INVESTIGATING THE EFFECT OF DIFFERENT ACIDITY CAUSED BY DIFFERENT SPOONS ON THE VOLUME OF SACCHAROMYCES ROSEI FORMED.

Extended Essay (Biology)

ABSTRACT :

It is known that when a vegetable such as tomato is pickled, certain volume of mold, fungi is formed. On the other hand when a metal spoon Is used instead of a wooden one, the volume of fungi is less. This might be because, when a metal and acid (vinegar solution in the pickle juice) reacts, H_2 gas is released and the dissolving of this H_2 gas results in a decrease in pH. It should be stated that the kind of fungi formed 'Saccharomyces Rosei' can only reproduce in an optimum pH of 6 to 7. But when a metal reacts with the pickle juice, the pH will be lower than the optimum value for the 'Saccharomyces Rosei' thus fungal reproduction will be inhibited to some extent. The objective of the study was to investigate, whether the brand of spoon used made a significant difference in the volume of fungi formed. In order to test the effect of silver nitrate, nickel, plastic and wood, 4 different data sets of 3 trials were created as well as a control group and observed for 14 days. After 14 days, it was statistically significant that less volume of fungi was formed in the data set containing nickel samples, when compared with the other data sets including the control group.

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INTRODUCTION :

Every year, I hear my mom talk about the fungi she saw on the pickle jars. What is the reason for the formation of a coat of organisms on top of the jar of a pickled vegetable? I decided to test the effect of the usage of metal spoon with pickles on the amount of organism, in this case an anaerobic fungi formation. This subject came to my mind after I heard from a friend that if one used a metal spoon instead of a wooden spoon while dealing with the pickles, they would last longer and the formation of mold on the top part of the jar would be prevented. After the moment that I heard such theory, I became curious and decided to test it for my own. So I started to be more careful and choose my utensils with more attention and used a metal spoon instead of a wooden spoon when dealing with pickled food.

As we all know, environments with a high amount of acidity are not convenient for organisms like fungi to either reproduce or live. And when an acid reacts with a metal, it produces salt and hydrogen[1]. The increase in the amount of hydrogen dissolved in the pickle juice, causes the solution to become more acidic, in other words it lowers the pH of the solution.

The nature of a solution based on its acidity is described as pH. The pH scale is from 0, strongly acidic, to 14, strongly basic. Neutral solutions have a pH of 7.[2] Most fungi, including Saccharomyces rosei, require near neutral conditions for optimal growth with a pH of approximately 6.0.[3] Many organisms change the pH of their substrate by producing by-products during growth. They can change conditions such that the environment can no longer support their growth. When the concentration of ethyl alcohol is 18%, the rate of alcoholic fermentation decreases. Yeasts and molds are more tolerant of lower pH than the bacteria.[4]

According to this, since we know that vinegar is an acid, we can't say that this environment is not convenient for fungal growth. The pH of vinegar is approximately 2,4.[5] But when we disturb the acidity of the solution by inserting a metal, in this case the spoon, different ionic salts and hydrogen atoms can be obtained.

Metal + acid → salt + H_2 (g)

And when the produced hydrogen atoms dissolve in the water of the pickle juice, as the concentration of hydrogen increases the pH decreases. This causes the solution to become more acidic and according to the literature value, Saccharomyces rosei can't reproduce and live in such low pH.[6]

On the other hand, when a wooden or plastic spoon is used instead of a metal one, none of these processes is expected to take place, thus the formation of mold, other organisms and reproduction of possible pre-existing fungi or other organisms is not prevented. Since there are no fungi or any other organisms present in the pickle sample which is disturbed with a metal spoon, both the life length and the quality increases.

Different kinds of metals such as silver and nickel have different effects on the acidity change of a pickled tomato under the same temperature, pressure conditions, when a reaction is provided in a closed container which is permeable to light thus the amount of fungi is expected to vary since fungal growth is effected by the hydrogen concentration, acidity, of the environment.

Vegetables such as cabbage, unripe melon, cucumber, carrot, green pepper, beet and tomato have been pickled for a long time in order to prevent them for rotting and save them for later usage. The reason why I choose tomato is that tomato doesn't contain any metal ions such as silver or nickel within itself and it is peeled, cut into small pieces and then pickled unlike cucumber and cabbage[7]. By this way it is easier for the pickle juice to diffuse inside the pieces of vegetable, in this case the cubic tomatoes. There are several benefits of using pickled tomatoes in this experiment. One of these benefits is that they can be divided and cut into smaller pieces which will increase the surface area suitable for diffusion. Besides, there aren't any extra metal ions inside tomatoes and this allows the examination of only the extra substance that we insert.[8] Since there won't be any reaction other than we allow, we will not attain misleading data.

I chose to use silver and nickel as my metals because both silver and nickel can be used to plate kitchen appliances. Despite the metals, I choose to use wood and plastic as my

other variables since both plastic and wood aren’t expected to react with acids found in pickles.

REASEARCH QUESTION :

Is the volume of Saccharomyces rosei, which is responsible from the mildewing of 'Solanum lycopersicum'[9], affected by different spoons which lead to different acidity when they react with the pickle juice under same temperature and pressure conditions?

HYPOTHESIS :

Saccharomyces Rosei is a type of fungi which can reproduce in an optimum pH of 6 to 7. Even though, vinegar which inserted into the pickles when the pickles are being set, since the amount very small, it doesn't act as an anti-fungal vector. To prevent any fungal reproduction in a jar of pickles, the pH of the environment should be disturbed and should be turned into an acid. The pH of a solution depends on the concentration of hydrogen ions present. This is the point where the effect of a metal comes in. It is proven that, when a metal reacts with an acid in a neutralization reaction, both a salt is produced and hydrogen gas is released, and when the released hydrogen gas is dissolved in the solution, the pH of the environment decreases and the acidity increases. When the pH of the pickle juice solution decreases, it no longer suits the optimum pH range of the Saccharomyces Rosei so reproduction stops, at least decreases to a level. Thus I am expecting to see the minimum amount of fungi in the falcons which I store with metals and the maximum amount of fungi in the control group and the test groups which I stored with wood and plastic pieces since there won't be any interaction between these substances and pickle juice itself.

It can be hypothesized that, since the volume of Saccharomyces Rosei is dependent on pH and since the pH is dependent on the reaction between an externally inserted substance and the pickle juice itself, the volume of fungi formed depends on the different spoons which lead to different acidity when they react with the pickle juice. Moreover, the maximum volume is expected to be observed in the cases where no reaction between the pickle juice and the spoon occurs, and the maximum volume in the cases in which metals will be used since there will be a reaction.

METHOD AND DEVELOPMENT :

Designing an appropriate method in order to support or reject the proposed hypothesis and answer the given research question brought many problems with it. The first problem was about how I was going to keep the amount of garlic, lemon juice, amount of salt and the size of tomatoes I was going to put into little containers in which I was going to insert different substances to test constant. This problem was solved when I decided to peel each tomato and cut them into small cubic pieces, of about $1cm^3$, which were approximately the same size and pickled these small pieces of tomatoes not in each little jar, in which I was going to do my experiment, but in a big common jar. In order not to have a problem while trying to equalize the amount of salt, garlic and lemon juice each experimentation jar is containing, I put 6 grams of garlic 3 milliliters of lemon juice and 50 milliliters of water in the common jar to prevent the tomatoes from rotting according to the recipe. I assumed that the juice I take and put in the falcons will contain equal amounts of each because before I divided the solution, I shook the jar in order to obtain a homogenous solution.

After pickling the tomatoes in the common jar I will put it in a cool environment approximately 15^oC in order to prevent it from rotting and in order to prevent the formation of fungi until I separate it into the falcons for experimentation. Because it is known that the optimum pH for reproduction of Saccharomyces Rosei is 30^oC to 65^oC. [10]

The second major problem was about what kind of container I was going to use. There was a possibility that the metal lid of glass jars would restrain the reliability of the data collected, so glass jars with metal lids couldn't be used. This problem was overcome when, by further research, I found about sterile, see through, graduated 50mL falcon tubes. These sterile falcon tubes had lids too, which was beneficial for preventing any sort of air flow or escaping of any possibly formed gas from the environment. The labels on the container made observing the amount of mold formed in each container easier. Labeling the containers was essential in order to prevent any kind of confusion. Since the containers were see through there was no need to open the containers every now and then to observe any kind of change, and taking photos became easier.

Not disturbing the pickles and preventing them from any possible vibration was an additional problem. Any kind of vibration was prevented by the usage of a rack placed on a small coffee table. I put my falcon tubes in this rack.

The amount of substance inserted was controlled by keeping the surface area of these substances constant. Besides keeping the amount of substance constant, the amount of pickle itself and its juice was kept constant by putting 3 cubes of pickled tomato and filling the falcon tubes until the juice level reached 35mL. (See method) After I put 3 cubes of tomatoes in each falcon, I am planning to use a small syringe to fill the falcon up to the 35 milliliter line so that the volume of Saccharomyces Rosei formed will be suitable for observation, because if I use less pickle juice, the volume of fungi will also decrease proportionally and I will have difficulty in collecting data.

It became important to make sure that all variables were being controlled. Light intensity, temperature and pressure was kept constant for all the containers just by putting them into the same rack and placing them in a room with no direct sunlight and with slightly higher temperature than the average room conditions which will possibly increase the rate of reaction. Another thing which had to be kept constant was the surface area of the solution in contact with the air within the tube. To ensure that the surface in contact with the air within the tube was the same, the tubes were placed inside a rack, perpendicular to the ground.

I planned to measure the dependent variable, in this case the amount of mold formed, by using the milliliter lines present on each falcon. By this way I will be able to compare the volumes of Saccharomyces rosei formed. I am planning to keep the duration of my experiment at least two weeks because reproduction of Saccharomyces rosei takes a bit time. I will photograph the falcons once every two days because I don't expect to observe any distinguishing difference over a day. I decided to continue my experiment until I attained same results in my two consecutive observations.

I am planning to have three trials for each test group, so it will be easier for me to compare and comment on the data I have collected. Repeating the experiment for more than once, will also give me the opportunity to use the mean values for each data set, when evaluating my results.

METHOD :

Materials and Apparatus :

- A big glass jar with a plastic lid
- A knife
- 3 tomatoes
- Garlic (6 grams)
- 3ml Lemon juice
- 50 ml of water
- Salt
- 15 x 50ml sterile falcon tubes
- 3 equal pieces from a plastic spoon (±2cm²)
- 3 equal pieces from a wooden spoon (±2cm²)
- 3 equal pieces of nickel (±2cm²)
- 6 grams of silver nitrate
- A permanent marker
- 15ml Syringe
- Heat source (central heating unit)
- Toothpick
- A bench top rack for 50mL falcon

To begin with, the tomatoes are washed, peeled and cut into small cubic pieces. These pieces are placed inside the big jar and 2 cloves of garlic, each about 3 grams, are added besides 3mL's of lemon juice. After the pickles are ready the lid is tightly closed and the jar is placed in a cool dark environment, approximately 15ºC, in this case in the basement until the tomatoes were pickled. The jar is never opened for approximately 10 days after it is put in a cool and dark place.

After the tomatoes were pickled, 3 cubes of tomatoes are placed inside the sterile tubes by the help of toothpicks and the tubes were filled with pickle juice until the 35mL point by the help of a 15ml syringe. The tubes were divided in to 5 groups (silver nitrate,

nickel, wood, plastic, control group) and the tubes were placed inside the bench top rack. A different predetermined variable was put in each set of tubes, in one of the groups, nothing was added, it was made the control group and the lids were tightly fastened and labeled according to the substance they contained.

The rack was then placed close to a heater with normal light. The tubes were observed and were pictured once two days to make observing the amount of mold formed easier. I kept taking the pictures of the graduated falcons and noting down the volume of mold until I got an identical data in two consecutive measurements in all of the categories.

When it came to measuring the amount of mold formed, I used the milliliter lines present on each falcon. So I was able to calculate the amount of mold formed in volumes. I repeated this experiment 3 times.

DATA COLLECTION :

			The substances which were inserted into each falcon for experimentation for 14 days				
			Wood (±0.5 cm^2)	Plastic (±0.5 cm^2)	Silver Nitrate (±0.5 ml)	Nickel(±0.5 cm^2)	Control Group
Volume of Mold in milliliters (±0.5)	Day 0	Trial 1	0.0	0.0	0.0	0.0	0.0
		Trial 2	0.0	0.0	0.0	0.0	0.0
		Trial 3	0.0	0.0	0.0	0.0	0.0
	Day 2	Trial 1	0.0	0.0	0.0	0.0	0.0
		Trial 2	0.0	0.0	0.0	0.0	0.0
		Trial 3	0.0	0.0	0.0	0.0	0.0
	Day 4	Trial 1	1.0	1.5	1.5	0.5	1.5
		Trial 2	1.5	1.0	1.5	0.0	1.5
		Trial 3	1.0	1.0	1.0	0.0	1.0
	Day 6	Trial 1	2.0	2.5	2.5	0.5	2.5
		Trial 2	2.5	2.5	2.5	0.5	3.0
		Trial 3	2.5	2.0	2.5	0.5	2.0
	Day 8	Trial 1	3.0	3.5	3.5	1.0	3.5
		Trial 2	3.5	3.0	3.5	1.0	4.0
		Trial 3	3.5	2.5	3.0	0.5	2.5
	Day 10	Trial 1	4.0	4.5	4.5	1.5	4.0
		Trial 2	4.0	3.5	4.5	1.0	4.5
		Trial 3	4.5	3.0	4.0	1.0	3.5
	Day 12	Trial 1	5.0	5.0	5.5	1.5	5.0
		Trial 2	5.0	4.5	5.0	1.5	5.0
		Trial 3	5.0	4.5	5.0	1.5	4.0
	Day 14	Trial 1	5.0	5.0	5.5	1.5	5.0
		Trial 2	5.0	4.5	5.0	1.5	4.0
		Trial 3	5.0	4.5	5.0	1.5	5.0

Table 1 : The table representing the change in the volume of mold, for each trial of each test group and the control group, at each measurement in milliliters.

	The substances which were inserted into each falcon for experimentation for 14 days				
	Wood (±0.5 cm^2)	Plastic (±0.5 cm^2)	Silver Nitrate (±0.5 ml)	Nickel (±0.5 cm^2)	Control Group
Trial 1	5.0	5.0	5.5	1.5	5.0
Trial 2	5.0	4.5	5.0	1.5	4.0
Trial 3	5.0	4.5	5.0	1.5	5.0

Table 2 : The table representing the final volume of mold in each trial of each externally inserted substance in milliliters (±0.5) at the end of 14 days.

	The substances which were inserted into each falcon for experimentation for 14 days				
	Wood (±0.5 cm^2)	Plastic (±0.5 cm^2)	Silver Nitrate (±0.5 ml)	Nickel(±0.5 cm^2)	Control Group
Mean value of the final volumes of mold in ml (±0.5)	5.0	4.67	5.17	1.5	4.67

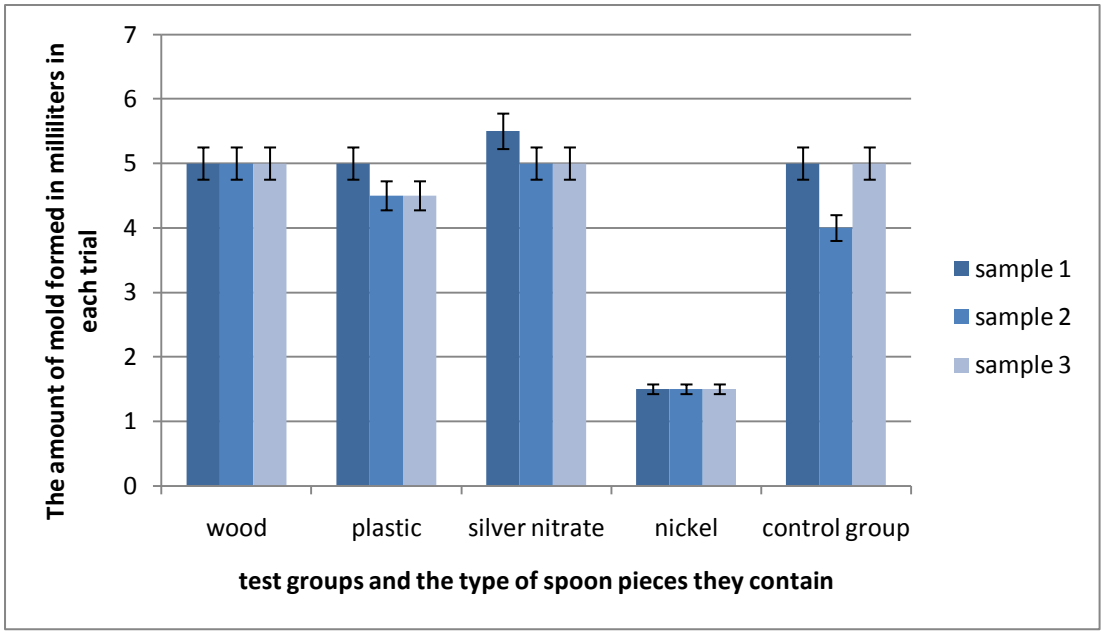
Table 3 : The calculated mean values of the volume of mold formed for every test group and control group in milliliters.

Standard Error Calculation :

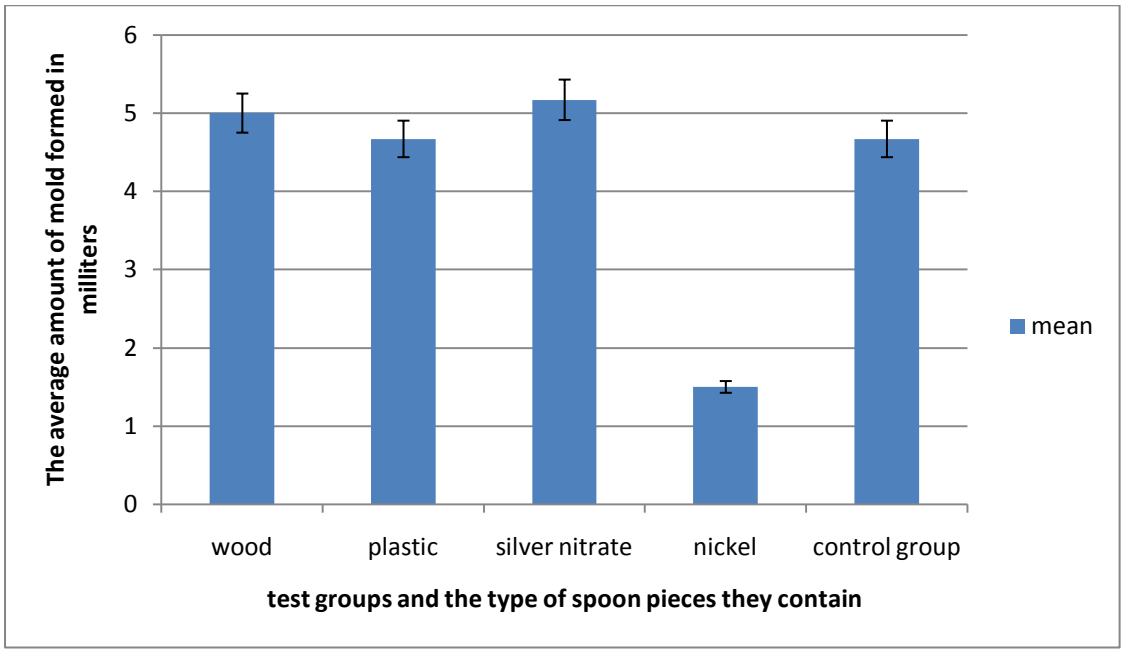
$$SE = \frac{Standard\ Deviation}{\sqrt{number}\ of\ trials} \qquad SD = \bar{x} \pm \sqrt{\frac{\sum x^2 - [(\sum x)^2/n]}{n}}$$

	The substances which were inserted into each falcon for experimentation for 14 days				
	Wood	Plastic	Silver Nitrate	Nickel	Control Group
SD	0.00	0.29	0.29	0.00	0.58
SE	0.00	0.17	0.17	0.00	0.33

Table 4 : The table representing the calculated standard error and standard deviation values for each externally inserted substance and the control group in milliliters.



Graph 1 : The graph representing the amount of mold formed in each falcon for each trial with the standard error calculations in milliliters.



Graph 2 : The graph representing the average amount of mold formed in each test group and control group with the standard error calculations in milliliters.

ANALYSIS :

- The Student's t-Test

	The substances which were inserted into each falcon for experimentation for 14 days				
	Wood	Plastic	Silver Nitrate	Nickel	Control Group
SD	0.00	0.29	0.29	0.00	0.58
SE	0.00	0.17	0.17	0.00	0.33
# of samples	3	3	3	3	3

Table 5 : The table representing the standard deviation, mean calculations of the 5 data sets and the number of trials conducted in each of these individual data sets.

Null Hypothesis (H_O) : There is no effect of external substance. The differences in the data sets are the results of chance variation only and they are not really different.

$$t = \frac{\overline{x1 - x2}}{\sqrt{\frac{SD1^2}{n1} + \frac{SD2^2}{n2}}}$$

Df = n1 + n2 – 2

	L_1 : Wood L_2 : Control Group	L_1 : Plastic L_2 : Control Group	L_1 : Silver Nitrate L_2 : Control Group	L_1 : Nickel L_2 : Control Group
t value	-1.00	0.00	-1.34	9.5
Level of significance (p)	*0.05*	*0.05*	*0.05*	*0.05*
Degrees of freedom (df)	4	4	4	4

Table 6 : The comparison of each test group with the control group by using student's T test. The critical value of 't' for 4 degrees of freedom is : 2.13 at *P= 0.05*

- 95% Confidence Interval

  95%CI = SE x $t_{P(n-1)}$

	The substances which were externally inserted into each falcon				
	Wood	Plastic	Silver Nitrate	Nickel	Control Group
SE	0.00	0.17	0.17	0.00	0.33
# of samples	3	3	3	3	3
Value of 't' at n-1	4.303	4.303	4.303	4.303	4.303
95% CI	0.00	0.73	0.73	0.00	1.42

Table 7 : The calculated 95% CI values for each data set.

- Analysis of Variance Between Groups (ANOVA)

Source	SS	df	MS	F	P
Treatment	27.9	4	6.975	69.75	<.0001
Error	1	10	0.1		
Ss / Bl					
Total	28.9	14			

Table 8 : Comparison of each data set with the help of anova calculation

The critical value of 'F' for 4 degrees of freedom for 15 samples is : 5.86 at *P= 0.05*

CONCLUSION AND EVALUATION :

The aim of this experiment was to show that, the usage of different brands of spoon could also be the reason for the formation of colonies of Saccharomyces rosei on top of a jar of pickled tomato. I started this experiment out, by deciding what may be one of the human based reasons, which caused the pickles to become moldy faster. When I heard from a friend that, there was a common belief, that the pickles became moldy slower, when a metal spoon was used when the pickles were being served. I learnt that people preferred a metal spoon instead of a wooden one to prevent the pickle from molding. To test whether the usage of a metal spoon made an obvious difference or whether it was just a rumor, I planned an experiment. I decided to measure the amount of mold formed in a constant amount of pickle with different brands of spoon. I put equal amounts of pickled tomato and it's juice and placed equal sized plastic, wood, nickel and silver nitrate pieces in different falcons. The reason why I choose plastic, wood, nickel and silver was because these materials can be used to make spoons. I placed the falcons in same and convenient conditions for fermentation and watched them carefully and took notes. After 16 days, the data I was collecting became stationary and I was able to make a comment on them.

According to my hypothesis, I was expecting to see the lowest volume of mold in the falcons which contained the metals silver nitrate, nickel ;and; the highest volume of mold in the falcons which I either put wood, plastic or the falcons I labeled as my control group. As it can be seen in table 1, the data I have collected supported my hypothesis.

As a result of the calculations in which I compared the data sets of wood, plastic, and silver nitrate with the control group, my "t" value was smaller than that of the expected "t" value which corresponded to the level of significance value of p= 0.05 and to the degree of freedom value of df=4.

Opposing to this, the "t" value I calculated when I was comparing the data sets of nickel and the control group was bigger than the literature value of "t" at p= 0.05 and df=4.

According to the rules of the student's "t" test, if the t value calculated is smaller than the literature value of the "t" value required, we accept the suggested null hypothesis (H_0) but on the other hand, if the calculated "t" value is greater than the literature value, we reject the null hypothesis and except the firstly suggested hypothesis.

Under the light of these facts, I can say that, for the data sets of wood, plastic and silver nitrate, we except the suggested null hypothesis. The differences in the data were the

results of chance variation and that they weren't actually affected by the external substance. But for the nickel data set, we can't reject the null hypothesis, and we except that the external substance had a remarkable effect.

Analysis of variance between groups (ANOVA) is used when the result of an experiment consist of more than two mean values. ANOVA is better and more relevant compared to the student's "t" test. Because in the students t test we except on error of 0.05% in each comparison and as the number of data sets we deal with increases, determining the total error becomes almost impossible. But in the case of ANOVA, since we only do a single calculation, it's easier to determine the error.

After the ANOVA calculation, I found my F value to be 69.75 and I saw that my expected F value for 15 samples and at 4 degrees of freedom was equal to 5.86 at a level of significance of p=0.05. Just as in the student's t test, if the calculated F value is greater than the literature value we reject the suggested null hypothesis. But according to my calculations, I saw that my F value was bigger than the literature value, so we reject the null hypothesis and conclude that, in at least one of the cases, the effect of the external substance was observed and that the differences in the results didn't rise from chance variation. And according to the t-test results shown in table 7 and mean results in table 3, I conclude that the case in which I found that the effect of external substance was important, is the data set of Nickel.

The volume of mold formed in all of the trials were close to each other in the cases of wood, plastic, nickel and in the control group. But in the case of silver nitrate, the data was not reliable. This resulted from the fact that silver nitrate was used instead of silver. Since silver nitrate precipitates when it reacts with salt (salt (NaCl (aq)) is present in pickle juice), using silver nitrate was a mistake, since it didn't act as a metal inside the pickle, it just precipitated and turned into an irrelevant salt AgCl.

$$AgNO_3 + NaCl \rightarrow AgCl + NaNO_3$$

Secondly, since I conducted the whole experiment in my house, I wasn't able to carry out the experiment with full success. I wasn't able to keep the temperature constant, since the central heating system was working on determined time intervals. In order to solve this problem an incubator could be used to keep the temperature of the experimentation environment constant at a specific temperature.

Third of all, before I started to test the effect of different substances on the pickles, I cut tomato pieces of 1 cm^3 size and waited for them to be pickled in a common jar. But during the period of time they waited in that jar, their edges may have melted away, which may have caused a change in the surface area of the tomatoes. By this way, the area where diffusion can occur decreased and this may have led to a decrease in the volume of mold formed. To prevent any possible surface loss, the tomatoes should either be pickled first and then cut into $1cm^3$ pieces, or they can be grated first and then pickled.

Fourth of all, when I was taking the falcons from the rack, to take their photographs, the pickles in the falcons shook, and some of the mold formed mixed with the juice and disappeared. In order to prevent any mold loss, separate and see through racks should be used for each data set. And these racks should be placed on a smooth surface to prevent the falcons from any possible vibration.

After I filled the falcons, when I was closing their lids, some amount of oxygen gas was left in the falcons. Since Saccharomyces rosei is a fungus which does anaerobic respiration, the presence of oxygen inside the falcons may have inhibited the respiration of the fungi to some extent, and this may have resulted in formation of less mold compared to the mold we would get if the experiment was conducted in an oxygen proof falcon. To overcome this error source, after filling the falcon, liquid oil can be poured inside the falcon until the surface of the pickles is covered. By this way the interaction of oxygen and fungi can be prevented.

We do not have any idea about the metal percentages in the spoons we use at home since they are alloys. Thus we can't be sure which metal contributed to the formation of mold and which metal didn't. To be sure of this, pure silver, pure nickel etc. should be used.

Lastly I was only able to do 3 trials for each substance inserted. If there were more trials, I would have been able to talk about more definite facts.

For further experimentation, one can test the effect of the metal which was inserted into the pickles in the first stage of pickling.

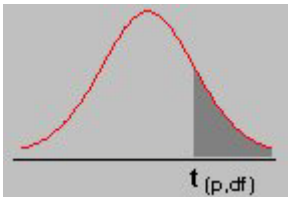
Since the temperature of the environment has a positive effect on the rate of reaction, one can question why the pickles aren't being stored in refrigerators.

Other than these, one can also conduct this experiment with different vegetables and without any external substances, so see the amount of contribution of the ions found internally in the vegetables to the amount of mold formed.

APPENDICES :

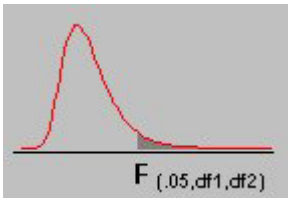
- APPENDIX 1 : Statistics Tables

t table with right tail probabilities



df\ p	0.40	0.25	0.10	0.05	0.025	0.01	0.005	0.0005
1	0.324920	1.000000	3.077684	6.313752	12.70620	31.82052	63.65674	636.6192
2	0.288675	0.816497	1.885618	2.919986	4.30265	6.96456	9.92484	31.5991
3	0.276671	0.764892	1.637744	2.353363	3.18245	4.54070	5.84091	12.9240
4	0.270722	0.740697	1.533206	**2.131847**	2.77645	3.74695	4.60409	8.6103
5	0.267181	0.726687	1.475884	2.015048	2.57058	3.36493	4.03214	6.8688

F Table for alpha=.05 .



df 2/ df 1	1	2	3	4	5	6	7	8	9	10	12	15	20	24	30	40	60	120	INF
1	161.4476	199.5000	215.7073	224.5832	230.1619	233.9860	236.7684	238.8827	240.5433	241.8817	243.9060	245.9499	248.0131	249.0518	250.0951	251.1432	252.1957	253.2529	254.3144
2	18.5128	19.0000	19.1643	19.2468	19.2964	19.3295	19.3532	19.3710	19.3848	19.3959	19.4125	19.4291	19.4458	19.4541	19.4624	19.4707	19.4791	19.4874	19.4957
3	10.1280	9.5521	9.2766	9.1172	9.0135	8.9406	8.8867	8.8452	8.8123	8.7855	8.7446	8.7029	8.6602	8.6385	8.6166	8.5944	8.5720	8.5494	8.5264
4	7.7086	6.9443	6.5914	6.3882	6.2561	6.1631	6.0942	6.0410	5.9988	5.9644	5.9117	**5.8578**	5.8025	5.7744	5.7459	5.7170	5.6877	5.6581	5.6281
5	6.6079	5.7861	5.4095	5.1922	5.0503	4.9503	4.8759	4.8183	4.7725	4.7351	4.6777	4.6188	4.5581	4.5272	4.4957	4.4638	4.4314	4.3985	4.3650

- APPENDIX 2 : Photographs

Figure 1 : Test group of silver nitrate at day ‘0’.

Figure 2 : Test group of silver nitrate at day ‘6’.

Figure 3 : Test group of silver nitrate at day ‘14’.

Figure 4 : Test group of nickel at day ‘0’.

Figure 5 : Test group of nickel at day ‘6’.

Figure 6 : Test group of nickel at day ‘14’.

Figure 7 : Test group of wood at day ‘0’.

Figure 8 : Test group of wood at day ‘6’.

Figure 9 : Test group of wood at day ’14’.

Figure 10 : Test group of plastic at day ‘0’.

Figure 11 : Test group of plastic at day ‘6’.

Figure 12 : Test group of plastic at day ‘14’.

Figure 13 : Control group at day ‘0’.

Figure 14 : Control group at day ‘6’.

Figure 15 : Control group at day ‘14’.

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