Growth of mould in different conditions

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Background information

Moulds belong to kingdom fungi. Fungi are eukaryotic organisms and can develop both indoors and outdoors. Based on the spore case (sporangium) in which the spores are produced fungi are classified into four divisions: 1. Sac-fungi (*Ascomycota*) – ex. is truffles; 2. Club fungi (*Basidiomycota*) – ex. is *Agaricus* (mushroom); 3. Zygote forming fungi (*Zygomycota*) – ex. is *Rhizopus* (the bread mould) and 4. Imperfect fungi (*Deuteromycota*) – ex. is *Peniccilium*. Most of the mould species are zygote forming fungi, usually found on cheese, bread, and other decaying food. The exact number of existing species of mould is unknown. One of the most well-known mould species is Rhizopus, the bread mould. Moulds spread and reproduce by making spores. These spores can be found everywhere around us but we cannot see them. Moulds, as well as other members of fungi kingdom, do not contain chlorophyll, so they cannot make their own food. For nutrition, they settle down in the places where they can find energy that is - on other organisms (plants and animals), this makes them heterotrophic.[1]

Moulds can grow on many types of food. Most often, we can find them on bread, because bread is a very rich source of nutrients for mould. Moulds also grows more often on bread because it is usually kept in warm and moist places and these are the desirable conditions for mould growth. A moist environment helps mould spores grow and reproduce. Warm environment is also desirable for mould because the enzymes inside the mould cells are able to work faster, while directing biochemical processes of the cells, when the temperature is higher.[2]

Mould can be both beneficial and harmful for us. They are widely used in medicine, as they naturally produce antibiotics, to kill and inhinit the growth of bacteria. Humans started producing antibiotics made from mould, which have been very valuable for the human population as a whole. One type of mould called *Penicillium* is used for the production of antibiotic called Penicilin. It is also involved in the ripening of many cheeses. Another well-known type of mould that is used by humans is *Saccharomyces cerevisiae*, also known as "baker's yeast", an important ingredient in making bread.[3]

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1 Kingdom fungi: http://biology.tutorvista.com/organism/kingdom-fungi.html
Retrieved on 13 November, 2015

2 Why is it so that bread mold grows quicker in dark, wet and warm conditions?
http://www.madsci.org/posts/archives/1997-05/863449273.Mi.r.html
Retrieved on 13 November,2015

Moulds can also be harmful for our health. It can cause a variety of symptoms. These symptoms can differ from person to person and from age to age; also, sensitive people who have allergies are more likely to be affected. Mould, depending on its type, is able to cause infections in our bodies. Some types of diseases like asthma can also be caused by mould (*Aspergillus*); however, it is very rare. These reasons suggest that we should have strict control over mould growth.[4]

Aim

In this investigation, we will find out how fast mould grows on bread if it is placed in room temperature, moist, warm, or cold conditions and if the pH is lowered to two by adding lemon juice. It is common knowledge that mould is a type of fungi, which can sometimes appear harmful to us, especially if it grows on the products that we consume every day. For this reason, we need to control it. More importantly, we need to know where to store our food to keep it safe. After this experiment, we will know in which conditions we need to keep bread in order to avoid fast growth of mould.

Design

Research question

In which condition does mould grow fastest on white bread?

Hypothesis

The bread will be stored in five different conditions to see which of them is most suitable for mould growth. We hypothesize that mould will most likely develop in warm and moist conditions because high temperature makes the enzymes inside the mould cell work faster and a moist condition allows

3 Importance of Fungi in Human Life https://www.boundless.com/biology/textbooks/boundless-biology-textbook/fungi-24/importance-of-fungi-in-human-life-153/importance-of-fungi-in-human-life-601-12945/
Retrieved on 13 November,2015

4 Mold->general information->basic facts http://www.cdc.gov/mold/faqs.htm#mold
Retrieved on 13 November,2015

cells to reproduce faster, since water is necessary for growth and it makes up most of the cell. Lowering the pH slows down mould development, so it is unlikely that the mould will grow fastest in this condition. Dry and cold conditions will also be less likely to result in mould production as they also slow down the enzymes functioning inside the cell.

Variables

Independent variables - conditions the bread slices are put in.

- Room temperature 21°C
- Moist
- Warm 40°C
- Cold 4°C
- Acidic - pH 2 (lemon juice)

Dependent variables - The speed at which mould is growing (measured by the change in area on the bread slice covered in mould)

Controlled variables – It is essential to be sure that the independent variable (IV) is the only factor that can affect dependent variable. All the other variables that might affect the IV must therefore be controlled. Controlled variables in this experiment include type of bread, lighting of the place, bread slices were put in, period of time during which the changes were measured.

- Bread used for sampling was white bread (made from white flour)
- How fresh the bread is. Bread should be newly baked, so that the conditions it was in before, would not affect the experiment
- No light
- Size of bread slice. They were cut into equal sized cubes. Length- 10 cm, Width- 7 cm, and Height- 1.5 cm.
- Development and growth of mould on bread was investigated for 10 days.

Controlled variables need to be kept constant because the can affect the results of the experiment.

Materials used

Necessary materials for conducting this experiment are 15 slices of white bread, a few drops of lemon juice (8 grams) and water (8 grams). A clean knife is needed to cut bread into slices. A

thermometer must be used to measure the temperatures in different conditions. Plastic bags (each bread slice was placed in a separate plastic bag). Hand sanitizer is recommended after contact with moulded bread to make sure that the fungi does not spread. To measure the width of each bread slice and to measure the mould grown on it, a ruler is essential. A teaspoon (4 grams) is needed to measure the amount of water and lemon juice put on bread. The other essential objects are the refrigerator and warm incubator, which are usually present in biology laboratories.

Method

1. Cut bread into slices, equal in size.
    - Using a ruler measure and mark height of each slice, to make sure that the size of the slices will be equal (1.5 cm)
    - Start cutting the bread into slices
    - After you have slices that are equal in height, using a ruler and a knife, make them equal in length (10cm) and width (7cm) as well.
2. Take out plastic bags and take three slices of bread per condition
    - In total you should have 15 slices of bread
    - Put 3 slices in each condition
    - Put each slice in a separate plastic bag, to avoid spreading mould, and also to make it easier to measure
3. Put bread slices in room temperature, and in warm and cold conditions.
    - First clean all the places you will leave bread in, to avoid any moisture from the outside getting in touch with bread slices
    - Put first 3 slices in dry cupboard , temperature inside the cupboard must be equal to 21°C
    - For warm condition you need a warm incubator, set the incubator to 40°C and put the next 3 slices in it
    - Put the other 3 slices in the refrigerator set to 4°C
    - Use a teaspoon (tsp.) to add 2 tsp. (8 grams) of water to the slices which will be in wet condition
    - Add 2 tsp. (8 grams) of lemon juice to the remaining 3 slices of bread
    - Put these 6 slices in the cupboard where the temperature was 21°C
4. The temperature of the place for acidic, moist and dry bread slices should be the same (21°C), so that is does not affect the results. This can be controlled by putting the slices in the same place (cupboard), since they are all in separate plastic bags and will not affect each other in any way
5. Make sure that all the slices are in the dark

- For dry, moist, and low-pH conditions use cupboards that can be closed to prevent light from coming in
- For cold condition, turn off the light of the refrigerator
- For warm condition, as the door of the incubator is made from glass, and the light can easily pass through, you will need to cover it with pressboard or with something else, that will not let the light to pass through

6. Check the bread slices every day to see whether the mould started growing.
7. Measure the temperature of the place where bread slices are, every day. It should be kept at 21°C, 40°C, and 4 °C
8. Once you see mould on any slice of the bread start measuring it every third day.[5] As it is expected that the mould on each bread will grow on different days, the experiment might last longer than expected
    - Use plastic gloves when touching the slices
    - Put the slices in a place, covered with an oilcloth
    - Using a ruler, measure the size (length and width) of mould grown on the bread
9. After contact with moulded bread, clean your hands with hand sanitizer
10. Make notes of your measurements and other observations.
11. Your experiment should last 9 days
12. After 9 days, measure the size of mould and throw away moulded bread
13. Clean all the places you put the bread in to prevent spreading the fungi spores
    - Use a clean sponge and a disinfectant

Data Collection and Processing

Collected raw data:

The colour of mould differed in each condition, from light green to dark grey. In the acidic condition, the color was dark green; in the wet condition it was light green with white around it; at room temperature (21°C) the colour was grey. These observations were same for all three slices in the

5 The reason why we start measuring mould growth from the 1st day of growth, and not from the day the bread was baked, is because we are interested in the rate of growth, and that is the same throughout the whole process, so it makes no difference which period you choose during the process

same condition. The texture of the mould grown could be compared to cotton, it was soft and had little spores on it.

Table 1 shows days when first mould growth could be noticed on bread slices. This was measured from the day when bread slices were put in every condition.

Table 1. *Observations of mould growth in different conditions*

Conditions	**Did mould grow?**	**Day of first growth**		
		Slice 1	**Slice 2**	**Slice 3**
Room temp. 21°	Yes	Day 4	Day 6	Day 7
Wet	Yes	Day 3	Day 3	Day 4
Cold 4°C	No	No answer (N/A)	N/A	N/A
Warm 40°C	No	(N/A)	N/A	N/A
Ph2. Lemon juice	Yes	Day 3	Day 4	Day 6

The surface area of each bread slice is $70 cm^2$. This is calculated by multiplying length (10cm) by width (7cm).

Table 2 shows the values of the area of mould that grew on each slice of bread on the third, sixth, and ninth day, since the day of its first growth, again, these were different for every slice.

Table 2. *Area of the mould grown on each bread slice, in each condition.*

	Wet			**Room temp. 21°**			**pH 2.**		
	Day3	**Day6**	**Day9**	**Day3**	**Day6**	**Day9**	**Day3**	**Day6**	**Day9**
	Uncertainties: ± $0.2 cm^2$								
Sample 1 (cm^2)	4	6	60	1	4	10	3	40	64
Sample 2 (cm^2)	10	14	25		3	8	1	8	20

				$\frac{1}{2}$					
Sample 3 (cm^2)	10	18	32	3	8	21	10	28	48

Data processing

We need to calculate the ratio of the total area of the bread slice to the area of mould grown on the surface of bread in percentage for each sample. For this purpose, each value from table 2 needs to be divided by $70\,cm^2$ (are of the bread slice) and multiplied by 100. The results obtained from these operations are shown on table 3.

Table 3. *The ratio of the area of mould grown on a slice to the total surface area of bread slice, given in percentages*

	Wet			**Room temp. 21°**			**pH 2.**		
	Day3	**Day6**	**Day9**	**Day3**	**Day6**	**Day9**	**Day3**	**Day6**	**Day9**
	Uncertainties: ± 0.2								
Sample 1 (%)	5.71	8.57	85.71	1.43	5.71	14.29	4.29	57.14	91.43
Sample 2 (%)	14.29	20.00	35.71	0.71	4.29	11.43	1.43	11.43	28.57
Sample 3 (%)	14.29	25.71	45.71	4.29	11.43	30.00	14.29	40.00	68.57

We will use this formula to calculate growth rate of mould in each condition:

$$growth\,rate = \frac{V\,present - V\,initial}{V\,initial} \quad .$$

Where:
$V_{Present}$ = Present Value for day 9 of each condition.
$V_{initial}$ = First values, from days 3 and 6.

New values are listed in table 4.

Table 4. *Growth rate of mould on each bread slice(sample), in wet condition, at room temperature and in acidic condition*

	Uncertainties: ± 0.02		
	Wet	**Room temp. 21°**	**pH 2**
Sample 1 (%)	5.00	1.00	0.49
Sample 2 (%)	0.04	1.29	1.22
Sample 3 (%)	0.14	0.91	0.26

Now, we need to calculate the average growth rate of mould in each condition. This can be done by using the formula for mean value. After finding the mean, we will know in which condition mould grows fastest. Average values for each condition are shown in table 5.

Table 5. *Average values for the growth rate of mould in each condition*

	Uncertainties: ± 0.02		
	Wet	**Room temp. 21°**	**pH 2**
Average value (%)	1.73	1.07	1.97

From table 5, we can see that the highest average value is obtained in acidic condition. This means that the mould grew fastest in that condition.

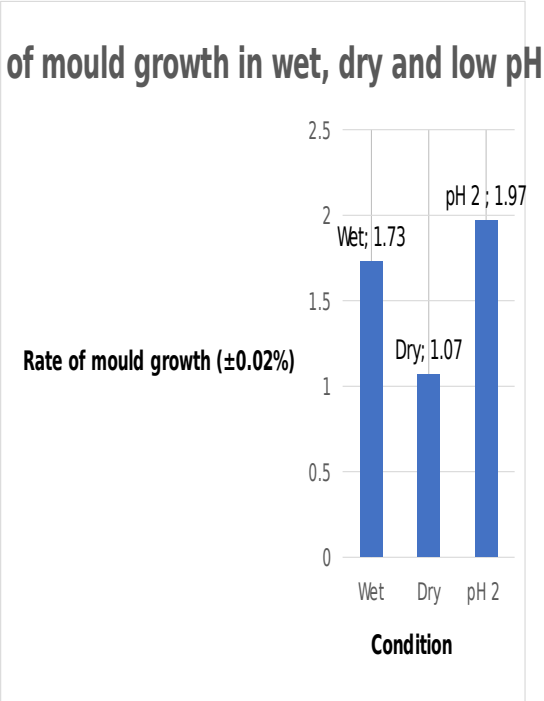
Now, standard deviation for each condition needs to be calculated. Standard deviation indicates how spread the values are around the mean. It will tell us whether the results we obtained are reliable. To calculate standard deviation, this formula is given:

$$\sqrt{\frac{\sum(x-\mu)^2}{n-1}}$$

In this formula, *x* stands for each value per each condition from table 4; and *n* stands for the number of these values per each condition. So when the standard deviation (st) for the data we obtained is calculated, the results are as follows: for wet condition – st = 2.84; for acidic condition - st = 1.68; and at the room temperature- st = 0.20. These results indicate that the values were the most accurate for acidic (pH2) condition, whereas for wet condition, they were furthest away from the mean.

The results can also be shown on the histogram, as in Graph 1. As we said before, the fastest average growth rate, could be found in acidic condition.

Presentation



Graph 1.

The results can also be shown on the histogram, as in Graph 1. As we said before, the fastest average growth rate, could be found in acidic condition.

Conclusion and evaluation

Limitations of experimental design

The data which was obtained from this experiment was not reliable at some point, because when the standard deviation was calculated, it gave us an answer which was more than one for both, room temperature and wet condition. This means that the values were too far from the mean. For this to be improved, changes in the area of mould must be measured every day.

This experiment was carried out at a school. This made it difficult to control all the confounding variables, also it limited the access to the experiment, so the changes could only be measured at a certain time during the day. These factors could affect the results which were obtained.

There was lack of apparatus, for example there was no hygrometer to measure humidity of the places where wet and dry slices of bread were put in. As we cannot touch the mould, it was difficult to take the exact measurements, which led to uncertainties.

Suggestions for improvement

To improve the experimental design, the conditions need to be stricter. Meaning that the controlled variables need to be kept under control all the time, for example, there can be some temperature changes during the night and it can affect the mould growth, if so, this needs to be avoided by limiting air access to the cupboard where bread slices are put in. Changes in area of mould, must be measured every day. The materials, which are necessary, need to be obtained in advance. The period of time can also be longer than 9 days for each condition, this would give more accurate data. The total time spent on the experiment can be extended to see whether and when mould will start growing in cold and warm conditions.

Conclusion

This experiment, was conducted in order to find out the most suitable conditions to store bread where the mould growth is less likely to occur in a short period of time. For this purpose, slices of white bread were put in five different conditions, where they were kept for nine days from the day when the first mould growth occurred. Out of five conditions which were investigated, the mould grew fastest in the acidic condition. That was where 2 tsp. of lemon juice were poured on bread. This was

shown by using tables and formulas to process the data. Other results we received during the period of nine days are the following: in the wet condition, the average growth rate of mould in percentage was 1.73; in room temperature 1.07; and in low pH condition, it was 0.97. In the two other conditions, which were cold (4°) and warm (40°), no mould grew on bread. In the warm condition the bread dried completely, in the cold condition everything remained the same. According to our results, the second fastest growth occurred in the wet condition and the third in room temperature (21°).

As a conclusion, it can be said that the bread should not be kept in acidic or wet conditions. The best place to keep bread is at room temperature (21°C) or, if you need to store it for a longer time, it can be put in the refrigerator.

The hypothesis was not supported by the experiment taken place. This can be a result of some uncertainties which were involved during the experiment, they might have had an effect on the results, for example the temperature changes during the night were not controlled. This makes the obtained results, unreliable to some extent.

References

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