The Investigation of the Preservative Effect of the Stretch Film on Spoilage of Boiled *Bos primigenius* ‘Cattle’ Meat

Extended Essay (Biology)

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Abstract

We use plastic packagings in our daily life to store our food, making it to keep its freshness for longer. Plastic packages main function is to preserve food from spoilage by cutting off the contact with oxygen as it causes organisms to react with food, leading to deterioration and after some point, spoilage. I chose to study with 'stretch film' as it is mostly used in daily life. The focus of this study is to test that if stretch film is preventing food from spoilage or not. It is not possible to investigate a specific bacteria in deterioration, so that total colonization is used. As meat is one of the products which is widely consumed and able to deteriorate faster than others, I used 'boiled meat'(boiled to make it sterilized) to test in this experiment. The aim of this study is to investigate whether stretch filming preserve food from spoilage, by counting total colonization, on meat. In the experiment, the 'boiled meat' divided equally to 14 containers, 7 open and 7 wrapped by sretch film, which will be analyzed at different days to see deterioration as the time passes. Meat samples are homogenized via serial dilution and incubated on agar medium over 30 ^{0}C for 72 hours, total colonization were counted. These values were then processed and microbiological deterioration of boiled meat were obtained. As the time passes, meat samples in open containers were spoiled while the ones that in containers wrapped with stretch film preserved from spoilage.

In this investigation, it was found that wrapping stretch film is effective in protection of food. No spoilage of boiled meat is observed in the containers wrapped by stretch film while the most spoilage is observed in the open containers. Overall, the results of this study advocates the hypothesis that stretch filming is protective against the spoilage of food.

Word Count: 307

Contents

Introduction

I usually come across with that most of the people would wrap their food before refrigate it. I wonder if its really protecting food or not, so I decided to investigate it. When I asked about it to my elders, I had the answer 'if it wasn't, why would we've been using it' but none of them could explain it scientifically. They also told that it prevents scent mixing of foods even if it hasn't any protective affect. I thought that if there isn't any difference between using it or not, it would be a waste of money while defiling the environment with plastic products.

Food deterioration is a series of continuous degradative changes occurring in a food item which may affect the product's wholesomeness, result in a reduction of its quality, and/or alter its serviceability.[1] Deterioration mostly occurs by the effects of microorganisms. Microorganisms live in every part of the biosphere, including soil, hot springs[2] so that they are able to contact with food easily. If enough water, oxygen and nutrient content is available, microorganisms can grow and multiply. As they react with food, they will grow on food causing deterioration of it.

The process in which food deteriorates to the point in which it is not edible to humans or its quality of edibility becomes reduced is food spoilage.[3] Foods become permable in nature (when harvested) if its not isolated from environment and they start to decompose by the affects of enzymes and microorganisms. When microorganisms increase in population they use organic matter of food much more and more chemical reactions occur, affecting the quality and structure of food, like its flavor, color and nutritional value. The chemical reaction of this decomposition may produce waste products that can be harmful for the consumer. Most common illness caused by the consumption of spoiled food is 'foodborne illness' which may also lead to severe diseases as(if) the bacterias and viruses affect serious organs.[4]

Most food products can be consumed before spoilage occurs even if they are deteriorated so the food that spoilt does more harm to human while deteriorated ones

[1] http://www.preppers.info/uploads/us_army_cc_md0723_food_deterioration.pdf
[2] http://www.naturalhealthresearch.org/microorganism/
[3] Tull, Anita (1997), *Food and nutrition* (3 ed.), Oxford University Press, p. 154, ISBN 978-0-19-832766-0
[4] http://food.unl.edu/food-poisoning-foodborne-illness

may have not. In this experiment I investigated spoilage of food as the purpose of using stretch film is to preserve food from spoilage.

The type of food used in this investigation was boiled meat. Meat is one of the most consumed food in daily life as it contains protein, vitamins and minerals which is needed. As meat is easy to deteriorate and spoil in standard conditions even when its refrigrated, it must be well preserved and stored which is the main reason why I choose meat to investigate with. Boiled meat is more tendent than other food to be spoilt or deteriorated in less time and it is one of the most commonly consumed food which will lead a general result. Cooked meat can be stored in the refrigerator 3 to 4 days.[5] It is also able to cause serious health issues, illnesses when spoiled, so that it must be treated carefully, protected from outer infections. To eliminate the possibility of the meat being infected, it is boiled to make it sterilized and contacted with no other surfaces than sterilized container which it would kept in.

There are several illnesses and disadvantages that spoiled meat may cause when consumed. The organisms spoiling meat may infect the animal either while still alive ("endogenous disease") or may contaminate the meat after its slaughter ("exogenous disease").[6] There are numerous diseases that humans may contract from endogenously infected meat, such as anthrax, bovine tuberculosis, brucellosis, salmonellosis, listeriosis, trichinosis or taeniasis.[7]

There are various ways to keep food unspoilt and edible for human consumption. Meat can be kept edible for a much longer time – though not indefinitely – if proper hygiene is observed during production and processing, and if appropriate food safety, food preservation and food storage procedures are applied.[8] Food preservation may be defined as the set of treatment processes that are performed to prolong the life of foods and at the same time retain the features that determine their quality, like color, texture, flavor and especially nutritional value.[9] Also there is a huge usage of

[5] http://www.foodsafety.gov/keep/charts/storagetimes.html
[6] J. Microbiol. Biotech. Res., 2012, 2 (4):529-532 ISSN : 2231 –3168
[7] Lawrie, 158, R. A.; Ledward, D. A. (2006). *Lawrie's meat science* (7th ed.). Cambridge: Woodhead Publishing Limited. ISBN 978-1-84569-159-2.
[8] M. T. Usman, *, A. S. Tanko, A. J. Alhassan , International Journal of Chemical and Biomolecular Science, Vol. 1, No. 3, October 2015 Publish Date: Aug. 17, 2015 Pages: 129-133
[9] http://www.fao.org/docrep/006/ad379e/ad379e02.htm

chemical preservatives as additives which makes foods' shelf life longer; for meat, nitrite and nitrate are used.[10]

One of the most popular ways to protect food is packaging, as wrapping with stretch film which is widely used. It is prefered than any other packagings as it is cheap and disposable; no need to clean, just throwing it away. I used stretch film in this investigation as long as it is the easiest and cheapest way known to preserve food, which makes it most common around the world.

I wanted to investigate that if stretch film truly preserve food or not because if its not, it would be such a waste of plastic products. **Does wrapping stretch film over the containers with boiled meat inside, inhibit the spoilage of boiled meat compared to the ones stored in open containers by counting colonies?** This research question and the detailed biological aspects of preservation by stretch film will be discussed throughout this paper.

[10] Msagati, Titus A. M. (2012). The Chemistry of Food Additives and Preservatives.

Hyphothesis

Many ways were used to preserve food from bacteria growth, spoilage like keeping it in a cold place. But it would be more efficient, cutting off contact with air which interrupts the burning reaction (oxidation) with food, also inhibiting bacteria to have optimum medium to multiply. As the food is wrapped with stretch film and than refrigrated, the contact of food with air would be cut off. In light of these information, it can be hypothesized that **wrapping with stretch film will preserve food from spoilage which also kept in 4^0C.** The boiled meat which wrapped with stretch film is expected with a decrease in deterioration rate and the activity of microorganisms on it even on the ones that kept most before investigated while the ones without wrapping will be deteriorated.

Method Development and Planning

To test the research question “Does wrapping stretch film over the containers with boiled meat inside, inhibit the spoilage of boiled meat compared to the ones stored in open containers by counting colonies?”, some of the boiled meat pieces must be kept as wrapped with stretch film and compared by spoilage to those which had not closed, investigated at a period of time.

I investigated this research at Etlik Veterinary Control Central Research Institute and acquired laboratory access from Dr. Özhan TÜRKYILMAZ, director of the Institute. The solutions and the equipment used in the experiment supplied by the Institute.

In order to observe the effect of stretch film on spoilage or preservation of food, one should compare numbers of microorganisms present on samples, leading the deterioration rate and spoilage. There are many types of bacteria which could be found on deteriorated, spoiled meat products but for my investigation, as I used boiled meat, most common bacteria that could be found on it; *Salmonella* and *L. monocytogenes.* I used CFU counting because it wasn’t able to look for a specific bacteria so I would compare the samples by the colonies that counted.

The food that I planned to use is boiled meat as meat is rich in nutrition which makes it essential for health so that it is widely consumed. Cooked meat (boiled also) has a time period of spoilage from 3 to 4 days after it cooked, which means that it must be well preserved and quickly consumed. Other food may have longer period of time to spoil and some of them even don’t need any preservations (honey) so that I used boiled meat which will make my investigation to conclude quickly and provide general result.

I used stretch film in this investigation as it is one of the most common packaging used at home because of its cheapness and easy usage that it is thrown away after used, no need to clean. The main function (admitted by most) of stretch film is to cut the contact of air with food so inhibiting the burning reaction (oxidation) of food and multiplication of bacteria on it, preserving food from spoilage. I investigated whether it is preserving or not by comparing the ones (boiled meat samples) wrapped with stretch film and the ones which were not.

Before I prepare my procedure, I thought 2 meat samples (one in closed container, one in open) will be enough for the experiment, as I would wait for a long time before performing CFU counting, waiting for them to be spoilt. Then I percieved that I need to investigate for many times in a period of time (before the food fully spoils, to see the spoilage process) so I should take samples from both of them in different times. To do that, I need to open stretch film to take samples from meat, leading a failure that the purpose of the investigation could not be obtained as closed container will take air in it as I open stretch film. As final solution, I prepared 14 samples (7 open, 7 closed), experimented in different times to observe deterioration and spoilage as time passes, 1 open and 1 closed would be experimented each time.

The first 4 steps (boiling meat and distributing to 14 containers, wrapping 7 of them with stretch film and refrigrating for 48 hours) were held at home to observe the protective effect of stretch film which we use daily at home; not at laboratory with being fully sterile (the rest of the method would have held at laboratory). If whole experiment were processed in laboratory, it would be fully sterile and no colonies would have found on samples so that the investigation would become meaningless.

The agar medium that used in the experiment is Plate Count Agar, as it is a nonselective media, not address to a specific group of microorganisms, so that it is the most appropriate media for total colony counting. Also Maximum Recovery Diluent is used in the experiment for serial dilution as it is used for in vitro standard microbiologic analyzes.[11] The reason for performing serial dilution is to investigate samples in more detailed concentration; gaining detailed values,results.

The procedure of the investigation also contains (benefited from) ‘EN ISO 4833’ procedure of evaluating meat spoilage used by Ministry Of Food, Agriculture And Livestock for cooked meat products. The calculations done to find number of colonies present on meat samples have taken from EN ISO 4833 procedure and numbers of colonies below 30 and above 250 have not taken into calculation, they will be assumed 0 according to it, to obtain accurate results and a valid conclusion.

The method will be not be repeated as the trials have been done while planting a sample.

[11] www.mikrobiyoloji.org/genelpdf/920020001.pdf

Method

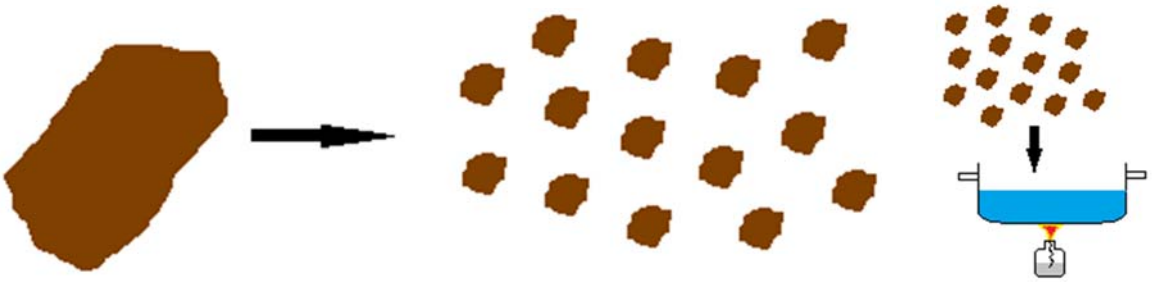
Materials Used in the Experiment

- SERA® stretch film
- 140 grams of *Bos primigenius* 'Cattle' meat
- Autoclave
- Pressure cooker
- Incubator (30 ± 1^{0}C)
- Analytical balance (0.01 mg precision)
- Bain-marie (water bath) (44-47 ^{0}C)
- Stomacher and 15 sterile bags
- Bunsen burner
- Sterile forceps, spoon, lancet
- Vortex mixer
- 375 Sterile pipets
- Automatic pipets and sterile pipet tip
- Microfuge tube rack
- Glass tubes, erlenmeyer flask, graduated cylinder and petri dish
- Plate Count Agar
- Maximum Recovery Diluent

Procedure

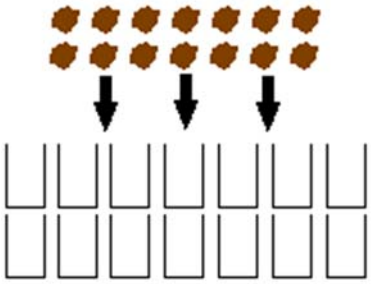
First 4 steps held in home.

1. 140 grams of *Bos primigenius* 'Cattle' meat cut with sterile lancet into 14 similar pieces (with approximately 10 gr each). Those pieces boiled at 125 ^{0}C for 30 minutes in pressure cooker. This process provided meat to be sterile.

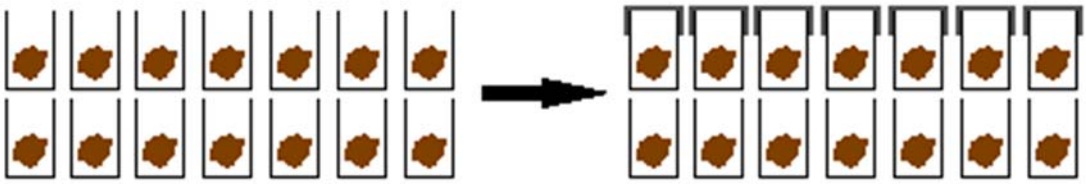


Meat

2. Pieces taken with sterile forceps and distributed to 14 sterile containers.



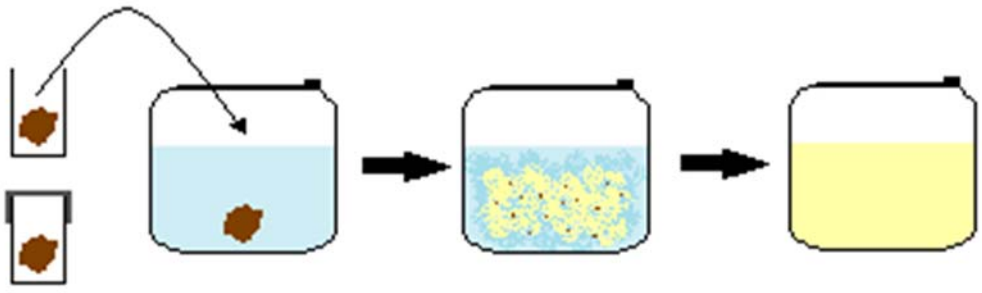
3. 7 of containers wrapped with SERA® stretch film and the others will stay open.



4. All 14 containers put at the same row of refrigrator and waited for 48 hours.

5. All containers taken to laboratory and 12 of them (except 1 open and 1 closed) will be refrigrated with other meat products (sausages and sucuk which were investigated -by Ministry of Food, Agriculture and Livestock- their edibality for human, testing if they have harming effect, with the procedure that we used TS 7703 EN ISO 4833[12]). 1 open and 1 closed container wasn't refrigrated because they were the first samples which planted and counted.

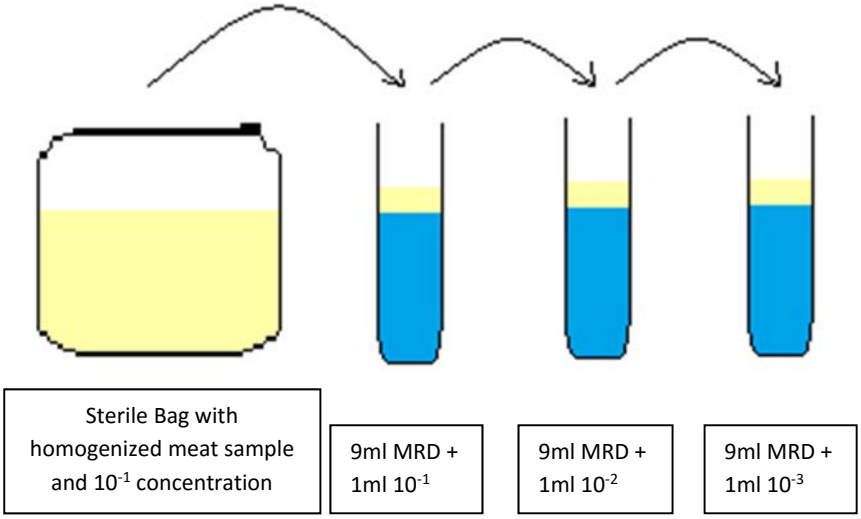
6. Meat sample in open container taken and put in a sterile bag (for stomacher) and 90 ml Maximum Recovary Diluent (MRD) added (10^{-1} concentration prepared). Then homogenized at stomacher.



Sterile Bag *Homogenization*

[12]https://intweb.tse.org.tr/standard/standard/Standard.aspx?081118051115108051104119110104055047105102120088111043113104073083101120089082100078069117115121

7. 1 ml of sample taken from bag and put into a glass tube, 9 ml MRD added (10^{-2} concentration prepared) (place the tube at the first position on rack or number it not to confuse with other ones) (All serial dilution processes done with sterile pipets (switched with new one after dilution), automatic pipets and sterile pipet tip).



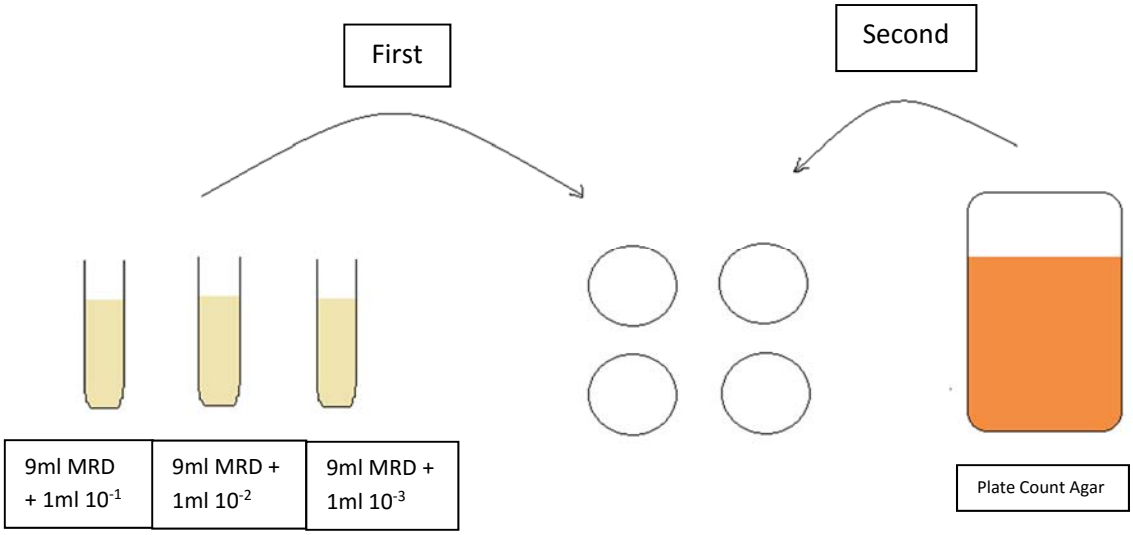
8. 1 ml of sample taken from first glass tube and put into another glass tube with addition of 9 ml MRD (10^{-3} concentration prepared).

9. Same process (8) done for second glass tube (10^{-4} concentrations obtained).

10. 500 ml Plate Count Agar (PCA) 10 gr weighed and melted, then autoclaved. pH set to 7±0,2 after autoclave. Sterilized at 121±1 ^{0}C for 15 minutes.

11. Samples in tubes homogenized with vortex mixer.

12. 1 ml taken from all tubes and put at empty petri dishes, then 15 ml 45^{0}C, cooled, PCA poured on petri dishes and stirred.

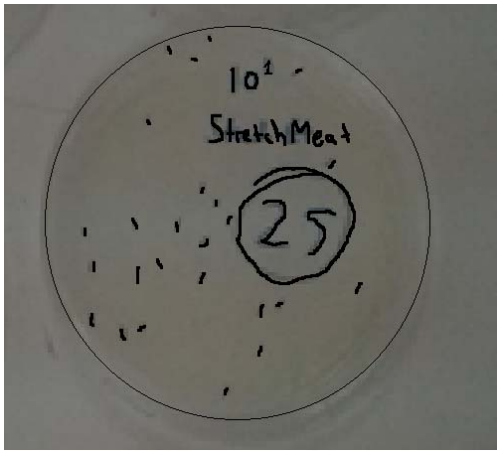


13. Same process (from 6 to 12) done for meat sample in closed container.

14. After samples in petri dishes cooled, they incubated at $30^0C \pm 1^0C$ in incubater for 72 hours.

15. Same process (from 6 to 14) done for 4 more times.

16. After incubation, CFU (colony forming unit) counting performed for samples.



17. Open 1 of each container (both open and closed) in different times (no need a certain time schedule, may decide according to colonies counted before (at previous samples)), perform steps from 6 to 16, to investigate the process of deterioration and spoilage.

Results

				CFU counting, number of colonies per concentration of serial diluted samples				Controlled Variables				
	Planting Samples / Dates	Trials	Grams of *Bos primigenius* 'Cattle' meat	1/10	1/100	1/1000	1/10000	Time of Cooking (Boiling) Bos primigenius 'Cattle' meat (±2 min.)	Cooking (Boiling) Temperature of Bos primigenius 'Cattle' meat (°C)	Temperature of the refrigrator (home)(±0.1 °C)	Temperature of the refrigrator (lab)(±0.1 °C)	Equipment used and conditions
Open Container (Stretch film not used)	1 (26.12.2014 - 29.12.2014)	1	10	13	10	4	4	30	125	7.2	4.3	CO2 Incubator (30 ± 0.1 °C) , Plate Count Agar (as agar medium) , Maximum Recovary Diluent (diluent used at serial dilution)
		2	10	14	11	5	2	30	125	7.2	4.3	
		3	10	14	12	6	3	30	125	7.2	4.3	
		4	10	12	9	5	2	30	125	7.2	4.3	
		5	10	11	8	4	1	30	125	7.2	4.3	
	2 (29.12.2014 - 31.12.2014)	1	10	14	12	10	7	30	125	7.2	4.3	
		2	10	17	15	11	8	30	125	7.2	4.3	
		3	10	15	13	9	6	30	125	7.2	4.3	
		4	10	15	12	11	7	30	125	7.2	4.3	
		5	10	14	12	10	8	30	125	7.2	4.3	
	3 (05.01.2015 - 08.01.2015)	1	10	19	16	13	6	30	125	7.2	4.3	
		2	10	18	15	12	9	30	125	7.2	4.3	
		3	10	20	16	13	7	30	125	7.2	4.3	
		4	10	22	18	15	7	30	125	7.2	4.3	
		5	10	21	16	14	8	30	125	7.2	4.3	
	4 (08.01.2015 - 12.01.2015)	1	10	23	20	15	11	30	125	7.2	4.3	
		2	10	25	21	16	12	30	125	7.2	4.3	
		3	10	23	20	16	11	30	125	7.2	4.3	
		4	10	24	21	17	13	30	125	7.2	4.3	
		5	10	25	22	16	12	30	125	7.2	4.3	
	5 (13.01.2015 - 16.01.2015)	1	10	29	25	19	16	30	125	7.2	4.3	
		2	10	35	30	23	18	30	125	7.2	4.3	
		3	10	30	26	21	14	30	125	7.2	4.3	
		4	10	38	34	28	24	30	125	7.2	4.3	
		5	10	28	22	16	13	30	125	7.2	4.3	
	6 (19.01.2015 - 22.01.2015)	1	10	88	51	41	22	30	125	7.2	4.3	
		2	10	67	43	27	19	30	125	7.2	4.3	
		3	10	68	56	38	24	30	125	7.2	4.3	
		4	10	71	54	36	21	30	125	7.2	4.3	
		5	10	103	83	40	22	30	125	7.2	4.3	
	7 (22.01.2015 - 26.01.2015)	1	10	105	82	30	25	30	125	7.2	4.3	
		2	10	95	59	29	23	30	125	7.2	4.3	
		3	10	98	61	39	25	30	125	7.2	4.3	
		4	10	265	150	70	29	30	125	7.2	4.3	
		5	10	273	144	98	30	30	125	7.2	4.3	
Closed Container (Wrapped with SERA® stretch film)	1 (26.12.2014 - 29.12.2014)	1	10	4	2	0	0	30	125	7.2	4.3	
		2	10	3	2	0	0	30	125	7.2	4.3	
		3	10	4	2	1	0	30	125	7.2	4.3	
		4	10	5	2	1	0	30	125	7.2	4.3	
		5	10	4	2	1	0	30	125	7.2	4.3	
	2 (29.12.2014 - 31.12.2014)	1	10	8	5	3	1	30	125	7.2	4.3	
		2	10	9	6	3	2	30	125	7.2	4.3	
		3	10	7	5	3	1	30	125	7.2	4.3	
		4	10	8	6	3	1	30	125	7.2	4.3	
		5	10	9	6	4	2	30	125	7.2	4.3	
	3 (05.01.2015 - 08.01.2015)	1	10	12	8	4	2	30	125	7.2	4.3	
		2	10	9	6	3	1	30	125	7.2	4.3	
		3	10	10	7	5	2	30	125	7.2	4.3	
		4	10	11	8	6	3	30	125	7.2	4.3	
		5	10	10	7	4	2	30	125	7.2	4.3	
	4 (08.01.2015 - 12.01.2015)	1	10	15	11	7	4	30	125	7.2	4.3	
		2	10	14	10	5	2	30	125	7.2	4.3	
		3	10	16	12	6	3	30	125	7.2	4.3	
		4	10	13	10	5	2	30	125	7.2	4.3	
		5	10	14	10	4	1	30	125	7.2	4.3	
	5 (13.01.2015 - 16.01.2015)	1	10	18	14	10	7	30	125	7.2	4.3	
		2	10	19	15	11	8	30	125	7.2	4.3	
		3	10	17	13	9	6	30	125	7.2	4.3	
		4	10	18	14	11	7	30	125	7.2	4.3	
		5	10	19	15	10	8	30	125	7.2	4.3	
	6 (19.01.2015 - 22.01.2015)	1	10	23	17	13	6	30	125	7.2	4.3	
		2	10	21	15	14	9	30	125	7.2	4.3	
		3	10	22	17	13	7	30	125	7.2	4.3	
		4	10	20	17	13	7	30	125	7.2	4.3	
		5	10	21	16	12	8	30	125	7.2	4.3	
	7 (22.01.2015 - 26.01.2015)	1	10	25	20	15	10	30	125	7.2	4.3	
		2	10	24	19	14	10	30	125	7.2	4.3	
		3	10	25	20	16	11	30	125	7.2	4.3	
		4	10	26	19	15	10	30	125	7.2	4.3	
		5	10	24	19	16	12	30	125	7.2	4.3	

Table 1 shows the raw data of number of colonies, collected from CFU counting for each dilution and plant. The controlled variables like temperature and time of boiling of meat, temperature of the refrigerator that meat samples kept in are also shown.

At the experiment, some qualitative changes observed:

- Desiccation occurred for the meat samples that kept in open container and their color become dark brownish after some point.
- No moist loss observed in closed container and meat samples kept in it had a change in color to grey and after some point, because of chemical (microbiological) deterioration, its color turned to green.

Calculations:

Calculations done for the colonies that counted per concentration of serial diluted samples to obtain final number of colonies present on the sample. If the values are below 30 or above 250, they won't have taken into calculation and they will count as 0 as long as the calculation addresses to a range of number of colonies to make the results accurate.

Calculation of CFU counted colonies:

$$N = [\sum C\,/[(1 \times \mathrm{n1}\ ) + (0.1 \times \mathrm{n2}\ ) + (0.01 \times \mathrm{n3}\ ) + (0.001 \times \mathrm{n4}\ )]] \times \mathrm{d}$$

N : number of colony in grams or ml.

∑C : sum of the colonies that counted on petri dishes

n_1 : number of petri that planted in first dillution

n_2 : number of petri that planted in second dillution

n_3 : number of petri that planted in third dillution

n_4 : number of petri that planted in fourth dillution

d : coefficient of dillution of first petri that counted

Calculation of CFU counted colonies at fourth trial of seventh open container:

CFU counting, number of colonies per concentration of serial diluted samples			
1/10	1/100	1/1000	1/10000
265	150	70	29

$N=[\sum C/[(1\times n_1)+(0.1\times n_2)+(0.01\times n_3)+(0.001\times n_4)]]\times d$

10^{-1} and 10^{-4} will not be taken into calculation as they don't match the range "30-250" $n_1=n_4=0$, $n_2=n_3=1$ $d=10^{-2}$

$\sum C= (150+70)= 220$

$N=[220/[(1\times 0)+(0.1\times 1)+(0.01\times 1)+(0.001\times 0)]]\times 10^{-2}$

$N= [220/0.11] \times 10^{-2}= 220/11=$ **20**

Numbers of colonies counted and processed by calculation							
	Open Container						
Plants / Trials	1	2	3	4	5	6	7
1	0	0	0	0	0	15	17
2	0	0	0	0	1	10	14
3	0	0	0	0	0	12	18
4	0	0	0	0	2	12	20
5	0	0	0	0	0	18	22
	Closed Container						
Plants / Trials	1	2	3	4	5	6	7
1	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0

Table 2: Processed data of the colonies that counted by performing CFU and calculated by "$N=[\sum C/[(1\times n1)+(0.1\times n2)+(0.01\times n3)+(0.001\times n4)]]\times d$". Table 2 shows that there are colonies observed from the meat samples in open containers while there is no threating of microorganisms for health in closed container.

Mean:

$$\overline{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

where;

n is the largest number of trial (n=5 for this experiment)

X_i is the number colonies that calculated from CFU counted values

Standard Deviation:

$$\sigma = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(x_i - \bar{x})^2}$$

where;

n is the largest number of trial (n=5 for this experiment)

Xi is the number colonies that calculated from CFU counted values

$\bar{X}$ is the mean value of number of colonies that calculated from CFU counted values

Standard Error:

$$SE_{\bar{x}} = \frac{s}{\sqrt{n}}$$

where;

n is the largest number of trials (n=5 for this experiment)

Xi is the number colonies that calculated from CFU counted values

σ is the standard deviation of the corresponding data

	plants	mean	median	range	variance	Standard Deviation	Standard Error
open	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0.6	0	2	0.8	0.8944272	0.4
	6	13.4	12	8	9.8	3.1304952	1.4
	7	18.2	18	8	9.2	3.03315	1.3565
closed	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	0	0	0	0	0	0
	5	0	0	0	0	0	0
	6	0	0	0	0	0	0
	7	0	0	0	0	0	0

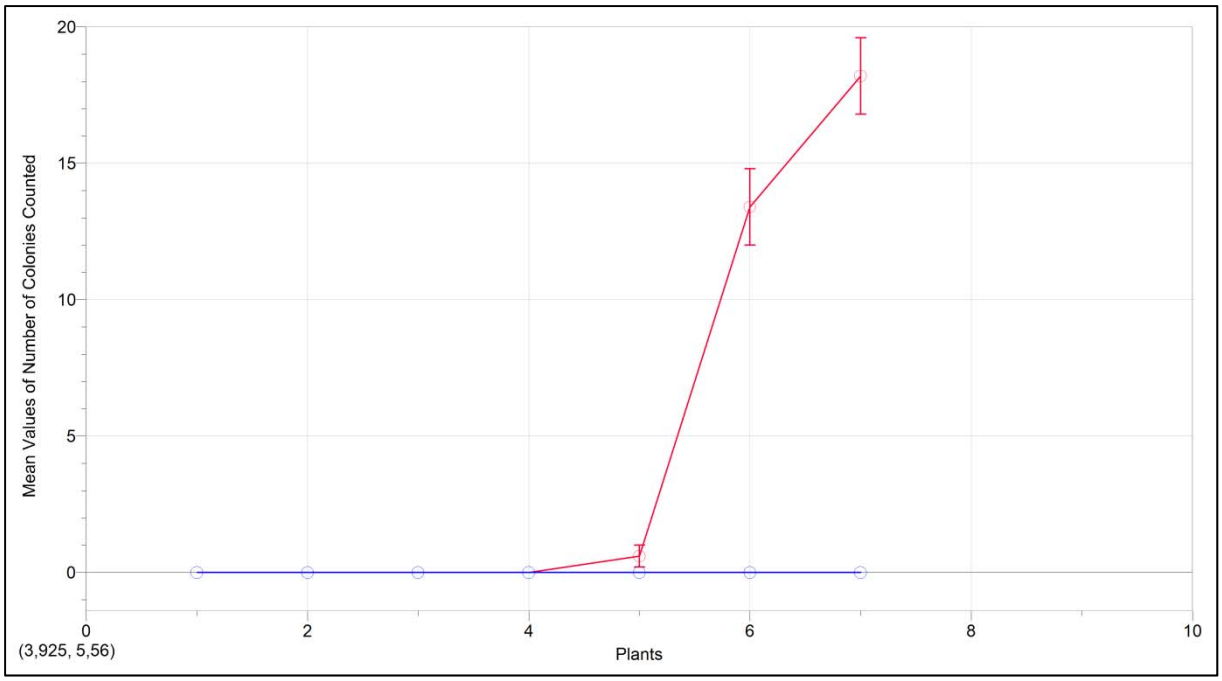
Table 3: shows the mean, median, range, variance, standard deviation and standard error values of processed data of the numbers of colonies counted in 14 samples with 5x4 plants (5 trials, 4 dilution) and processed by calculation.

Numbers of colonies counted		
Plant	Open Container	Closed Container
1.	0	0
2.	0	0
3.	0	0
4.	0	0
5.	0,6	0
6.	13,4	0
7.	18,2	0

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	Variable 2
Mean	15,8	0
Variance	11,52	0
Observations	2	2
Hypothesized Mean Difference	0	
df	1	
t Stat	6,583333333	
P(T<=t) one-tail	0,047984063	
t Critical one-tail	6,313751515	

Table 4 and 5

Table 4 shows the mean of number of colonies that calculated (See Table 2) and t-test which is processed from mean values shown in Table 5. Last two values of numbers of colonies counted have taken to t-test (plant 6 and 7) as they show that spoilage occurred which is our main purpose. As P value of one-tail is below alpha value (0.05) , it can be concluded that the experiment is valid.



Graph 1 shows the mean values of number of colonies counted on open container and closed container. The deterioration of meat samples in open container can be seen from graph, (as the time passes) as numbers of colonies present on it increase. For values of meat samples in closed container (and first four plant of open container) have 0 standard error values so that error bars are not included.

Conclusion and Evaluation:

In this experiment, it is investigated that stretch film whether preserve food (cooked meat) from spoilage or not. Meat samples were seperated to 14 identical sterile containers and 7 of them is wrapped with stretch film. They have been taken in pairs (1 open 1 closed in each plant) in different times to observe whether spoilage occurs as the time passes.

Taken meat samples are investigated as they homogenized first and diluted serially with MRD. 1 ml sample taken from the dilutions and planted to agar medium (PCA). After incubation, CFU (colony forming unit) counting performed and results have collected. After calculations, number of colonies that formed on meat samples are obtained. Statistycal analysis of the values showed that wrapping stretch film has a preserving effect on food as no colonies counted (no colonies which can be called harmful) on meat samples kept in closed container (wrapped with stretch film)(See table 2, 4).

The results of the experiment shows that as time passes, more colonies are formed on the meat samples which kept in open containers as they reacted with air and oxidized. Stretch film inhibited air exchange or any contact of air with meat samples in the container so that they didn't reacted and low number of colonies formed on them. The mean values of number of colonies that calculated from the values of counted colonies in dilutions are 0 at closed container and 4,6 at open container (See table 5) which means that stretch film is protecting food from microbiological deterioration (but not %100) and spoilage.

There are also colonies that counted in closed container (See Table 1) but they do not cause any harm to consumer so that they are ignored by the calculation which assumes the number of colonies below 30 and above 250 as 0. It can be said that deterioration occurs in both containers while spoilage occurs in open container. Deteriorated food does not cause harm until it spoils so that stretch film can be used to avoid spoilage.

The spoilage process can be seen from the Graph 1, as number of colonies that counted in open container is increasing while that counted in closed container remains same (0).

Additionally, the hypothesis “wrapping with stretch film will preserve food from spoilage which also kept in 4^0C.” is verified as spoilage occurred in open containers while no spoilage observed in closed containers.

T-test performed for the mean values of number of colonies that calculated from values of CFU counting and the P value for one-tail is found 0.048 which is below alpha value (0.05), concluding that the experiment is valid.

The purpose of this investigation is to found whether stretch film has a protecting effect on food or not, it can be stated from the results of the experiment that it is preserving from spoilage. Stretch film is widely used (SERA® in Turkey) and it is protecting but they are only used once (disposable) and thrown away, causing environmental pollution as it needs thousands of years to be fully dissolved in nature.

I also did the same experiment for a meat sample kept in container with plastic lid and there is no spoilage observed in it while deterioration rate is low, as in closed container. The reason for me to do this is that plastic lid containers are also preserving food from spoilage and it would be more environmental friendly and ergonomic as it is bought for once and used many times with cleaning, without polluting environment by throwing plastics.

The Error and Uncertainty

In the investigation, some errors occurred ,caused by equipment used or the environment the experiment was performed in. The digital devices like vortex mixer, refrigrator and electronic scale have low uncertainties, systematic error tried to be minimized but some other factors caused error in the experiment. It can be seen that the standard errors are very small in value from the statistical analysis done (even zero for closed containers), showing the accuracy of the results in the experiment. It was hypothesized that 'wrapping with stretch film will preserve food from spoilage' and no spoilage and any harmful effect seen in closed container, it can be concluded that the experiment is accurate.

The number of colonies that counted is increasing as time passes shows that deterioration (and after some point spoilage) is occuring which means that the experiment is resulting and results are reliable as there is not a value of number of colony which is lower than the one in previous steps, showing an increase in regular pattern.

The statistical analysis also gives information about precision. As there is no spoilage occured in closed container, mean values of number of colonies that calculated after CFU counting is 0, standard error and standard deviation for the values of samples in closed container is also 0. For the data obtained from the samples in open containers, the standard deviation is 0.9, 3.1, 3.0 and standard error is 0.4, 1.4, 1.3 for the last 3 plant, as these values are low it can be stated that the results are accurate and precise, also the investigation is reliable. There are both random and systematic errors in the experiment that can be seen by the error bars in Graph 1.

The errors and the uncertainties in the experiment were minimized by stabilizing the controlled variables such as:

- The stretch film which 7 containers wrapped with is SERA® and wrapped only for one layer, to stabilize the non-permeability of air, inhibiting it to enter closed container.

- The food used in the experiment is *Bos primigenius* 'Cattle' meat, 10 gr in each container, measured with analytical balance (0.01 mg precision).
- 14 containers kept in 7.2 ^{0}C for 48 hours in refrigrator at home, time needed for the deterioration to initiate.
- The type of agar medium kept constant for all meat samples (Plate Count Agar).
- The diluent used in serial dillution (Maximum Recovary Diluent) and dillution concentrations are same for each plant.
- The conditions of CO_2 incubator are stabilized as they effect multiplying colonies, present on sample.

In order to minimize the limitation of the experiment and obtain more reliable results, some improvements could be done;

- Between plant 5 and 6, since drastic inrease in colonization occured, there must be more samples collected at that period (Plant 6 and 7 must have processed earlier.) in order to observe more detailed deterioration and spoilage and get more accurate results.

Further Investigation

After obtaining results and attaining an answer to my research question “Does wrapping stretch film over the containers with boiled meat inside, inhibit the spoilage of boiled meat compared to the ones stored in open containers by counting colonies?”, a new question arises: Does wrapping stretch film protects all food from spoilage? Research can be done to investigate the preservative effect of stretch film on other food like fruits or vegetables.

Additionally, one can increase the layer of stretch film while wrapping or try other plastic packagings to investigate the ability of non-permeability of air. There are lots of types of food with different times of spoilage and lots of plastic packagings that can be experimented for further investigations.

Appendices

Plate Count Agar

Plate Count Agar is a nonselective microbiological growth medium commonly used to assess "total" or viable bacterial growth of a sample.[13]

Its main components with percentage are;

- 0.5% peptone
- 0.25% yeast extract
- 0.1% glucose
- 1.5% agar
- pH adjusted to neutral at 25 C.

Maximum Recovary Diluent

Maximum Recovery Diluent (Peptone Saline Diluent) is a protective and isotonic diluent for maximal recovery of micro-organisms (ISO/DIS 6649). It combines the protective effect of peptone in the diluting solution with the osmotic support of physiological saline1,2. The low concentration of peptone does not cause multiplication of the organisms within 45 minutes (@ 20-25°C) of dilution of the sample. The isotonic strength of the diluent ensures recovery of organisms from various sources which may be vulnerable in distilled water or aqueous suspensions.[14]

[13] Atlas, R.M. (2004). Handbook of Microbiological Media. London: CRC Press. p. 1390. ISBN 0-8493-1818-1.
[14] ISO/DIS 6649. Meat and Meat Products-Detection and Enumeration of Clostridium perfringens.

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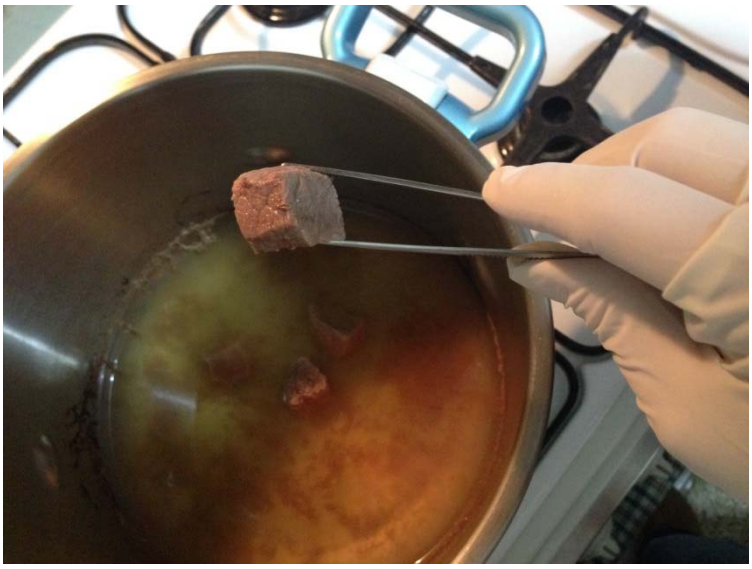

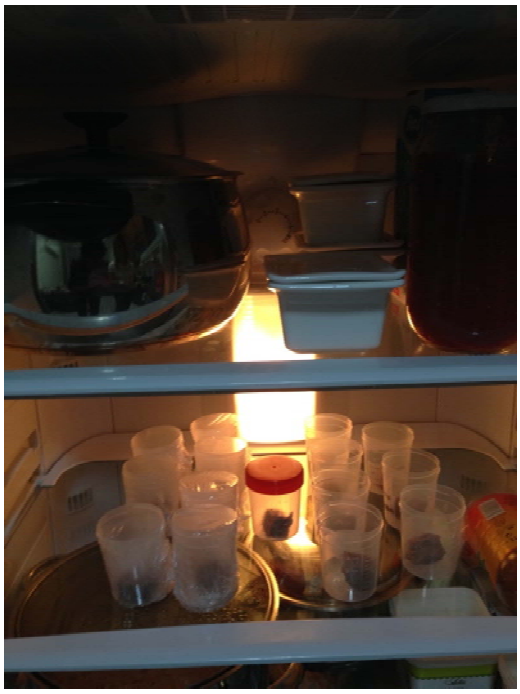

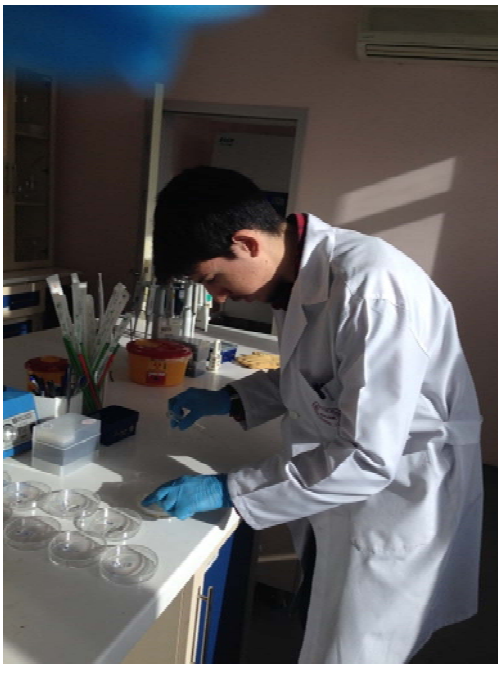

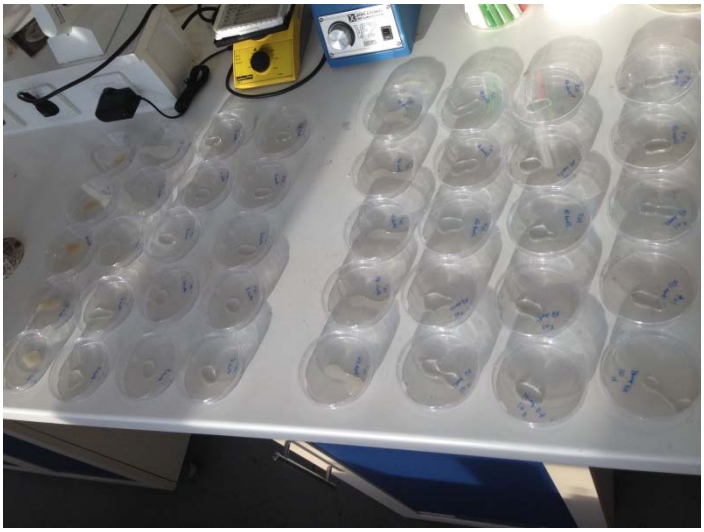

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