and he is a 2 year old boy and he is eating baby food for nutrition. One day he and his family came to us. After a time, he got hungry and because of that his mom prepared his meal. She heated his meal to give it to him. Later on, my cousin started to puke and no one could understand why he puked. After they went out my home, I started to think what can make my cousin sick. After a quick research, I thought maybe a microorganism can make him sick and I found a very detrimental microorganism called *Bacillus cereus* and I learned that it can proliferate in different temperature rates.

Nutrition and health problems of the babies have an important place in growing a healthy generation, which looks safely to the future. Having *Bacillus cereus* among the gastroenteritis factors recently, which is composing the huge part of the baby sicknesses, and insufficiency of the studies on this topic caused me to research presence of *Bacillus cereus* in baby food.

The recent studies showed that *Bacillus cereus* might have an important place among gastroenteritis factors in addition to the classical gastroenteritis factors.[1]

First time a researcher, named Lubenau drew attention to presence of these microorganisms as factor for poisoning in nutritional agents on 1906.[1]

You can see the shape of *Bacillus cereus* under microscope via gram dyeing.

Bacillus cereus is mainly present in environment, soil, milk and milk powder, baby foods, fruits and vegetables as well as various nutritional agents in vast numbers. It reproduces in suitable conditions which are moisture and temperature.

Although *Bacillus cereus* is determined in poisoning cases, its mechanism is still a topic of debate today.

Food poisoning, occurred due to *Bacillus cereus*, were frequently concurred foods with roasted and baked rice within Chinese restaurants of Europe. It is also reported that it is rarely seen in canned food, vegetable soups, milk, ice-cream, cooked vegetables, yoghurt, minced meat, etc. prepared food.[2]

Its incubation period generally varies between 15 minutes and 16 hours. When it enters in your body with a food the symptoms that will occur are stomachache, nausea, stomach cramps, watery diarrhea, from time to time vomiting and fever.[3]

It is determined that intensity of *Bacillus cereus* in foodstuff varies depending on the conditions related with consumption of such foodstuff, temperature, time and moisture rates.

Bacillus cereus is easy multiplying bacteria in mediums however as isolation and identification of *Bacillus cereus* in foodstuff, including complicated micro-flora, various selective mediums were developed.[4]

Presence and importance of *Bacillus cereus* were started to be investigated in our country during 1970s. Food poisoning due to bacillus cereus was firstly reported on 1000 soldiers of a military unit, located near İzmir.[5]

It is reported that factor for food poisoning was *Bacillus cereus* in various countries of the world, Far East and especially in Europe. In most of these cases, isolation, identification and counts of the microorganisms were reported quantitatively. Submission of foodstuff for consumption under inappropriate conditions within our country, failure to maintain conditions related with food hygiene sufficiently cause gastroenteritis and from time to time cases ending with death.[6]

In my study, I tried to determine presence of *Bacillus cereus* within local and foreign baby foods with various brands and structures, which are commercially available, quantitatively. I particularly wanted to draw attention to the potential presence of *Bacillus cereus* in gastroenteritis, occurred on babies with baby foods. I wish this study, which was implemented by me with limited number of samples, would be continued more extensively and would be integrated in form of reaching to this microorganism (factor) starting from gastroenteritis factors.

As a result, this paper will focus on the research question: How does temperature rate affect the number of bacteria, *Bacillus cereus* reproduction in baby food?

> Hypothesis: There is a coincidence that after my cousins' s mom heated his meal, he started to puke so when she increased temperature level of the baby food, the microorganism, *Bacillus cereus* could proliferate. Because of that it can be hypothesized that as the temperature rate increases the number of *Bacillus cereus* colonies will increase.

Material

Study was implemented for each of the 10 baby food samples, composed of ao one local baby foods, within private Doğa laboratory. All of the samples received and studied in their original packages. Brand, serial numbers, introduction dates and expiry dates of received samples were inspected and recorded.

A. TOOLS-EQUIPMENTS

a. Glassware

Tube(x50)

Petri dish(x1)

Pipette

Erlenmeyer (300 milliliters)

Lam(x1)

Pasteur pipette(x1)

b. Devices

Incubator (28-35°C) (x1)

Autoclave(x1)

Pasteur oven(x1)

Fridge(x1)

Microscope(x1)

Balance(x1)

c. Other devices

Spores

Baby food

Plug(x1)

Filter paper(x1)

Pincers(x1)

Paraffin(x50)

d. Medium

Phenol-Red Egg-Yolk Polymxin Agar(x50)

Above mentioned agents, except Phenol-Red, were boiled and melted and after cooling down to 50-60°C, pH was adjusted to ± 7.2, and sterilized in autoclave for 15 minutes at 120°C.

METHOD DEVELOPMENT:

At the very beginning of the experiment, I heated all the glassware, to be used during experiment, at 165°C because glassware should be decontaminated from bacteria and virus, etc. microorganisms, formed within them, and I should have a more sterile environment. In order to increase the number of microorganisms, which might form within the baby foods, I watered baby foods via water with peptone because this water makes the reproduction of bacteria higher. I mixed this formed mixture in the vortex device, because I need to make the mixture homogeneous, to be discharged into tubes with equal concentration of baby food and peptone. The reason for me to put baby food samples within the tubes onto this medium within environments with different temperatures after spreading them onto phenol-red egg-yolk polymixin agar medium was to try to determine the most appropriate reproduction place with considering the cultural features as isolation and identification of *Bacillus cereus* is difficult within foodstuffs, including mixed micro-flora (mixed microorganisms), and baby foods. Studies were performed on various mediums however in most studies phenol-red polymixin agar medium was selected as the best medium in connection with its reproduction because in the phenol-red polymixin agar, there is a sulphate. Sulphate is inhibating the reproduction of other microorganisms. Another reason for us to use this medium is that if there is *Bacillus cereus* within the baby food, we used, and if it's reproduced, reproduced microorganisms form lecitinase ring in this medium.[7] Microorganism groups, which do not form this ring, are eliminated during experiment by this way. The reason for me to use horizontal agar medium in order to purify the lecitinase positive colonies and for the purpose of being ensured that it is *Bacillus cereus*, is to be able to have microorganisms to stand so as to have distance between microorganisms on the medium and thus to see which one is the *Bacillus cereus* microorganism. The reason for to re-incubate this medium for another 24 hours at 30°C during experiment is to determine whether there is still a different microorganism remained within this microorganism in the lecitinase ring. The reason for dropping a drop of serum physiologic water onto a single colony, which was taken onto lam, and for homogenizing with extract, is the use of this water for electrolyte environment instead of pure water, and this shows us that it shall not disturb the structure of the microorganism. The reason for us to pass this preparation, formed on lam, over Bunzen Beck is to evaporate serum physiologic water, present within this homogenized mixture, and to maintain full attachment of microorganism onto lam and thus to facilitate dye taking without disturbing the features during dyeing. We used crystal violet, acid-alcohol mixture and aquafucsine during gram dyeing because I wanted to be sure whether isolated

microorganism is a genuine bacillus cereus or not. If microorganism is painted with crystal violet, microorganism turns into violet, and thus microorganism is gram positive. If these inspected colonies did not give positive values as a result of crystal violet dyeing, this colony is not *Bacillus cereus*. Distilled water is being used after crystal violet dyeing because if bacteria are gram positive, paint is not dropping of the microorganism during rinsing. This process measures whether microorganism is gram positive or negative. If these measurements are also giving the same results in Lugol and acid-alcohol mixture, gram positive value, and aquafucsine dye did not attach onto the microorganism, this shows that microorganism is possibly Bacillus cereus. 1 drop of immersion oil is being dropped onto this preparation, which is dried as a result of this process, and then it is being inspected under microscope, and final decision of which preparation is Bacillus cereus is taken here. The reason for the preparations examined under the microscope immersion oil pour preparations of micro-organisms are micro-organisms allows us to see more clearly and all the external display properties. We have chosen the very beginning of the experiment at 25°C, 4°C, 37°C because of being micro-organism growth survey conducted at 37°C was found to be most at this temperature. The reason we have chosen other more readily available in the environment or the degree of preparation. Another reason for choosing this temperature Bacillus cereus average growth temperature 28-37 ° C, although urea may be between 10-48°C. Bacillus cereus main surrounding soil, water, milk and milk powder, baby food, the many nutrients, such as fruits and vegetables, it is seen that plenty of urea. These micro-organisms enter pathogenic effect has been seen in many cases but emphasized hard not content with just food poisoning this affects appropriate conditions. It has also been shown that the micro-organism to cause putrefaction of foodstuffs with strong fermentative property. Our dependent variables were the different temperatures are in effect during the test argument to prune the growth of Bacillus cereus. Our controlled variables we kept constant during the test if the falling amount of food each tube, each tube falling peptone water content of the food pH, made by pressure medium, having the same brand of food, the volume of the tubes we put mixtures, heavy materials of which the feed location and feed volume location , agar solid broth made in materials and volume, the essence that we use to spread the mixture over medium, we use in painting crystal violet, iodine, acid-alcohol, we keep constant the amount of aquafucsine mixture. We also waited 24 hours that equal time for reproduction of fattening foods in place. We dropped grams of coloring matter, and we waited on the breeding of micro-organisms in 1 minute with each drop.

METHOD:

1. In order to sterilize the glassware, put them into Pasteur oven and heat to 165°C. Put water with 13.5 ml peptone into a pot of 1000 ml.

2. Measure 1.5-gram baby food, brand “Bebelac” on balance and add all of the 1.5 gram into water with peptone.

3. Cover this formed mixture with parafilm and mix it with using vortex device.

4. Distribute this homogenized solution with a pipette with 2 ml scale so as to put 1 ml per each tube.

5. Put distributed tubes into a spore of 10 tubes per each measurable degree and separate these tubes so as to have 5 groups.

6.In order not to mix tubes during experiment, number each of them.

7.After completion of grouping process, get phenol-red egg-yolk polymixin mediums for each tube and divide each one of them into 6 equal divisions.

8. Pour solutions within each tube to phenol-red egg-yolk polymixin mediums via pipette with a scale of 1 milliliter so as to have 0,2 ml per each part and then spread these poured solutions onto extract, sterilize with heating it via bunsen burner.

9. After spreading, put 5 different groups into their environments so as to have 1℃, 13℃ 25°C, 37°C and 249°C temperatures, and wait for 24 hours.

10. Leave the groups with 25°C,37℃ and 49℃ into incubator, group with 1°C into fridge and group with 25°C at room temperature for 24 hours.

11. After 24 hours, calculate of overall colony number on the agar medium (with considering 10^{-1}watering rate).

12. Choosing the microorganism colonies which are surrounded with precipitates on the purple – red base of the medium.

13. For the purpose of verifying that these colonies are *Bacillus cereus*, take the colony of microorganisms, formed with precipitate ring by reduction, via extract, and replant via reduction method instead of horizontal agar medium so as to have one microorganism.

14. Put solid agar medium plates into incubator for 24 hours at 30°C, and incubate them.

15. After 24 hours, take single colony, which produced at agar medium, and plant it to a separate normal horizontal tube agar medium.

16. Leave these tubes into incubator for another 24 hours at 30°C.

17. After incubation, take any colony, which is single in the horizontal agar plate, and homogenize it with using extract via a drop of serum physiologic water on the lam.

18. Then, pass dried preparation three times from Bunsen burner flame of 50°C to make it sterilize.

19. In order to make gram dyeing, get crystal violet, Lugol, acid-alcohol mixture and aquafucsine.

20. As numbers of colony of microorganisms vary for each irrigation, ml value differs.

21.First, pour a drop of crystal violet mixture from prepared gram dyeing set onto the preparation on the lam and wait for one minute.

22. After one minute, rinse preparation with distilled water.

23. Repeat same dyeing process for Lugol, acid-alcohol mixture and aquafucsine respectively.

24. During gram dyeing dispose the preparations, which do not take the paint, via elimination.

25. Leave the ones with dye for drying.

26. Pour 1 drop of immersion oil onto preparation on the dried lam via dropper.

27. Inspect the preparations, on which immersion oil was dropped, under microscope and determine which ones are *Bacillus cereuses*.

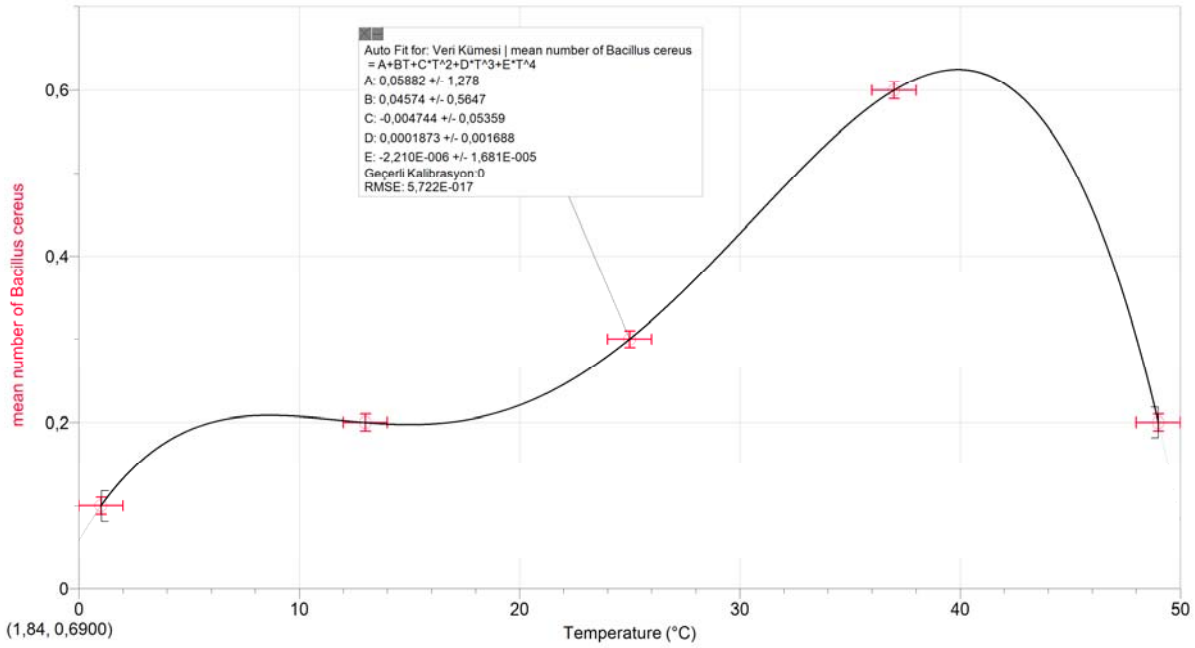
28. After these processes complete for all groups count the number of Bacillus cereus colonies.

Analysis.

Temperature(°C±1)	Trials	Number of bacillus cereus colonies reproduced at a certain time $\sqrt{n}$
49	1	0
	2	0
	3	0
	4	0
	5	1
	6	0
	7	0
	8	0
	9	0
	10	0
37	1	0
	2	1
	3	0
	4	1
	5	1
	6	1
	7	0
	8	1
	9	1
	10	0
25	1	1
	2	0
	3	0
	4	0
	5	0
	6	1
	7	0
	8	1
	9	0
	10	0
13	1	0
	2	0
	3	0
	4	0
	5	0
	6	1
	7	0
	8	1
	9	0
	10	0
1	1	0
	2	0
	3	0
	4	0
	5	1
	6	0
	7	0
	8	0
	9	0
	10	0

Table1. Raw data table of number of *Bacillus cereus* observed with exact same conditions except for temperature rate.

In order to see the relation between temperature and the number of *Bacillus cereus* produced at a certain time, the following graph is plotted.



Graph1: The graph shows the mean of number of *Bacillus cereus* produced at a certain time depending on temperature rates.

Raw Data Table.

Temperature rates	mean of number of *Bacillus cereus* colony reproduced	Standard deviation	Standard error	Confidence interval
1	0.1	0.316	0.100	0,277
13	0.2	0.422	0.133	0,370
25	0.3	0.483	0.152	0,423
37	0.6	0.516	0.163	0,453
49	0.1	0.316	0.100	0,278

Table2. Vivid statics for number of *Bacillus cereus* production for different temperature rates.

Calculations

In order to calculate the mean of number of *Bacillus cereus* colony reproduction for 37℃.

$$\mu = \frac{1}{N}\sum_{i=1}^{N} x_i = \frac{1}{N}(x_1 + \cdots + x_N) \quad (8)$$

$\frac{0+1+0+1+1+1+0+1+1+0)}{10} = 0.6$

In order to calculate the standard deviation of mean of the number of *Bacillus cereus* colony reproduced for 37℃.

$$S = \sqrt{\frac{\Sigma (x-\bar{x})^2}{n}} \quad (9)$$

$\frac{(0.6)2+(0.4)2+(0.6)2+(0.4)2+(0.4)2+(0.4)2+(0.6)2+(0.4)2+(0.4)2(0.6)2}{\sqrt{10}}$=0,516398

In order to calculate the standard error of mean of the number of *Bacillus cereus* colonies reproduced for 37℃.

$$\text{Standard error} = \frac{\sigma_x}{\sqrt{N}} \quad (10)$$

$\frac{0.516}{\sqrt{10}}$= 0,453

Table2: Anova One Table for Reproduction of *Bacillus Cereus* Colony

H_0: There is not a significant difference between mean number and the data occupied through each *Bacillus cereus* colony reproduction in different temperature rates.

H_1: There is a significant difference between number and the data occupied through each *Bacillus cereus* colony reproduction in different temperature rates.

Anova: One Value

Summary

Groups	*Count*	*Sum*	*Mean*	*Variance*
Temperature(°C±1)	5	125	25	360
Number of bacillus cereus produced at a certain time	50	13	0,26	0,196327

ANOVA

Source of variance	*SS*	*df*	*MS*	*F*	*P-value*	*F limit*
Between Groups	2782,125	1	2782,125	101,7181	6,24E-14	4,023017
Within group	1449,62	53	27,35132			
Total	4231,745	54				

Results: H_1 can be considered as true because the p-value I gained is lower than the level α value which of 0.05.

Conclusion and Evaluation:

In this investigation, I observed the effect of the temperature on reproduction of *Bacillus cereus* and I observed the proliferate it. In my experiment I used 5 different temperature rates which are 1℃, 14°C, 25°C, 37°C and 49℃ to see the effects of the temperature rates. As we show in the Table1. The mean of number of *Bacillus cereus* colony reproduced for these groups in order are 0.1, 0.2, 0.3, 0.6, 0.1. These results show that Bacillus cereus colonies are reproducing better at 37℃. The standard deviation of the groups are 0.316, 0.422, 0.483, 0.516 and 0.316. These results show that number of Bacillus cereus colony reproduction for this experiment is reliable because these standard deviations are not a great number. The standard error for these groups are 0.100, 0.133, 0.152, 0.163 and 0.100 and these results are not a great numbers again this experiment is relaible for counting the number of *Bacillus cerues* colony reproduced.

In Table2. P-value of this experiment is 6,24E-14 and it is lower than 0.05 so H_0 rejected and H_1 accepted which is "There is a significant difference between number and the data occupied through each *Bacillus cereus* colony reproduction in different temperature rates." and this make result which is temperature has an effect to the number of Bacillus cereus colony reproduced at a certain time.

There are some variables that I controlled during this experiments which are glassware tube, petri dish, pipette, Erlenmeyer (300 milliliters), lam, Pasteur pipette, incubator (28-35°C), autoclave, Pasteur oven, fridge, microscope, balance, spores, plug, cotton, pH paper, filter paper, pincersi, paraffin and medium.

Phenol-Red Egg-Yolk Polymxin Agar These are the variables that controlled to see the effect of different temperature rates on the number of *Bacillus cereus* reproduced at a certain time.

As shown as a result of my experiment, I observed that temperature has impact on reproduction of *Bacillus cereus* named microorganism and I proved my hypothesis which is the temperature rate increases the number of *Bacillus cereus* colonies will increase.

We did not have a digital balance in hand for weighting the baby food samples, therefore, normal sensitive balance, used in the Laboratory, was employed and a value was weighted.

In order to distribute water with peptone and baby food mixture so as to have 10 cc in each tube, we used pipettes of 1 and 2 ml, and as we did not use automatic pipettes during these measurements, I saw that there is a tolerance for measurement. Besides, the impacts, caused by adhesion and cohesion forces of the mixture during measuring, hindered me to have an accurate measuring but I did not have the chance to prevent from this.

0.1 ml of mixture that spread uniformly over the surface of the mixture of Egg Yolk Polymyxin Agar medium alone because we could not stir this mixture automatically in the vortex of a portion of the food I saw the bat. Besides, I'm used to deploy the wire loop instead of fattening may have no exact sterile operation in contrast to heating every time.

I count the number of colonies of calculating the number of micro-organisms isolated after waiting Egg Yolk Polymix Agar mediums for all the temperature rates for 24 hours because formed in it was not possible to count the micro-organisms so I counted the number of *Bacillus cereus* colonies.

A loop is formed around the colonies. Ones that do not have loops are not picked as a microorganism. Breeding colony of the broth and precipitate ring forming a single colony picked up by the loop but I received is estimated to have more than one colony in the colony was not possible.

Preparations tried to be kept 10 cm from migrating over Bunsen burner, but the top tube burst through the glass while it did not exactly flush rapidly migrating distance of 10 cm. This caused a random error. Furthermore, the upper side and the lower side of the mixture in the tube cannot be heated at the same rate.

While the Gram stain preparation process, as colonies affected by temperature in different ways, it was not possible to use the same volume of alcohol in dying process

I dropped drips of paint on top of the immersion oil I poured every drop of holding drugs that has led to an equal volume of pruning 10x10 *Bacillus cereus* it has been hampered our selection of micro-organisms under the microscope. If we pour the drops were determined less by micro-organisms it cannot be seen clearly.

After my investigation, I believe that I have enough knowledge and data to see the effect of temperature rate on the number of Bacillus Cereus produced at a certain time. Also I believe that I got sufficient data and results to accomplish to the end.

As I allude in my hypothesis, when the temperature increases, the number of *Bacillus cereus* colonies reproduction increases.

It is not possible to isolate baby foods and other dried foodstuffs from *Bacillus cereus* . Because *Bacillus cereuses* are reported in the performed studies that they are mainly enduring to environmental conditions. (It can endure to +75°C temperature, -5°C moisture)[6]. Therefore,

Bacillus cereus spores are being seen even in the foodstuffs, produced under full compatibility to technological rules.

Being *Bacillus cereus* one of the active microorganisms in food poisoning, attention to the presence of *Bacillus cereus* in foodstuffs and number of studies, performed on this microorganism, were increased. Insufficient cooling of especially cooked foodstuffs with protein in humid environments cause this microorganism to reproduce rapidly. Poisoning occurs only microorganism is taken in huge amounts with foodstuff. Incubation at 30°C for 18 hours is enough for reproduction of microorganism at a level of causing poisoning.

It is determined that presence of *Bacillus cereus* in foodstuffs alone has no great importance but in case of presence of reproductive environment, it might cause a risk formation. Reaching to sufficient number for poisoning for this microorganism when reproductive conditions are met depends on the initially available number of microorganisms.

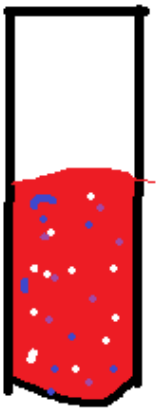
When general characteristics of the *Bacillus cereus* are considered, one can observe that it is present in the baby foods, and in case of not complying with rules during its preparation and storage, various enteritis factor can be potential sources. Because like within various developing countries, in our country, especially in rural areas, baby foods are being prepared once per day in amounts equal to daily consumption of babies. These are mostly stored at inappropriate temperatures and babies are being fed from these baby foods. However, general rule for baby foods is to prepare the amounts, enough for one meal for the baby. Otherwise, an environment, appropriate for reproduction of toxic microorganisms such as bacillus cereus, is established.

If it is continued to cook baby foods at the temperatures, facilitating the reproduction of these microorganisms, major poisonings might be experienced in babies, and it might negatively affect their growing processes. Therefore, in order to prevent formation of *Bacillus cereus*, baby foods and foodstuffs should be consumed daily and they should be consumed without being cooked at certain temperatures.

Appendix1:

Horizontal agar medium: It helps to recognise the microorgnisms easily more than Phenol-Red Egg-Yolk Polymixin Agar.

Horizontal Agar medium



Phenol-Red Egg-Yolk Polymixin Agar

Choosing the *Bacillus cereus* in the Horizontal Agar medium is easier so in my Extended Essay I choose it to spot the *Bacillus Cereus* in the mixture

Vortex: It is a machine that mix the mixture, microorganisms scatter homogenous with this machine it cruns the mixture to doing this invident.

Peptone: With this medium, all the microorganisms in the mixture will reproduce more than without peptone so it helps to see more *Bacillus cereus* in a one medium.

Immersion oil: It helps to see the microorganisms in the microscope with dropping this oil onto the lam.

Phenol-Red Egg-Yolk Polymixin Agar: It is a jel or liquid. It feeds the microorganisms or cells to growing them.

References

1. Hobbs and Gilbert;

   Food poisining and Food Hygiene

2. Bacillus cereus Food poisoning, Public Health Laboratory Service (1973) British Medical Journal III. 647.
3. KNAYSİ, G: Effects of tempuratures on the ratio of germination in B. Cereus, Journal of Bact. 83/;619 (1965).
4. WALZ, E.; Die Bedeutung der aeroben sporenbildner fur die Fleischwirt Sehalft, inbesondere für Dauerwaren Halbkonserven und Rohwürste.
5. INAL, T. (Comparative Studies of selective media for qualitativ and Quantitive dedection of Bacillus cereus in foods.IV), (1972)
6. POLKHOVSKII, V.A.
   Bacterological diagnosis of food poisning caused by *Bacillus cereus* Laboratornoe Delo No 3-171-174 (1975)
7. WALZ, E.; Die Bedeutung der aeroben sporenbildner fur die Fleischwirt Sehalft, inbesondere für Dauerwaren Halbkonserven und Rohwürste.
   Habilitationschrift, Tierarztliche Hochsehule, Hannover (1967)
8. https://tr.wikipedia.org/wiki/Aritmetik_ortalama
9. https://saylordotorg.github.io/text_essentials-of-geographic-information-systems/s10-01-descriptions-and-summaries.html
10. http://qualityamerica.com/LSS-Knowledge-Center/statisticalinference/standard_error_of_mean.php