TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Biology Extended Essay

Investigating the effect of additives in fabric and homemade mayonnaises at 28^{o}C and 4^{o}C in conditions, by bacterial culture method.

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ABSTRACT

The purpose of this study was to determine the total amount of bacteria measurements for fabric and homemade mayonnaises due to the additives at 28^{o}C and 4^{o}C under the conditions. Bacterial culture method is used for analysis. Bacteria are chosen as Lactobacillus plantarum, because of being frequent in mayonnaise's normal microflora. It is cultivated into homemade and fabric mayonnaises by using Plate Count Agar in different dilutions. Results indicate that the rate of bacterial growth in homemade mayonnaise is more than the growth in fabric mayonnaise. When the effect of temperature is observed, it is seen that temperature plays an important role in bacterial growth. The growth of bacteria in homemade mayonnaise at 28^{o}C is more than the fabric mayonnaise's, compared to the results of 4^{o}C. This study's conclusions indicate that fabric mayonnaise is more everlasting to food spoilage because of containing additives as citric acid, EDTA etc., which affects the pH level, than homemade mayonnaise. Further studies must be done in order to minimize the effects of additives and increase the shelf-life in the same time.

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INTRODUCTION

Nowadays we hear news about harms and benefits of food additives frequently. Whether it is harmful or beneficial, additives are commonly used in food industry. Food additives are substances that are added to foods, to help flavour, colour or preserve them. Some additives play essential roles in keeping our food safe, but others may be responsible for unpleasant reactions. As the types of additives, usage of additives is important too. Same additive, prepared with different materials can be used to decrease the rate of food poisoning. For example, to prevent food poisoning, fabric mayonnaise is prepared with pasteurized milk[1]. Pasteurization process is heating the food and then cooling it immediately, which slows the spoilage of bacterial growth in food.[1] On the other hand, high acid content also slows bacterial growth[2]. Because of this news, I take a look at additives in every food I eat, especially every type and brand of mayonnaise. Mayonnaise draws my attention because there are many myths about mayonnaise spoilage. Furthermore, it is delicious, and is used in many recipes. Because of spoiling immediately, mayonnaise has a bad reputation as it causes food spoilage, though it is undeserved. One of those myths says that "mayonnaise is often the cause of food-illness" but actually, mayonnaise doesn't cause food borne, bacteria do. The growth of bacteria is a natural process, if the medium is at standard conditions which allows bacteria to grow, it will grow. Ingredients of mayonnaise especially materials like vinegar or lemon juice slows the growth of bacteria.[2]

As I heard from my grandparents, who know how to make homemade mayonnaise, it is not a big deal to make homemade mayonnaise, because ingredients are easy to find and process is simple. As my granddad said, materials to make homemade mayonnaise are; oil, egg yolk, lemon juice or vinegar with many optional herbs and spices. However, in fabric mayonnaise many additives are added to thicken, to extend the time to spoil or to make it more tasteful. There are many additives as EDTA, flavour enhancers, thickeners, citric acid, sucrose and corn syrup.[3] EDTA is a polyamino carboxylic acid, a water soluble and colorless solid, which is used to protect people from harmful metals that find their way into foods people eat by attaching and removing them[4]. Citric acid is an organic acid which is used to add an acidic taste and decrease the pH rate. Like all acids, EDTA& citric acid also slows the growth of bacteria that's why they are added to mayonnaise.[5] Flavour enhancers give food a taste, for example fennel leaves which have mouth refreshing properties, are usually used in mayonnaise. Sucrose and corn syrup are also used in mayonnaise as flavour enhancers. Sucrose is best known for its nutritional role. Corn syrup is made of the starch. It is also used in foods to soften texture, add volume besides enhance flavours.[6] On the other hand, high-fructose corn syrup has some harm which causes obesity, metabolic syndrome and type-2 diabetes.[7] These are long termed, and common diseases that people should be aware nowadays. This shows that, additives have harms as much as they have benefits and shouldn't be used frequently. Thickeners improve the emulsion/suspension of ingredients that increases stability of the product.[8] Generally all additives have the same aim, to extend the time of spoilage that's why most of them are acidic. However in homemade mayonnaise only lemon juice or vinegar is added to increase acidic level.

So I wondered the effects of other additives in mayonnaise and decided to make a comparison between homemade mayonnaise and fabric mayonnaise. I will compare the effects of additives in standard temperature conditions (which refers to 28^{o}C), and also in 4^{o}C to observe

whether it is important to store mayonnaise in a refrigerator or not, by observing the growth of bacteria that has been planted. There are many types of bacteria which can be harmful or beneficial. The most effective bacteria that lie behind food poisoning are salmonellae which are caused lack of cooking and preparing the food healthy. As it is written in AMERICAN DIETETIC ASSOCIATION web site, "Mayonnaise is not the culprit in food borne illness. The culprits are foods that are not prepared, served or stored properly." Other types of bacteria are natural and appear in much food spoilage, especially in mayonnaise, like yeast and bacteria. In this experiment it is expected that there will be bacterial growth in each type of mayonnaise (homemade and fabric). So my research question is "Do the additives in mayonnaise affect the amount of growth of bacteria, L. plantarum, that will be compared between homemade mayonnaise and fabric mayonnaise samples at 28^{o}C and 4^{o}C under the conditions, by using bacterial culture method?"

References:

1- http://www.foodsafetysite.com/educators/competencies/general/foodprocessing/processing2.html
2- http://www.dressings-sauces.org/Mayonnaise_Dressings.html
3- www.britannica.com/EBchecked/topic/212615/food-additive
4- http://www.naturalanswer.com/edta.htm
5- http://www.foodreference.about.com/od/Food-Additives/a/What-Is-Citric-Acid.htm
6- http://en.m.wikipedia.org/wiki/High-fructose_corn_syrup
7- KL Stanhope, PJ Havel - The American journal of clinical nutrition, 2008 - Am Soc Nutrition
8- www.recipetips.com/glossaryterm/t--36479/thickener.asp

HYPOTHESIS

There is evidence that additives in mayonnaise have an important role in detaining time of food spoilage in standard temperature conditions. Evidence suggests that, decrease of additives will increase the rate of bacterial growth.[9] The most important additives which effect bacterial growth are citric acid, vinegar and lemon, because of regulating the acidic level. As a scientist in Department of Food Science in America, John E. Rushing wrote that, properly acidifying to pH 4.6 or below will inhibit the growth and formation of toxins from the bacteria.[10] Other additives like EDTA and thickeners also affect food spoilage, however it is directly related with mayonnaise's acidity. That is because all organisms and bacteria need different conditions as high or low temperature, moist or dry medium, high or low acidic level to grow.[11] Bacterial growth can happen in two ways, one of them can be observed with bacterial culture, by taking results from lab, and the other way of observing bacterial growth is area of growth zone on mayonnaise by measuring its size.

It can therefore be hypothesized that, absence of additives in homemade mayonnaise, especially citric acid &EDTA, increases the rate of bacterial growth in standard temperature conditions. It is expected that in homemade mayonnaise more bacteria will be observed because of lacking acidity when it is compared to fabric mayonnaise.

References:

9- www.dressing-sauces.org/foodsafety_picnic.html Gibson, Traci, The Association for Dressing&Sauces

10- http://www.ces.ncsu.edu/depts/foodsci/ext/pubs/formulatingdresings.PDF

METHOD DEVELOPMENT AND PLANNING

Planning is one of the most important parts to think carefully because it is the main part to determine whether the hypothesis is true or not. Bacteria may grow in standard conditions however it takes long time and it is not the best idea for an investigation. There are two ways of measuring bacterial growth, the simpler way is measuring the growth zone with a sensitive ruler without touching the ruler to mayonnaise. The other way of measuring the bacterial growth and consistency, is taking bacterial culture in a lab. Culturing is a method which studies bacteria by growing them on medium containing nutrients.[11] In addition, second way is chosen because taking bacterial culture gives more information than naked eye, and makes results more precise. There are many bacteria everywhere, even in the medium that we breathe. So it would be wrong if mayonnaises were stored at the kitchen in open cups, because some amount of bacteria may settle. Therefore, the experiment will take place in a sterilized lab, in closed sterile Petri dishes. As it is mentioned before, it takes so long to bacteria to grow itself and it is uncertain, so bacteria will be planted to both homemade and fabric mayonnaises for more certain results.

The most essential spot to be careful in this experiment is the ingredients of fabric mayonnaise and homemade mayonnaise. Homemade mayonnaise's ingredients are; yellow part of egg (yolk), a pinch of salt, minimum amount of water (approx. 10 ml), sunflower oil (0.5 L), and a full lemon. Firstly, pouring the salt onto three egg yolks, adding 10 ml water to prevent solidification, and then pouring 0.5L sunflower oil and mixing them together in the same direction in order to obtain more consistent mayonnaise, and lastly adding lemon generates mayonnaise. It is the way of obtaining 550grams of mayonnaise. The difference between homemade mayonnaise and fabric mayonnaise is additives and the most important ones are citric acid, sucrose, EDTA and corn syrup in fabric mayonnaise, salt and lemon juice or vinegar in homemade mayonnaise because of regulating acidity, which effects the growth of bacteria. In homemade mayonnaise lemon juice or vinegar are used instead of using EDTA, sucrose or citric acid. Their roles are similar however vinegar salt and lemon juices are more natural. During the experiment, the same brand of fabric mayonnaise will be used in order to keep the amount of additives in equal standing. Besides additives, other thing to be careful is to keep the amount of both mayonnaises same. I decided to use 10 grams for each of them; it is the appropriate amount to observe differences. Actually the amount doesn't matter while it is used as an independent variable and the difference doesn't affect the rate or amount of spoilage unless there is a difference between amounts of fabric and homemade mayonnaise.

As I researched, the experiment should be done in a laboratory, therefore to find necessary cultivation materials and a sterilized laboratory I asked help from an academician of Ankara University, Turkey. With the help of her, I get access for autoclave, CASO Broth (which is the general medium for activating cultivated bacteria), Petri dishes and the other tools. During the experiment in Ankara University, Department of Food Engineering, I was supervised by the academician and my steps are followed in order to prevent making mistakes.

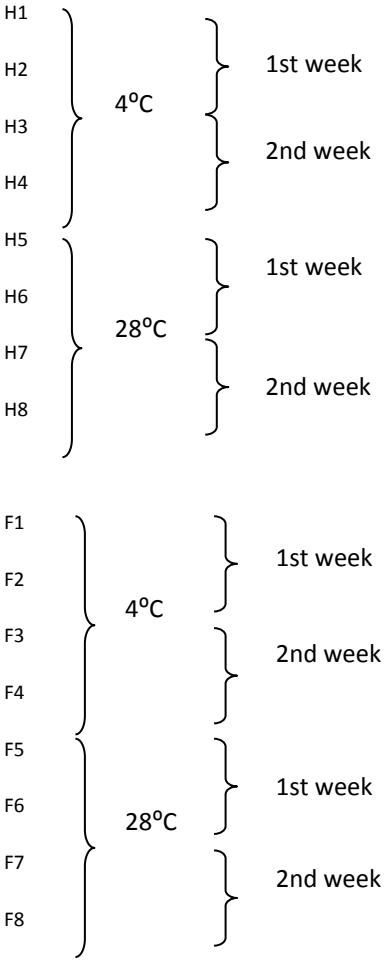
The experiment will last two weeks, in order to observe the process of reproduction of bacteria more clear. Two rounds will be made during the experiment and half of homemade and fabric mayonnaises will be observed for one week and the other half will be observed for two weeks. First of all, a zeroth day observation will be done because without knowing the normal range of the growth, the results at the end won't mean anything. 90ml of NaCl(aqueous)(an isotonic liquid that is

used in dilution process) and 10 g mayonnaise are mixed in order to prepare the material of zeroth day. This day is considered as the origin of the experiment.

Lactobacillus plantarum is used as the bacteria that will be cultivated because in mayonnaise's normal microflora, L.plantarum is the most concurred bacteria.

I expect that there will be much more bacterial growth in homemade mayonnaise than fabric mayonnaise.

Here is a visual to simplify the order of homemade and fabric mayonnaises where H is homemade and F is fabric. Two parallels are made in order to minimize probable error.



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11- http://www.disknet.com/indiana_biolab/b062.htm Harold Eddleman, Ph. D., President, Indiana Biolab, 14045 Huff St., Palmyra IN 47164

MATERIALS & APPARATUS

To obtain homemade mayonnaise, there is a description and some materials.

Salt

3 eggs (yolks)

0.5L Sunflower oil

10 ml Drinking water

Measuring cup (250 ml)

Mixer

A lemon

Squeezer

Knife

Graduated cylinder (100ml)

A bottle of fabric mayonnaise

Test tube x83

Plate Count Agar (PCA)

Physiologic Salt Solution (PSS)

Pure water (1600ml)

CASO Broth

Lactobacillus plantarum (bacteria)

Autoclave

Micro-syringe (1ml)

Micro-syringe (0.1ml)

Glass Petri dish x165

Gloves

Incubator (at $4^{o}C$)

Incubator (at $28^{o}C$)

Magnetic stirrer & a magnetic stir bar

Electronic stirrer

Flame source

A match

Electronic weigher

Metal spoon x2

Glass spatula x2

%76 concentrated ethyl alcohol solution (300 ml)

Acetate pen

Pure water (700 ml)

Beaker (100 ml)

Glass bottle (500 ml)

Sterile plastic test cups x17

METHOD

Part 1: Preparation of homemade mayonnaise

1. Crack 3 eggs and pour the yolks(yellow part of egg) in an empty 2L bowl
2. Add a pinch of salt onto the yolks and mix with a mixer until the salt disappears
3. Measure 10 ml of water with a graduated cylinder and add it to the previous mixture
4. Add 0.5 Sunflower oil to the mixture
5. Blend the mixture with a mixer always in the same direction, unless mayonnaise would be less consistent than expected
6. Cut the full lemon to half with a knife
7. Squeeze two half of lemon by a squeezer in order to obtain lemon juice
8. Pour the lemon juice to the mixture
9. Mix it for 15 minutes by a mixer, approximately 550 grams of mayonnaise will be ready.

Part 2: Activation of bacteria

1. Take 0.1ml of inactive Lactobacillus plantarum from stocks
2. Put 5 ml CASO Broth in a test tube
3. Put 0.1 ml of L. plantarum into 5ml CASO Broth
4. Put the test tube into incubator (at 28^{o}C) and wait 24 hours for the activation of bacteria

Figure 1: Active (cloudy) and inactive(clear) bacteria

Part 3: Preparation of broth & Petri dishes

1. Broths are chosen by the bacteria (L. plantarum) that will be analyzed
2. Put all glass materials that will be used in the experiment in sterilizer and set the temperature as 170^{o}C and wait for 2 hours
3. After 2 hours, take the glass materials out with a glove
4. Look for the amount of Plate Count Agar (PCA) to add into pure water which is written on the bottle of PCA. (In 1L, 22.5g)
5. Calculate the amount of PCA to add into pure water (500 ml will be needed, 1L -> 22.55 g, 500ml -> 11.25 g)
6. Measure 11.25 g of PCA with a weigher by pouring the PCA into a spoon
7. Add 11.25 grams of PCA into 500 ml of pure water in a 500 ml glass bottle
8. Use magnetic stirrer to stir the mixture

9. Place the glass bottle onto the magnetic stirrer
10. Sterilize the magnetic bar by submerging it in a 76% concentrated ethyl alcohol solution, and then putting it onto the flame until it dries
11. Put the magnetic bar into the beaker
12. Turn the stirrer on
13. Wait for 5 minutes
14. Put the glass bottle full of 500 ml broth into autoclave for 15 minutes at 121^{o}C
15. Burn the flame with a match
16. Take the autoclaved glass bottle full of broth and pour 12.5 ml of broth to each Petri dish
17. Try to be near the flame as much as possible, for maximum protection from the bacteria in medium

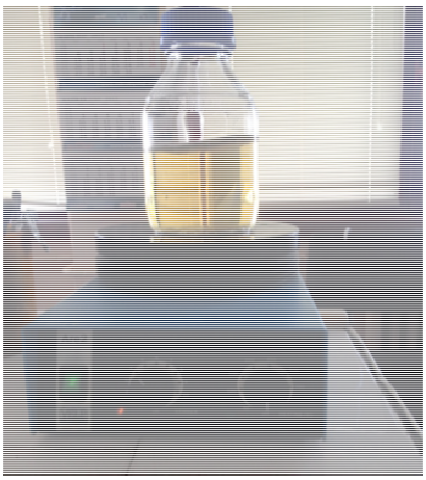


Figure 2: Illustration for the third part's 11st and 12nd steps

Figure 3: Glass materials, glass bottles with broth ready for autoclave process

Figure 4: Autoclave is set at 121^{o}C (step 14)

Figure 5: Broths are poured to Petri dishes, near to flame (step 16)

Part 4: Preparation of mayonnaises

This part will be done in two sections, because bacteria will be cultivated after waiting one or two weeks. Cultivation will be done on H1, H2, H5, H6, F1, F2, F5, F6 after one week and on H3, H4, H7, H8, F3, F4, F7, F8 after two weeks.

1. Take 1ml of activated L. plantarum and put it into homemade and fabric mayonnaise
2. Take two different metal spoons
3. Submerge the spoons into the 76% concentrated ethyl alcohol solution, and then put it onto the flame until it dries
4. Stir both mayonnaises with two different spoons to disperse the bacteria in mayonnaise
5. Separate both fabric and homemade mayonnaises into 10 grams with a weigher, and mark them as it is planned, as H1, H2, F1, F2 etc.
6. Dilute mayonnaises by taking 10g of mayonnaise with a metal sterilized spoon, and putting into 90 ml of Physiologic Salt Solution (PSS) in 100 ml beaker and note it is the 10^{-1}th dilution
7. Take 1 ml of 10^{-1}th diluted mayonnaise with a 1ml micro-syringe and put it into 9 ml of PSS in a test tube. Note that it is the 10^{-2}th dilution
8. Continue this dilution method with the 1ml micro-syringe for 10^{-3}, 10^{-4} and 10^{-5}th dilutions in test tubes for both homemade and fabric mayonnaises.
9. Take 0.1 ml of diluted mayonnaises from test tubes with a 0.1 ml micro-syringe and inject it into Petri dishes
10. Submerge the spatula into the 76% concentrated ethyl alcohol solution, and then put it onto the flame until it dries
11. Spread the mayonnaise in the beaker with the glass spatula without quelling too much
12. Place Petri dishes into 28^{o}C incubator and wait for 24 hours

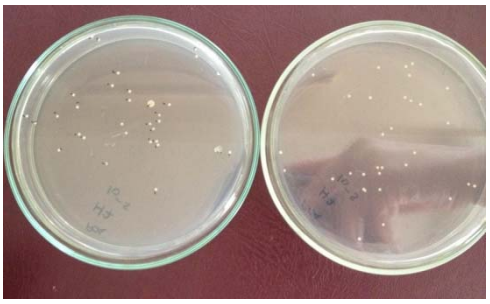
Figure 6: Visuals of diluted mayonnaises, for 10^{-1}, 10^{-2}, 10^{-3} dilutions

Part 5: Counting

This part will be done in two sections, for the first and the second week

1. Take an acetate pen
2. Count colonies that seem like white dots by marking them on the plate without opening the Petri dishes.
3. Note the results by making a table which shows all dilutions separately

Figure 7: Marked Petri dishes, with two different dilutions



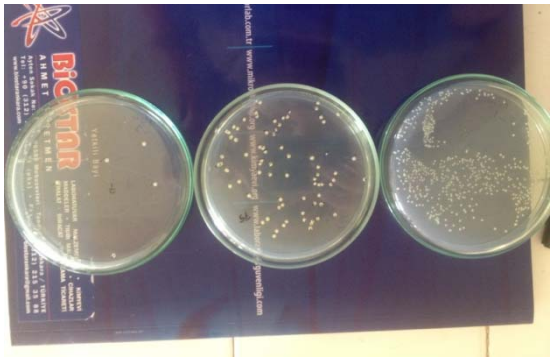


Figure 8: 10^{-5}, 10^{-4}, 10^{-3} dilutions

Figure 9: A visual of "unable to count" in tables, for homemade mayonnaise in 10^{-1} dilution

DATA COLLECTION AND PROCESSING

Results of the 0th day:

	10^{-1}	10^{-2}		10^{-3}		Total (kob/g)
TAMB(PCA)*	Unable to count	144	119	24	23	$1.41x10^5$

***Total amount of bacteria

Table 1: Results of the controlled mayonnaise sample (H9) in zeroth day in order to comprehend the growth of bacteria

These results are found with the formula;

$$\boldsymbol{N = \frac{c}{d * V(n1 + n2 * 0.1)}}$$

Where;

N is the total mesophyllic bacteria

C is the census data

d is the concentrated Petri dish's dilution rate

V is the transferred volume to the Petri dish

n_1 is the number of concentrated Petri dish

n_2 is the number of diluted Petri dish

As an example, to calculate PCA of the 0th day;

$$N = \frac{144 + 119 + 24 + 23}{10^{-2} * 0.1(2 + 2 * 0.1)} = 1.41 * 10\text{^}5$$

Results of the 1st week:

	Temperature /°C (±0.5°C)		Rate of dilution					
			10^{-5}	**10^{-4}**	**10^{-3}**	**10^{-2}**	**10^{-1}**	**Total(kob/g)**
TAMB	**4°C**	**H1**	6	87	992	Unable to count	Unable to count	$1.05*10^7$
			8	109	1112	Unable to count	Unable to count	
		H2	2	30	2888	Unable to count	Unable to count	$2.23*10^7$
			11	161	1804	Unable to count	Unable to count	
	28°C	**H5**	5	86	979	Unable to count	Unable to count	$1.17*10^7$
			9	112	1384	Unable to count	Unable to count	
		H6	3	63	844	Unable to count	Unable to count	$1.23*10^7$
			9	123	1664	Unable to count	Unable to count	
TAMB	**4°C**	**F1**	1	2	9	70	Unable to count	$6.4*10^4$
			1	1	7	50	Unable to count	
		F2	1	1	10	54	Unable to count	$6.5*10^4$
			0	1	12	64	Unable to count	
	28°C	**F5**	1	2	25	115	Unable to count	$1.3*10^5$
			0	2	14	129	Unable to count	
		F6	1	1	18	147	Unable to count	$1.6*10^5$
			1	3	28	155	Unable to count	

Table 2: TAMB (total amount of bacteria) results for H1, H2, F1, F2 in 4°C and H5,H6,F5,F6 in 28°C in the dilutions of 10^{-1} to 10^{-5} in first week.

Results of the 2nd week:

	Temperature/°C (±0.5°C)		Rate of dilution					
			10^{-5}	10^{-4}	10^{-3}	10^{-2}	10^{-1}	Total(kob/g)
TAMB	4°C	H3	29	270	Unable to count	Unable to count	Unable to count	$2.61*10^7$
			29	247	Unable to count	Unable to count	Unable to count	
		H4	23	168	Unable to count	Unable to count	Unable to count	$1.75*10^7$
			27	166	Unable to count	Unable to count	Unable to count	
	28°C	H7	32	223	Unable to count	Unable to count	Unable to count	$2.60*10^7$
			32	284	Unable to count	Unable to count	Unable to count	
		H8	37	319	Unable to count	Unable to count	Unable to count	$3.37*10^7$
			53	333	Unable to count	Unable to count	Unable to count	
TAMB	4°C	F3	1	2	28	176	Unable to count	$2.32*10^5$
			2	3	41	257	Unable to count	
		F4	0	3	26	151	Unable to count	$1.51*10^5$
			1	4	47	100	Unable to count	
	28°C	F7	1	4	39	240	Unable to count	$1.98*10^5$
			0	2	33	117	Unable to count	
		F8	1	2	23	126	Unable to count	$1.38*10^5$
			1	1	15	135	Unable to count	

Table 3: TAMB (total amount of bacteria) results for H3, H4, F3, F4 in 4°C and H7, H8, F7, F8 in 28°C in the dilutions of 10^{-1} to 10^{-5} in second week.

In order to calculate the average of H1 and H2, average values of H1 and H2 are taken from 10^{-1} to 10^{-5} separately. Because of having "unable to count" values in the rate of 10^{-1} and 10^{-2} dilution in H1 and H2, the graph doesn't show these values.

For instance, 10^{-5} values of H1 are 6 and 8, 10^{-5} values of H2 are 11and 2. Average of those 4 will be taken. H1 and H2 values can be taken together because of being at the same temperature and same rate of dilution.

$$\frac{6+8+11+2}{4} = 6.75$$

For	H1&H2			H3&H4		H5&H6			H7&H8	
Dilution of mayonnaise	**10^{-5}**	**10^{-4}**	**10^{-3}**	**10^{-5}**	**10^{-4}**	**10^{-5}**	**10^{-4}**	**10^{-3}**	**10^{-5}**	**10^{-4}**
Mean	6.75	96.75	1699	27.00	212.75	6.50	96.00	1217.75	38.50	289.75
Median	7.00	98.00	1458.00	28.00	207.50	7.00	99.00	1181.50	34.50	301.50
Range	9.00	131.00	1896.00	6.00	104.00	6.00	60.00	820.00	21.00	110.00
Variance	14.25	2942.92	756388.00	8.00	2879.58	9.00	724.67	141156.30	99.00	2404.92
SD	3.77	54.25	869.70	2.83	53.66	3.00	26.92	375.71	9.95	49.04
SE	1.89	27.12	434.85	1.41	26.83	1.50	13.46	187.85	4.97	24.52
t-value	3.18	3.18	3.18	3.18	3.18	3.18	3.18	3.18	3.18	3.18
%95CI(Excel)	6.01	86.32	1383.90	4.50	85.39	4.77	42.84	597.83	15.83	78.03

Table 4: Mean, median, range, variance, standard deviation, standard error, t-value and %95 Confidence Interval in Excel, according to rate of dilutions with respect to homemade mayonnaises are given.

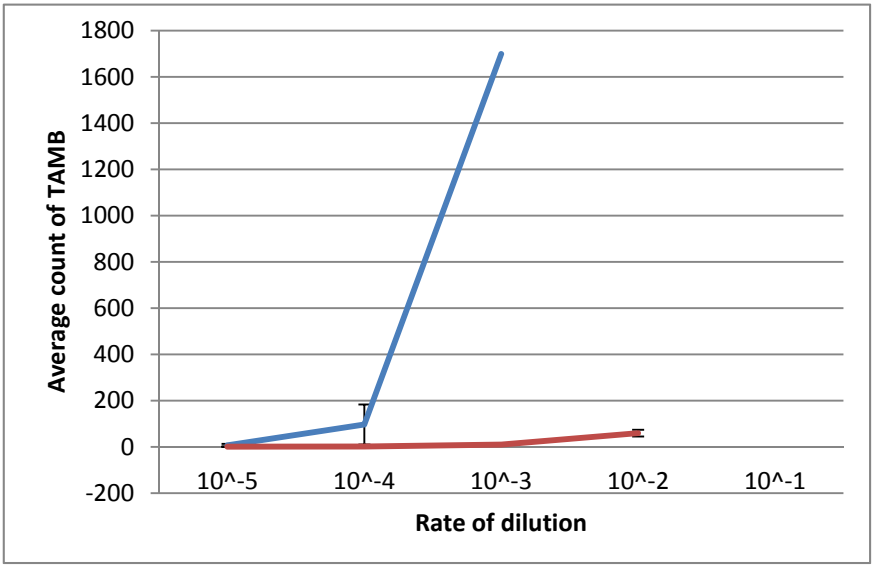
For	F1&F2			F3&F4			F5&F6			F7&F8		
Dilution of mayonnaise	**10^{-5}**	**10^{-4}**	**10^{-3}**	**10^{-5}**	**10^{-4}**	**10^{-3}**	**10^{-5}**	**10^{-4}**	**10^{-3}**	**10^{-5}**	**10^{-4}**	**10^{-3}**	**10^{-2}**
Mean	0.75	1.25	9.50	1.00	3.00	35.50	0.75	2.00	21.25	0.75	2.00	21.25	136.50
Median	1.00	1.00	9.50	1.00	3.00	34.50	1.00	2.00	21.50	1.00	2.00	21.50	138.00
Range	1.00	1.00	5.00	2.00	2.00	21.00	1.00	2.00	14.00	1.00	2.00	14.00	40.00
Variance	0.25	0.25	4.33	0.67	0.67	103.00	0.25	0.67	40.92	0.25	0.67	40.92	323.67
SD	0.50	0.50	2.01	0.81	0.81	10.15	0.50	0.82	6.40	0.50	0.82	6.40	18.00
SE	0.25	0.25	1.04	0.41	0.41	5.07	0.25	0.41	3.20	0.25	0.41	3.20	9.00
t-value	3.18	3.18	3.18	3.18	3.18	3.18	3.18	3.18	3.18	3.18	3.18	3.18	3.18
%95CI(Excel)	0.79	0.79	3.31	1.30	1.30	16.15	0.80	1.30	10.12	0.80	1.30	10.18	28.63
				4287.33									
				65.48									
				32.74									
				3.18									
				104.19									

Table 5: Mean, median, range, variance, standard deviation, standard error, t-value and %95 Confidence Interval in Excel, according to rate of dilutions with respect to fabric mayonnaises are given.

GRAPHS

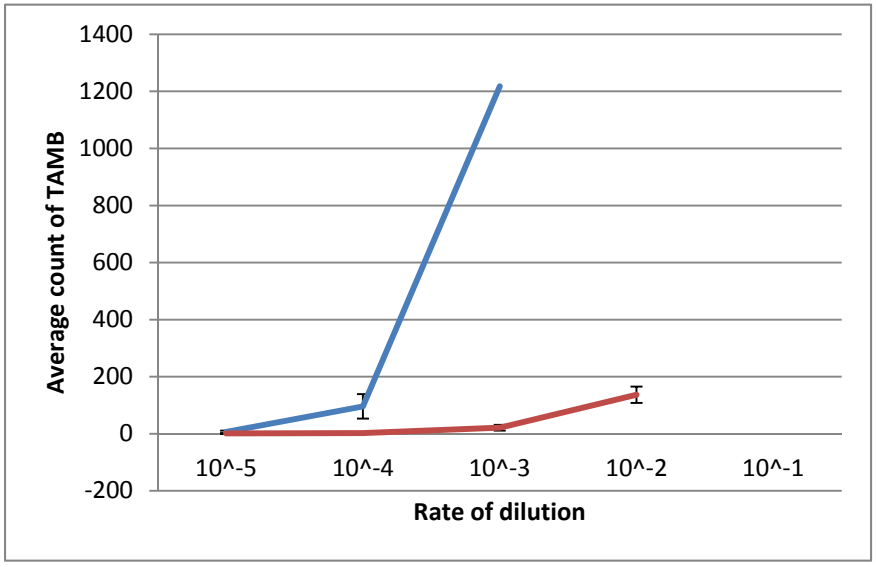
Line graphs of 1st week:

- **Results at 4°C**



Graph 1: Average counts of TAMB for homemade (H1 and H2)(blue) and fabric(F1 and F2)(red) mayonnaises at 4°C according to rate of dilution in first week

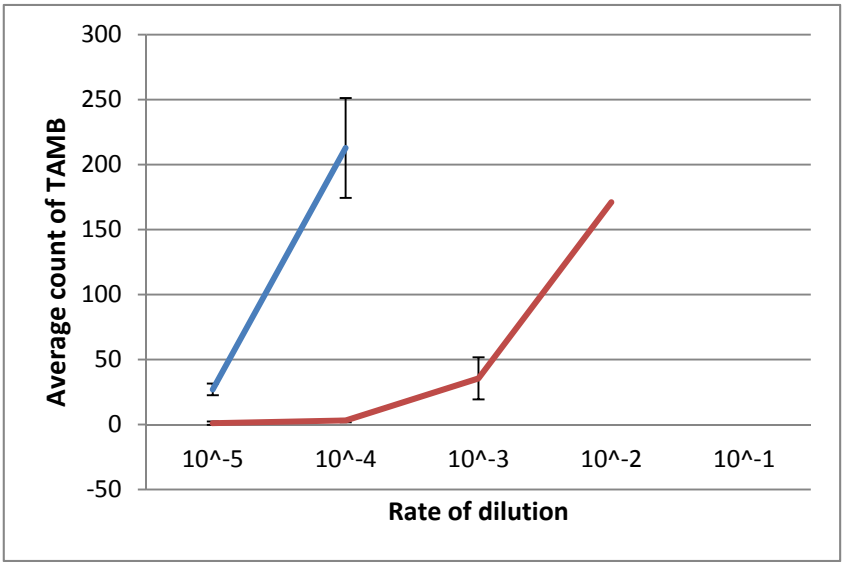
- **Results at 28°C**



Graph 2: Average counts of TAMB for homemade (H5 and H6)(blue) and fabric(F5 and F6)(red) mayonnaises at 28°C according to rate of dilution in first week

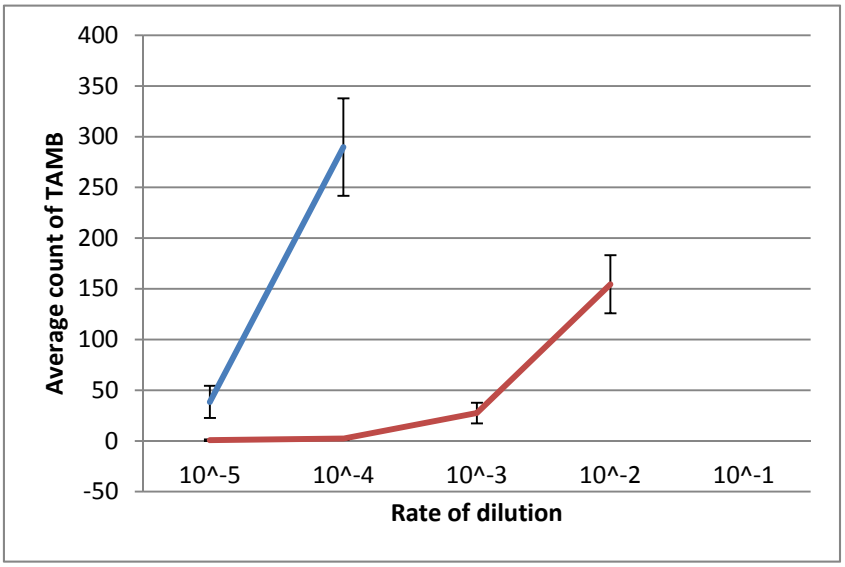
Line graphs of 2nd week:

- **Results at 4°C**



Graph 3: Average counts of TAMB for homemade (H3 and H4)(blue) and fabric(F3 and F4)(red) mayonnaises at 4°C according to rate of dilution in second week

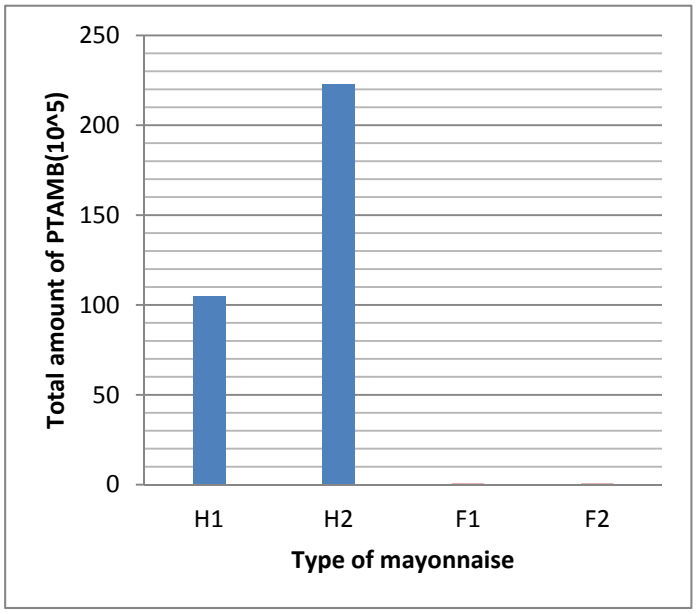
- **Results at 28°C**



Graph 4: Average counts of TAMB for homemade (H7 and H8)(blue) and fabric(F7 and F8)(red) mayonnaises at 28°C according to rate of dilution in second week

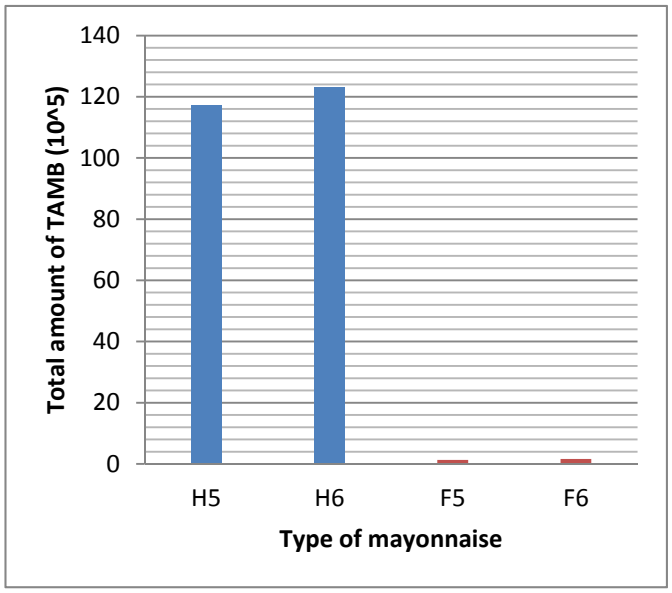
Bar graphs of 1st week:

- **Results at 4°C**



Graph 5: Total counts of TAMB for homemade (H1 and H2)(blue) and fabric(F1 and F2)(red) mayonnaises at 4°C according to rate of dilution in first week

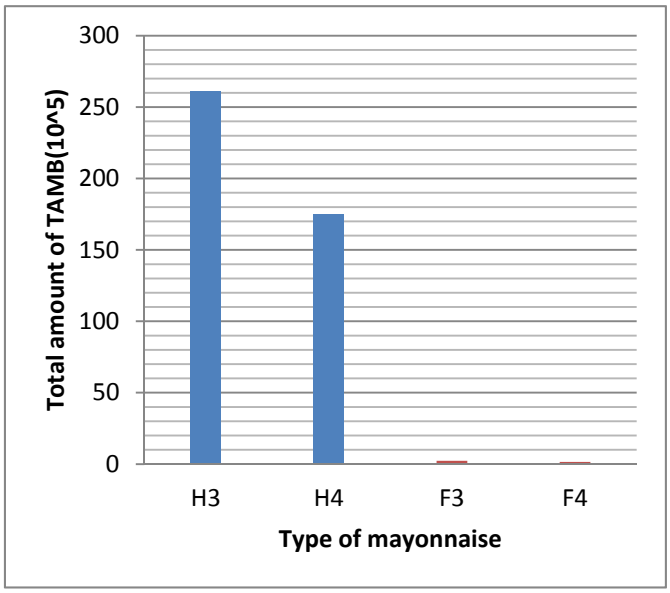
- **Results at 28°C**



Graph 6: Total counts of TAMB for homemade (H5 and H6)(blue) and fabric(F5 and F6)(red) mayonnaises at 28°C according to rate of dilution in first week

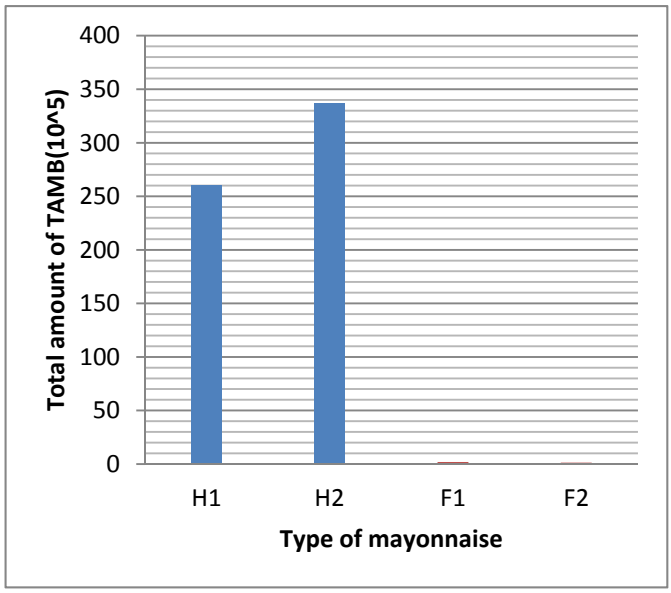
Bar graphs of 2nd week:

- **Results at 4°C**



Graph 7: Total counts of TAMB for homemade (H3 and H4)(blue) and fabric(F3and F4)(red) mayonnaises at 4°C according to rate of dilution in second week

- **Results at 28°C**



Graph 8: Total counts of TAMB for homemade (H7 and H8)(blue) and fabric(F7 and F8)(red) mayonnaises at 28°C according to rate of dilution in second week

CONCLUSION

The purpose of this study was to investigate the effect of additives by observing the difference of bacterial growth of fabric and homemade mayonnaise at 4°C and 28°C with bacterial culture method. I made mayonnaise at home, and bought another mayonnaise from a market in order to compare two mayonnaises. L.plantarum is chosen because of having a wide area in mayonnaise's microflora. Cultivation of bacteria is made in plenty dilutions in order to take coherent results. In homemade there was more bacterial growth so dilutions are made up to 10^{-5} for both mayonnaises, in order to count colonies easily. 2 weeks were required for this experiment, H1, H2, F1, F2 (at 4°C), H5, H6, F5, F6 (at 28°C) are held in different incubators for one week, cultivation is made and counting was taken. H3, H4, F3, F4 (at 4°C), H7, H8, F7, F8 (at 28°C) are held in different incubators for two weeks, cultivation is made and counting was taken. The results are calculated and seen that, homemade mayonnaise have more bacterial growth than fabric mayonnaise, and temperature difference (4°C and 28°C) doesn't make serious changes on fabric mayonnaise. Results of 10^{-5} dilutions will be compared because of having less error and most coherence. For instance, average TAMB on dilution 10^{-5} of F1&F2 at 4°C is 0.75kob/g, and average TAMB on dilution 10^{-5} of F5&F6 at 28°C is 1.00kob/g in first week. Furthermore, there are differences that can't be ignored in homemade mayonnaise between 4°C and 28°C. Bacterial growth of H1&H2 on first week at 4°C is 6.75kob/g, where H3&H4 on first week at 28°C is 27.00kob/g. This is caused because of not having any additives, except lemons' citric acid in homemade mayonnaise. pH level inhibits the growth of bacteria and fabric mayonnaise have EDTA, citric acid, sucrose and corn syrup which declines the pH level so temperature didn't affect the bacterial growth in fabric mayonnaise. Therefore, it can be said that, there is nothing wrong storing fabric mayonnaise in normal shelves, without cooler agent. When F1&F2 and H1&H2 are compared which belong to the first week and 4°C, as it is seen on Table 4 and Table 5, average TAMB of H1&H2 is 6.75kob/g and average TAMB of F1&F2 is 0.75kob/g in 10^{-5} dilution.

Homemade mayonnaise	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**
Total TAMB(kob/g)	$1.05*10^7$	$2.23*10^7$	$2.61*10^7$	$1.75*10^7$	$1.17*10^7$	$1.23*10^7$	$2.60*10^7$	$3.37*10^7$
Fabric mayonnaise	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
Total TAMB(kob/g)	$6.40*10^4$	$6.50*10^4$	$2.32*10^5$	$1.51*10^5$	$1.30*10^5$	$1.60*10^5$	$1.98*10^5$	$1.38*10^5$

Table 6: Total amount of bacteria (TAMB) kob/g for both homemade and fabric mayonnaise

Result of TAMB is given above, by the Table 6. There are more differences from first to the second week in fabric mayonnaise, first week's results for fabric mayonnaise were $6.40*10^4$, $6.50*10^4$, $1.30*10^5$, $1.60*10^5$ and second week's are $2.32*10^5$, $1.51*10^5$, $1.98*10^5$, $1.38*10^5$. There is an increase approximately by 10^1, therefore expiration date is an important factor to avoid food borne. The results of homemade mayonnaise is similar on both first and second weeks, the reason of this similarity may be caused by environmental resistance. In homemade mayonnaise, there is a major bacterial growth. Graphs on this study show the average counts of TAMB and total counts of TAMB. Line graphs show the average count of TAMB, which showed an exponential incline, except the results of H3&H4 and H7&H8's graphs, Graph 3 and Graph 4 because of taking results only in two

dilutions (10^{-4} and 10^{-5}). These graphs show exponential incline, because of being bacteria species, and growing rapidly. There is a major difference between homemade and fabric mayonnaise in average counts. It is seen that bacterial growth occurred rapidly and more in homemade mayonnaise than fabric mayonnaise. Bar graphs show the total amount of bacteria, and they are compared as the first week's homemade and fabric results at 4°C, first week's homemade and fabric results at 28°C etc. Results are compared as 10^{-5}, because of being a common number for both mayonnaises. As in line graph, there is also a major difference between fabric and homemade mayonnaise, which shows that there is an essential role of additives inhibiting growth of bacteria.

A study, named "Survival and growth of E.coli in ground, roasted beef, as affected by pH, acidulants and temperature" by, U.M. Abdul-Rouf, L.R. Beuchat and M.S. Ammar, shows that significant increases in populations occurred in salads containing 16 to 32% mayonnaise (pH 5.94 to 5.55) between 10 and 24 hours of incubation.[12] Death was more rapid as the pH of acidified beef slurries incubated at 5 degrees C was decreased from 5.98 to 4.70.[12] . The order of effectiveness of acidulants in inhibiting growth was acetic acid > lactic acid > or = citric acid.[12] From this study, it can be commented that as pH level decreases, inhibition of bacterial growth increases. Three types of acids are used in fabric mayonnaise, but only citric acid (in lemon) is used in homemade mayonnaise. This explains why TAMB of fabric mayonnaise is less than homemade mayonnaise.

In conclusion, with all these data, it can be said that my hypothesis is true. Additives are affecting the growth of bacteria, because of decreasing pH level of mayonnaise, generating an inappropriate medium for bacteria and increasing the shelf-life. On the other hand, these additives are harmful for human health if they are consumed in a high rate.

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U M Abdul-Raouf, L R Beuchat and M S Ammar, Department of Food Science and Technology, University of Georgia, Griffin 30223-1797.

13- http://aem.asm.org/content/59/8/2364.short
T.F. Brocklehurst, Mary L. Parker, P.A. Gunning, Heather P. Coleman and Margaret M. Robins, 11 MAR 2008

EVALUATION

At the beginning of this experiment, I decided to measure the diameter of the growth zone in mayonnaise, after keeping mayonnaise for one week, nothing but becoming yellower and greasy. I learned that mould zone occurs only when a tin is used, and only at the edges of the thin, therefore I tried to find a reliable method. The second method was to keep 2 samples of both mayonnaises in open cups in the kitchen for two weeks, then brought them to a lab, to take a bacteria counting with bacterial culture. Result of these cultivations failed because of having deficient number of colonies. Therefore, experiment has modified, and bacteria are cultivated at the beginning. With this method, growth of bacteria is observed week by week, for two weeks. It was hard to make a viscous mayonnaise, and the tip is to mix the mixture always in the same direction, because as I learned from my dad, lipid molecules enclose other lipid molecules, and a consistent mayonnaise is produced, otherwise, molecules can't enclose each other. Because of this problem, I made 3 mayonnaises, then found the right thickness. Mayonnaise is a hard food to work with, because E.coli, which is a harmful bacteria could grow. All precautions were made by working in a sterilized lab, and cultivating only one type of bacteria, which is found in mayonnaise commonly, and harmless.

Some improvements about this study can be done. pH plays a fundamental role in bacterial growth, so pH should be measured for both mayonnaises, with this way, more discussions may be made. This study investigated the growth of bacteria only in two different temperatures, more temperature values may be used in order to observe behaviors of bacteria more clear, for instance, growth at $0^{o}C$, $-10^{o}C$, $50^{o}C$ etc. should be observed. The effect of additives was investigated in this study, ingredients should be taken separately, and different effects of all additives should be investigated. I took same amounts of mayonnaise, and cultivated same amount of bacteria, however because of studying with a fabric mayonnaise, I couldn't fix the amount of additives. For instance, citric acid, which is found in both types of mayonnaise, should be taken in same amount and a comparison should be made. EDTA, corn syrup etc. should be investigated separately, by using them in different amounts again by cultivating L.plantarum. In the light of this experiment, improvements can be done in food industry, by decreasing the amount of additives, by finding the minimum amount of all additives that affects bacterial growth. In marketing, mayonnaises with shorter expiration dates should be sold, in order to minimize the additives. New and less harmful inhibitors may be found by evolving new experiments' results. My experiment showed that, there is nothing wrong to use salad dressings, if you know how long you will store it and how to store it.

APPENDIX 1

EXPLANATION FOR ABBREVIATIONS & SYMBOLS

Lactobacillus plantarum: Type of bacteria that is frequently found in mayonnaise's normal microflora.

Microflora: A group or colony of microorganisms present in a specific, localized location

TAMB: Total amount of bacteria

PCA: Plate count agar. A solid broth where all types of bacteria can grow.

CASO Broth: A liquid broth that is used for the activation of the bacteria

PSS: Physiological salt solution. It is used for the dilution process.

76% Concentrated ethyl alcohol solution: A solution that kills the all kind of bacteria for certain.

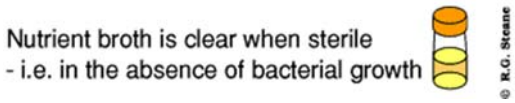
SD: Standard deviation

SE: Standard error

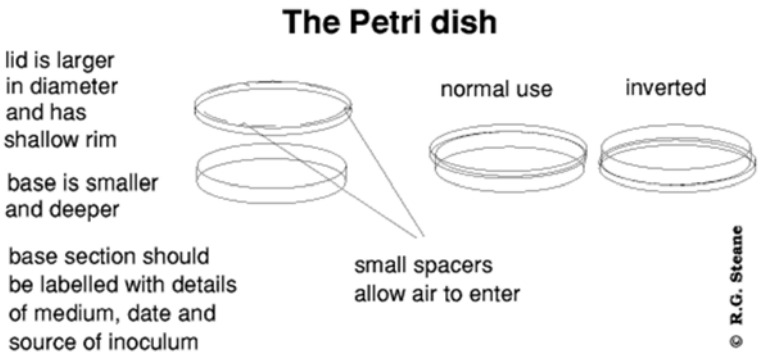
APPENDIX 2

Bacterial culture method will be explained below.

Bacteria will grow on practically any source of organic food which provides carbon compounds to be respired for energy, and nitrogen compounds to be incorporated into proteins for growth. These substances are normally provided dissolved in water. However, in nature, bacteria can break down solid and insoluble substances by releasing enzymes into the substrate in which they are growing. These substances are thus broken down or digested to simpler substances and the process is called extracellular digestion because it takes place outside the bacterial cells.



The two normal media used in bacteriology are a clear soup-like liquid nutrient broth, usually in tubes, and nutrient agar, which is set into a jelly by the addition of a seaweed extract called agar, and when melted poured into glass or plastic petri dishes - also known as "plates".



Combinations of chemicals (buffers) may be used to keep the pH stable. Measured amounts of the concentrates are added to water, and dissolved to reconstitute the media. Sometimes, substances are mixed into media, in order to suppress growth of other types of bacteria. There are many such selective media.

Sterilization, aseptic techniques, inoculation, incubation

These media must then be sterilized by heating in an autoclave at 121°C (pressure 1 bar or 15 lb/sq. in.) for 15 minutes, which kills all living organisms, including spores. All apparatus used from this point onwards must be sterilized by heat (at 160 °C for 2 hrs).

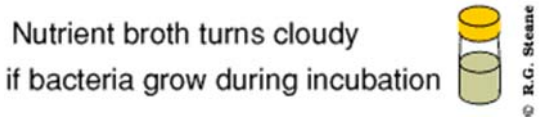
Aseptic techniques must be used to reduce the likelihood of bacterial contamination. This usually involves disinfection of working areas, minimizing possible access by bacteria from the air to exposed media, and use of flames to kill bacteria which might enter vessels as they are opened.

Sometimes bacteria in a liquid are introduced using a sterile pipette to the Petri dish before the agar medium is poured on top ("pour plates").

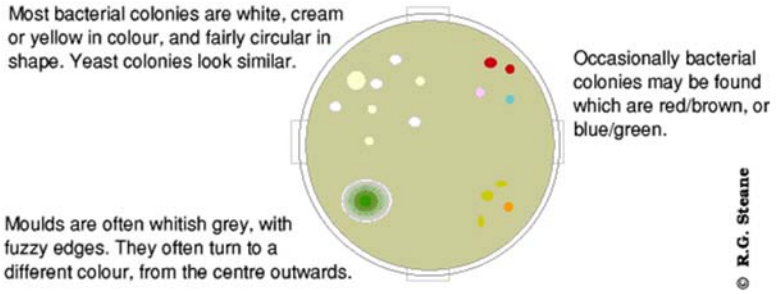
Then the Petri dishes containing agar or tubes containing broth are incubated, i.e. put in a special apparatus at a fixed temperature (usually 37°C - human body temperature, for possible pathogens - or 25°C for bacteria from the environment).

When growing bacteria, it is usual to invert the Petri dishes, so as to prevent condensation droplets from falling onto the surface of the agar.

Cultures are usually examined after 24 hrs incubation. Liquid media such as broth become cloudy if bacteria are present. This could be the result of only one bacterial cell originally entering the medium, then dividing repeatedly to produce millions.



Bacteria on agar "plates" become visible as distinct circular colonies; each colony should represent an individual bacterial cell (or group) which has divided repeatedly but, being kept in one place, the resulting cells have accumulated to form a visible patch.



By an extension of this method using serial dilutions in sterilized liquids, the number of bacteria in a given amount of sample, e.g. food, can be calculated.

After use, bacterial cultures, etc. must be sterilized by the use of heat, before disposal.

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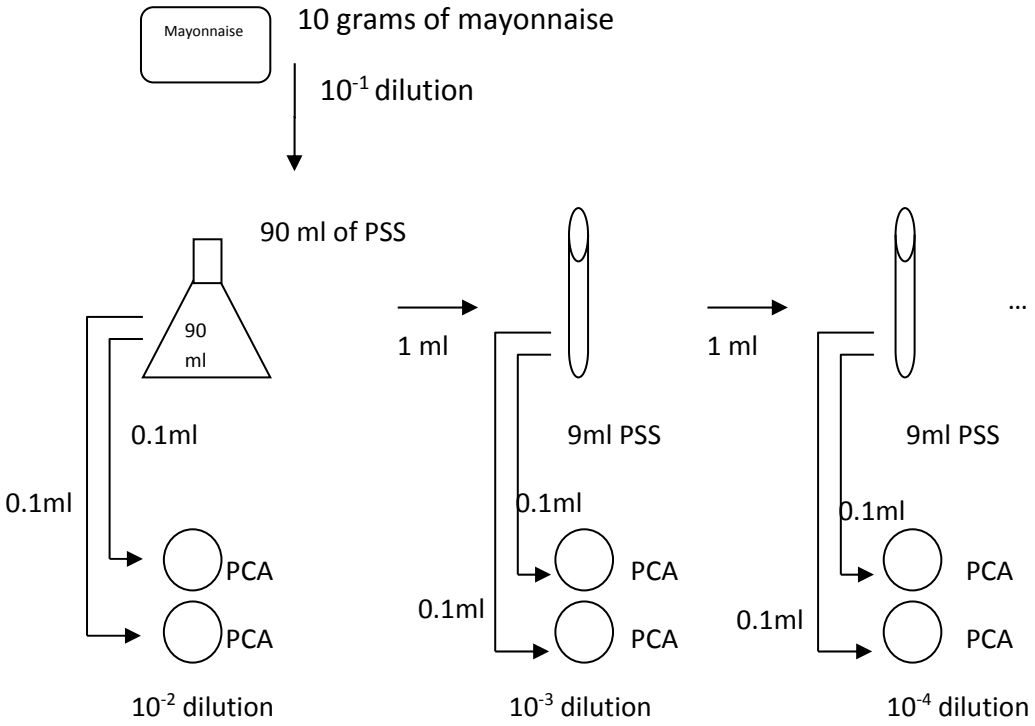
APPENDIX 3

Dilution method will be explained below.



Rate of dilutions can be increased by this way. 1ml micro-syringe is used in the rate of 10^{-1} dilution, and 0.1ml micro-syringe is used at the other rates of dilutions. Dilutions depend on the numbers of bacteria, so dilutions are used in order to obtain clearer results/counting. This scheme represents only one kind of mayonnaise for instance; it represents the dilution process of H1. Therefore, the process is repeated for every kind of mayonnaise and for each trial. 2 Petri dishes of PCA represent the first and the second trial for each trial and type of mayonnaise.

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