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AN INVESTIGATION IN

# MOULD GROWTH

UNDER THE EXPOSURE OF LIGHTS  
OF DIFFERENT WAVELENGTHS

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RESEARCH QUESTION:

To what extent do different wavelengths of the visible light spectrum inhibit food degradation by moulds specifically on 31% wholegrain bread within 14 days?

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# 1. INTRODUCTION

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## 1.1 Aims of Study

The presence of mould is something many people are familiar with in their everyday lives, especially in the form of food spoilage. Food degradation is defined as changes which render food unfit for human consumption where the most common sources are from microbial contaminations such as from yeast or fungi.<sup>1</sup>

Food spoilage is posing itself to be an increasing problem today due to how it contributes to food waste. One-third of the food produced globally goes to waste<sup>2</sup> and it is estimated that in the USA by the USDA that “21% of the total food supply is lost at the consumer level,”<sup>3</sup> In addition, spoilage is not only limited to the consumer level, but it also affects corporations and farmers alike. The Western European bread industries alone lose 200 million euros per year due to spoilage.<sup>4</sup> Therefore, it is safe to declare that spoilage is generally not beneficial, economically or otherwise, to anyone anywhere in the world. The amount of food wasted is astounding and thus, the purpose of this study is to attempt in discovering any simple solutions to a much larger problem in order to make this a more sustainable world.

To look further into food waste and food spoilage, I chose to explore how they can be minimised through experimenting on mould growth. There have been several studies in the past about mould prevention and many conclusions have been drawn about them. All in all, it is agreed that the main factors which able mould growth are humidity, pH and

temperature.<sup>4,5</sup> Therefore, in curiosity to investigate something less common that can still prove to be nonetheless important, the research question arrived at was:

***To what extent do different wavelengths of the visible light spectrum inhibit food degradation by moulds specifically on 31% wholegrain bread within 14 days?***

If a certain coloured light proves to be more efficient in inhibiting mould growth on bread, it can mean that, by applying the same concept of colour, adjustments can be made to the way of how people store their bread or food. For example, food can be stored through the use of coloured plastic bags, coloured containers or even colour-lighted cupboards. By doing this, more time can be granted for people to finish their food before it spoils, thus reducing waste. This can also be applied to a larger picture as corporates or farmers can use this concept to decelerate fungal growth on their goods that they wish to sell.

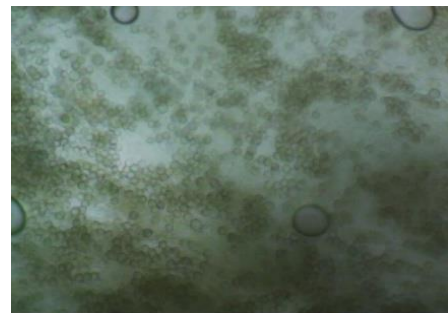
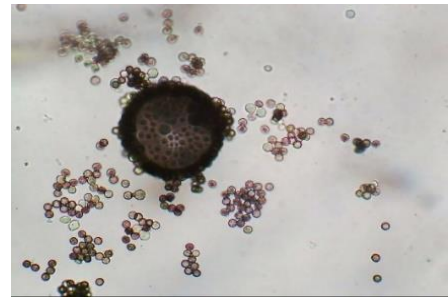
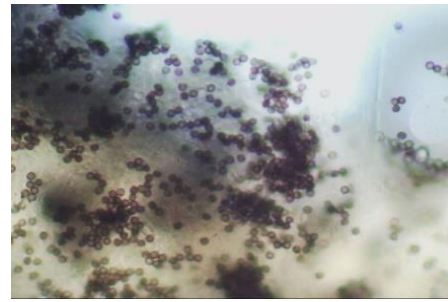
## 1.2 Background Information & Other Scientific Research

Mould is a common name for several species of the Fungi kingdom.<sup>6</sup> Thousands of species exist to the knowledge of humans and there are likely more which have yet to be discovered.<sup>7</sup> Most moulds are known saprotrophs which reproduce by releasing spores that land on decaying organic matter. They derive nutrients through the usage of digestive enzymes to break down large molecules into smaller molecules before absorbing them.<sup>6</sup> Moulds are very widespread due to their ability to reproduce in large numbers and how their spores can be transported through air.<sup>6</sup>

Mould is a mass of mycelium which consists of threadlike hyphae and fruiting structures<sup>6</sup> (*figures 1*).

They share several similar characteristics to plants, such as their composition of a stalk-like structure and cell walls. However, a large aspect which differentiates moulds from plants is the absence of chlorophyll in moulds,<sup>6</sup> meaning that they are unable to photosynthesise.

Despite that, it has been observed that light still has an effect on their behaviour and development. According to Luis M. Corrochano,<sup>8</sup> who wrote an article on fungal photoreceptors, “light regulates fungal development and behaviour and activates metabolic pathways”. He explained how fungi use light as signals to “perceive and interact” with their environment and how certain reactions are brought forth due to a certain light colour.<sup>8</sup> This



Figures 1 Pictures of fruiting structures of *Rhizopus stolonifer* and *Penicillium* taken from samples in the experiment at 40x magnification.

study proves that moulds or fungi are not “blind”. However, to predict which colour light would be most effective in inhibiting mould growth or terminate spore germination, it is possible to use information from other researches such as the usage of UV irradiation to treat mould. The usage of UV has been studied and proved that shorter wavelengths do inhibit mould growth on foods such as grapes<sup>9</sup> or baby spinach.<sup>10</sup>

Therefore, based on these studies, the hypothesis drawn about this investigation is that shorter wavelengths, such as blue light, would be the most successful in inhibiting fungal growth.

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## 2. METHODOLOGY

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### 2.1 Variables

The aim of the study is to determine if different wavelengths of the visible light spectrum will affect mould growth. The independent variable is therefore light colour; wherein this experiment was red (620-750nm), blue (450-495nm), yellow (570-590nm), white (400-700nm) and total



Figure 2 Usage of the gridded sheet to measure the moulded area.

darkness. The dependent variable is the area of mould ( $\text{mm}^2$ ) measured by placing a transparent sheet with  $1\text{mm}^2$  grids above the moulded area of the sealed bread sample. The squares with over 50% of mould coverage are then counted (*figure 2*).

Controlled variables include: the distance between the light bulb and the samples, kept constant at a 30cm distance. The time which the breads are kept under the lights is also kept constant by not removing the bread from its position or only removing it for a minimum period of time during measuring. Both these variables have the purpose of ensuring that all of the samples are exposed to the same quality and quantity of light.

The volume of water added to each slice of bread is kept constant by only dropping 2ml of water onto one bread surface. Where the purpose of adding water is to create a more suitable (humid) environment for mould growth in order to speed up the experiment and decrease the probability of no growth.

The time which each bread slices is exposed to the air during preparation is kept constant by timing preparation correctly to ensure that one sample does not have an advantage of being exposed to more mould spores than another.

The type of plastic bag used to seal each bread sample is kept the same by using those from the same package. It is important as different plastic bags can cause a difference in light exposure if one is opaquer than another. The temperature is also kept constant at room temperature by keeping the samples indoors at all time as temperature can affect how favourable mould growth would be.

The same loaf of bread is used to reduce errors such as the length of time which the bread was exposed to spores during its production. Finally, the surface where mould is measured from is always the same, in addition to that being the surface where water was dropped on.



## 2.2 Experimental Procedure

The preparation of the experiment occurred the day before the stated expiration date of the bread. One slice of bread was taken and cut in half before 2ml of water was dripped, with a pipette, carefully and equally onto one surface. The now damp 50mm to 110mm bread pieces were then placed into a plastic bag where it was sealed by tape and labelled.

Twenty-five pieces of bread were necessary in total as five replicates were used for each experiment of a different coloured light. Measuring for all samples to keep a distance of 30cm from the light source, they are then left under their respective controlled environment for a continuous of 14 days. The samples are checked for mould growth every 12 hours and those which did display growth is then taken from its position to be measured (using the gridded sheet) for a maximum of 5 minutes before being returned to its position.

*Figures 3* show the set up for all the experiments.



Figure 3.1 Set up for red and blue lighting



Figure 3.2 set up for yellow and white lighting

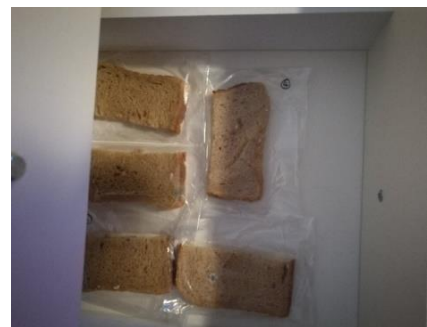


Figure 3.3 set up for no lighting (kept in a drawer in total darkness)

## 2.3 Safety Precautions

After the experiment has ended, none of the samples was taken out of the plastic but disposed of directly to prevent mould spores from escaping. Although bread mould is not usually considered dangerous, there had been health risks regarding mould spore inhalation which can cause respiratory problems.<sup>11</sup> In other instances, a few moulds may start to produce mycotoxins which can cause poisoning and can have long term effects on one's health if ingested.<sup>12</sup> As the purpose of the experiment is to allow heavy mould growth, the probability of the presence of mycotoxins was higher.<sup>13</sup> However, as long as the bread is not consumed, one would be safe from mycotoxins, but the same cannot be said for spore inhalation, especially to people who have mould allergies or are immune-compromised.<sup>14</sup> Inhaling mould spores are not dangerous to healthy people, but it is still best avoided.

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# 3. RESULTS & ANALYSIS

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## 3.1 Raw Data

(see appendix)

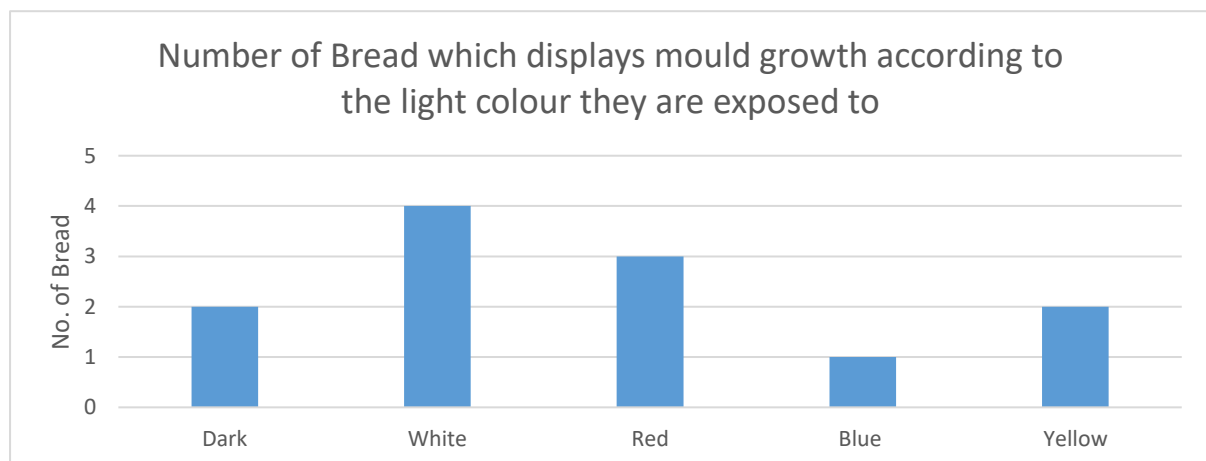


Figure 4 Bread samples under white light during the experiment displaying the various stages of mould growth with one displaying no mould growth.

## 3.2 Processed Data

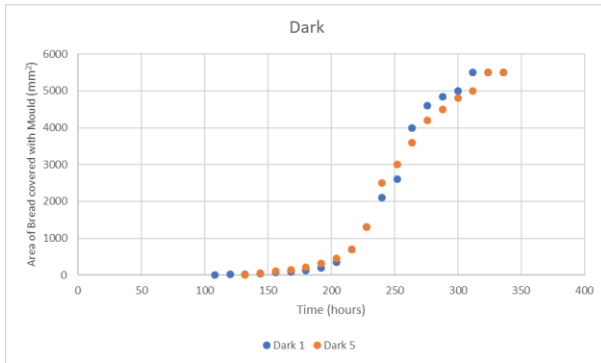
There was no growth on any of the bread slices for the first four days of the experiment and it was only in the morning of the fifth day, that the first signs of mould appeared. The experiment ended after fourteen days (336 hours) thus no more data is collected after that.

Out of five samples of each experiment, not all showed signs of spoilage (*Graph 1, figure 4*). For example, while the samples in the dark were the first to show signs of mould and the first to reach maximum growth, only two out of five moulded in total. Whereas for white light, four out of its five samples moulded in the end but the display of moulding only appeared after the samples in the dark.

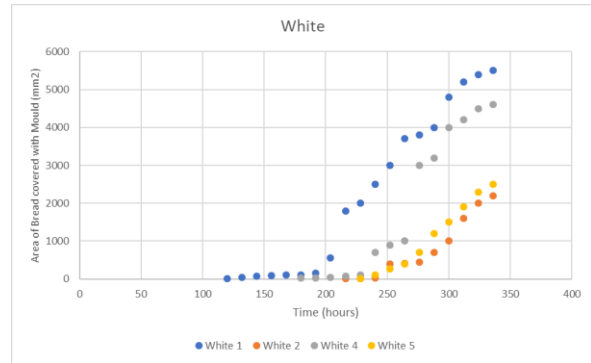


Graph 1 – overview of the number of breads (out of 5) from each experiment which showed signs of spoilage according to the light colour they are exposed to at the end of the experiment.

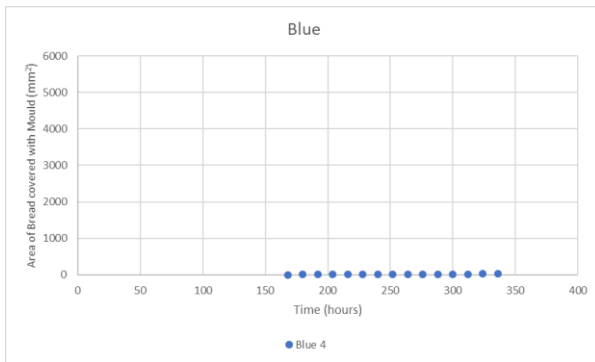
# Graphs of Mould Growth on Individual Bread



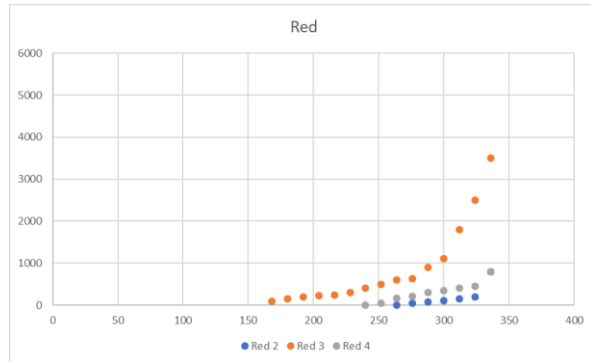
**Graph 2.1** Area of mould grown on individual bread in the dark over time



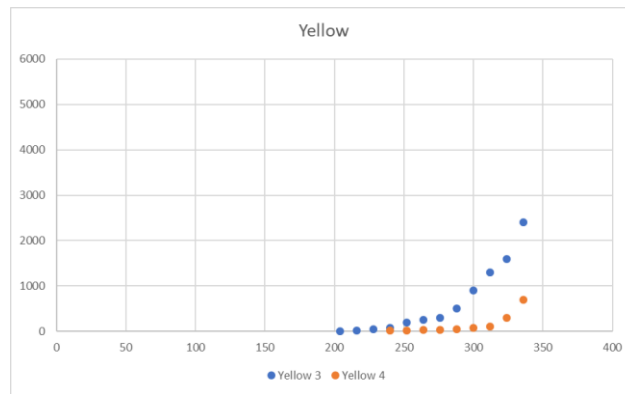
**Graph 2.2** Area of mould grown on individual bread under white light over time



**Graph 2.3** Area of mould grown on individual bread under blue light over time

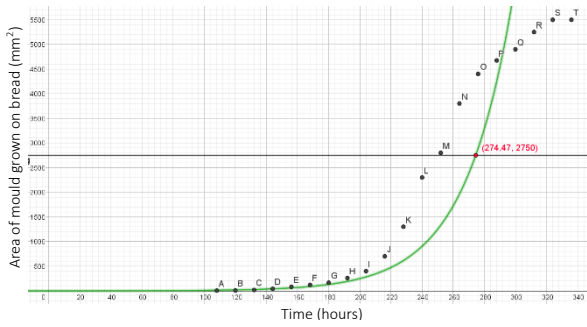


**Graph 2.4** Area of mould grown on individual bread under red light over time

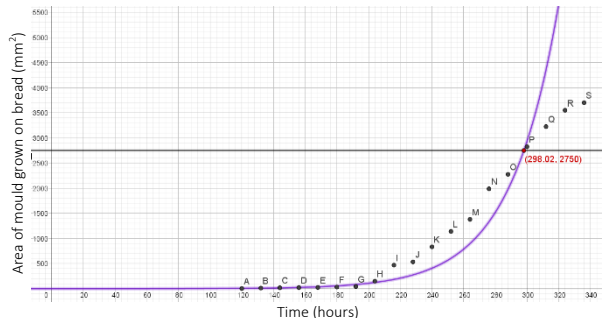


**Graph 2.5** Area of mould grown on individual bread under yellow light over time

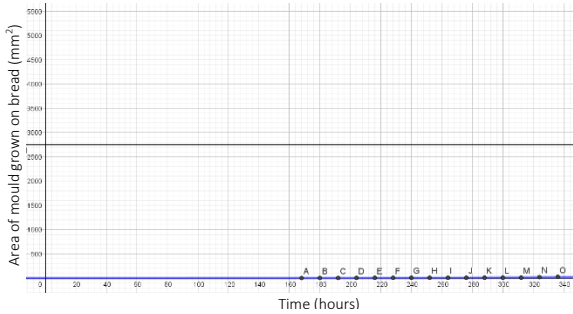
# Graphs of Average Mould Growth



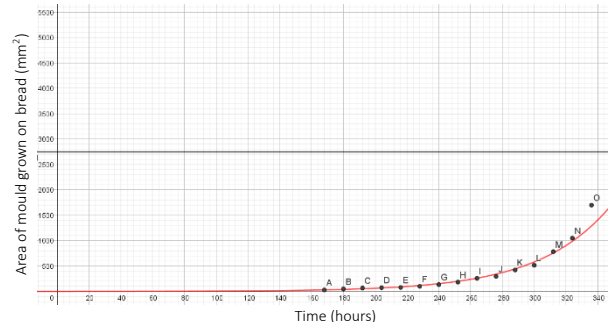
**Graph 3.1 Average area of mould grown on bread in the dark**



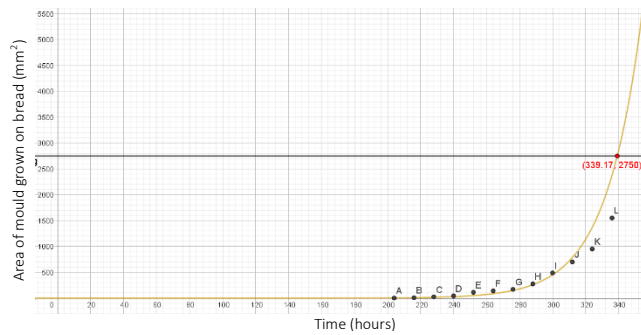
**Graph 3.2 Average area of mould grown on bread under white light**



**Graph 3.3 Average area of mould grown on bread under blue light**



**Graph 3.4 Average area of mould grown on bread under red light**



**Graph 3.5 Average area of mould grown on bread under yellow light**

The data collected of the bread which moulded were first plotted on graphs to show the mould coated area ( $\text{mm}^2$ ) against time (hours), referring to *graphs 2* (page 12). The graphs are divided by light colour and only samples which showed signs of mould growth are plotted. For most samples, there is a noticeable pattern of growth following the sigmoid growth curve. The population would start off by increasing slowly before accelerating and grows exponentially until they reached the carrying capacity where the curves plateau (seen very clearly on *graph 2.1*).

While *graphs 2* show the pattern of growth, *graphs 3* (page 13) was drawn in order to make a conclusion about which colour was most efficient in inhibiting mould growth. Each graph is differentiated by light colour and the dots show the average area which the mould has covered between the spoiled samples. From that, a trendline was drawn for each light for comparisons between them by using the time it took for the average area of mould to cover half of the bread surface.

Through the usage of this method of comparison, it can be seen clearly in both *graphs 2 and 3* that blue light was the most efficient in inhibiting mould growth. While on the other hand, it can be concluded that the absence of light and white light was the most inefficient in inhibiting mould growth. Bread kept in the dark was the first to cross halfway as gathered from *graphs 3* while bread kept under white light moulded the most, at the same time they also came second to the samples in the dark to cover half the bread surface.

Despite not being as efficient in inhibiting mould growth as blue light, red light came second to it out of the five different lighting conditions while yellow came third when using *graphs 3* in comparison. However, both red and yellow were very similar as a whole as only two out of five moulded for yellow while three out of two moulded on red.

### 3.3 Different Types of Moulds (Qualitative Data)

More than one type of mould grew during the experiment and was differentiated through its different colours and other visible characteristics. However, several moulds share similar colour and therefore it is very difficult to properly identify any species accurately without the correct equipment. For this analysis, assumptions are made based off their colours and probability of growing on bread. The most common bread moulds include *Penicillium* sp., *Aspergillus* sp. (and *Rhizopus stolonifer*) which were ones which were assumed to be identified in this experiment.

A fuzzy greenish blue-grey mould with the occasional white mycelia was the most common mould out of all others and was also the first to make an appearance in the experiment. Due to its blue-green colour,<sup>15</sup> it is assumed that it belongs to the genus *Penicillium* which is sometimes also called “blue mould”. *Penicillium* was present in the majority of the samples and after a long period of degradation, the bread samples soften and is visibly sunken.

The second mould to appear was likely from the genus *Aspergillus* (figures 5.2 and 5.3), it is identified from its yellow colour although some of the green patches can also belong to that genus as well.<sup>16</sup> It was found growing mostly under white



Figure 5.1 Beginning growth of *Penicillium* growing on sample under white light.



Figure 5.2 Beginning growth of *Aspergillus* on the top surface of the bread from the white light sample.

and yellow light, although there were also slight traces of it found also under red light. However, despite the visibility of yellow moulds on the top surface of the bread, there was a much heavier growth on the underside of the sample (figure 5.3). In addition, some of the samples with *Aspergillus* hardened, however, that could have been due to factors other than the species of mould.

Lastly, the mould with least appearance in the experiment was *Rhizopus stolonifer*, also known as “Black bread mould”. It was only seen on one sample surface (figure 5.4) and amongst so many other moulds, it was difficult to tell if it was even a new type of mould or simply a more concentrated or darker version of another. However, from the pictures taken from the microscope at 40x (figures 6, page 17), the colour of the fruiting structures was noticeably darker than the one of *Penicillium*.

Different moulds grow on different breads and in this experiment, it seems that *penicillium* was the preferred mould.

However, from a pre-experiment with white bread, *Rhizopus stolonifer* made the first appearance and was the only mould seen, proving that different moulds can prefer different types of bread.



Figure 5.3 *Aspergillus* (and *Rhizopus stolonifer*?) growing on the underside of a white light bread sample.



Figure 5.4 a blend of the three types of moulds present on a white light sample.



## Pictures Taken under Microscope

Figures 6 are pictures taken by a microscope at 40x where the circular fruiting structures of mould is visible. Figure 6.1 is a noticeable greenish colour and is assumed to belong to the *Penicillium* genus while figures 6.2 are a darker black colour and thus is assumed that it is the *Rhizopus stolonifer* species. Finally, the sample taken from *Aspergillus* can be seen on figure 6.3, however, due to its light colour and dry texture, it was difficult to take a clear picture but its circular fruiting structures can still be seen on the image.

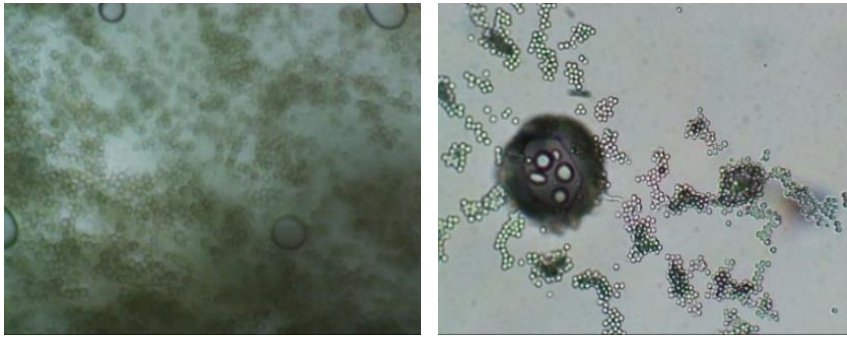


Figure 6.1 *Penicillium* sample taken from the experiment under 40x magnification with visible fruiting structures

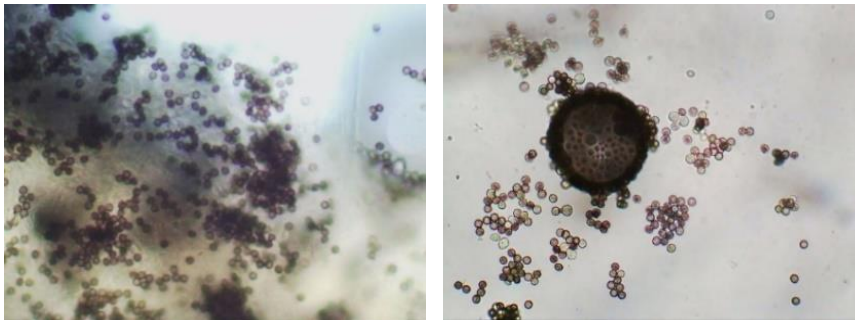


Figure 6.2 *Rhizopus stolonifer* species mould taken from the experiment under 40x magnification with visible fruiting structures.

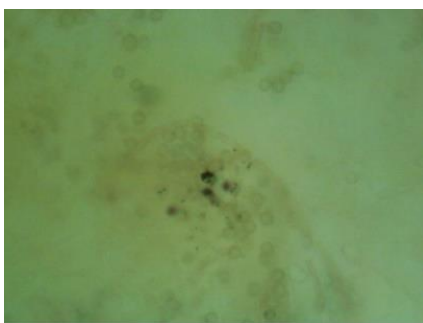


Figure 6.3 *Aspergillus* genus mould taken from the experiment under 40x magnification with visible fruiting structures.

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## 4. EVALUATION

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The methodology used gave sufficient results to draw a conclusion and had no complications throughout the whole experiment. Bread was chosen for the experiment because it is common in households and can be easily observed. Whereas coloured light bulbs were chosen instead of coloured films due to how light bulbs are capable of producing a shorter span of wavelengths that would lead to more reliable results.

A strength in the experiment is how it had several sources to support the direction of its results. In addition to the research regarding UV radiation which supported the theory that shorter wavelengths are capable of inhibition. Another research that was come across investigated a very similar issue regarding mould growth and light colour, where if found before this experiment was conducted would have greatly helped in giving additional insight.

The research was conducted by Markus Schmidt-Heydt of the *Max Rubner-Institute* who experimented on various types of moulds. His experiments yielded positive results between the relationship of mould and the colour of light. One of his articles stated that “wavelengths from both sides of the spectrum, e.g. red (long wavelength, 627 nm) and blue (short wavelength 470-455 nm) had the strongest inhibitory effects on [fungal] growth”.<sup>17</sup> Between the two, Schmidt-Heydt further states how “blue light generally had a stronger effect”. Moreover, his article also stated the moderate wavelengths (590-530nm) (yellow and green light) caused positive responses on the mould.<sup>17</sup>

Having several researches supporting the overall data that was collected from this experiment is a strength, however, there remain some factors that affected the results which were unaccounted for. Four different light sources were used in the experiment where their light quality was checked by a spectrometer. While there were no problems with the red, blue (both staying in their respective sides of the spectrum) and white lights, there was an inconsistency with the yellow light. Unlike the other coloured lights, it was not as pure and it ranged largely within the visible light spectrum. In addition to the poor light quality, the yellow bulb also radiated heat, unlike the other three light sources.

The heat radiated from the yellow bulb then further caused water to evaporate from the bread that could be seen clearly when condensation occurred and water drops were present on the inner side of the plastic. The evaporation could have then furthered affected the texture of the sample, causing it to harden. Both the heat and water evaporation does not assist the yellow light samples in growing mould as it is then disadvantaged in two of the largest factors of fungal growth; temperature and humidity. The difference in the environment could have also furthered caused the growth of a different species of mould therefore, instead of *Penicillium*, it was *Aspergillus* that dominated on that slice of bread.

On the topic of different environments, it is also possible that the other lights also gave off enough heat to inhibit mould growth slightly by decreasing its humidity. Despite no detection of any notable temperature changes, LED lights still emit heat.<sup>18</sup> When compared to the bread kept away from any light sources, the samples in the dark could have had an advantage of the complete absence of additional heat and no decrease in its humidity. Leading those samples to be the first to display mould growth.

Moreover, despite the easy overview which the usage of gridded sheets allowed, it was very prone to human error as the number of squares is hand-counted. Especially when the mould starts to cover a larger area. In addition, there were difficulties in identifying clearly where the mould has covered on the bread surface. Whereas green mould was quite easily identified, yellow and white moulds were hard to differentiate from the bread. However, the green mould was also the one which caused most clouding on the plastic when smudged. All these factors could have then led to miscalculations and thus inaccurate data.

Lastly, the methodology of using the two types of graphs to compare which light was the best at inhibition is also flawed due to how they do not fully take all the factors into consideration, such as the number of samples moulded. Therefore, causing it to be difficult to judge clearly which is better at inhibiting than another. While it is easy to conclude that blue light was the best at inhibiting mould growth, it was difficult to conclude simply with the graphs if white light or the dark was worst in inhibiting mould growth.

Despite all the weaknesses in this investigation, there were still strengths. On top of the support of several researches, the blue and red lights experiment was successful in yielding the expected data. By conducting the experiment for 14 days and measuring the growth every 12 hours, sufficient data were collected to draw reliable graphs showing the relationship between light colour and the mould covered area. Where the results were prominent enough to allow visual comparisons between all five sets of experiments.

## 4.2 Future improvements

Altogether, the experiment went according to plan, however, for future experiments, improvements can still be made. For a larger overview of how the visible light spectrum can affect food degradation through moulds, more variety of wavelengths, such as green, can be incorporated into the experiment. Due to the lack of equipment for this investigation, only the blue and red lightbulbs were suitable out of the three coloured lights, therefore, reliable observations were only made of the two ends of the spectrum. Without a complete overview, it is difficult to draw an accurate conclusion.

Other methods that could have been applied included using a control group. For example, two-tailed student t-tests could have been used to identify any significant differences between a control group and the other sets of experiments. Though in the end, they would only yield results of any significant differences and not the extent of the differences. Such as if blue light showed more inhibition in food degradation than the control group and not if blue was better at inhibiting fungal growth than white light. Furthermore, a t-test requires a minimum of 6 replicates which this experiment did not have (although it can be incorporated) and is usually done with continuous numbers.

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## 5. CONCLUSION

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Mould is an ever-present aspect of human life, predominant in food degradation. Mould spores are inescapable yet vital for the ecosystem despite being a nuisance to a human household. They are responsible for returning nutrients back into the earth but they are a leading issue to food waste and cause economic loss to both industries and individuals alike. Regardless of their function, moulds are still unwelcome and unhelpful in a human home which is where this investigation comes in; to explore ways which mould growth can be inhibited or minimised through different wavelengths.

To answer the research question: the blue colour was the most successful light to inhibit mould growth based on this investigation, backed up by outside researches on the effects of UV radiation on mould growth and the research done by the *Max Rubner-Institute*.

The second-best colour to have inhibited mould growth was red light while white light and the dark were the most ineffective in inhibiting food degradation. Instead, they could have promoted its growth. Although white light may contain wavelengths that inhibit, it also contains wavelengths which promote mould growth and that seems to be more apparent in this case. Based on previous studies, the medium wavelengths (yellow light) should have promoted mould growth, however, it was not evident in this experiment due to factors such as the radiated heat or decrease in humidity.

In the end, this experiment has yielded positive results of the effects which light colour has on mould growth. Despite its many limitations, it can at least state with quite a

certainty that the 31% wholegrain bread used in this experiment would be safe from spoilage for a longer period of time under blue light.

## 5.1 Extensions

To further look into the investigation of mould growth, an extended experiment on different types of bread can also be conducted to observe if different wavelength would yield the same results of the same effects. With different types of bread, there is a likelihood of the presence of other mould species as it was concluded that *Penicillium* was the dominating mould for the bread of this experiment.

Other than that, an extension which would have been interesting to continue investigating would be why opposite ends of the visible light spectrum were the most successful at inhibiting mould growth while the moderate wavelengths promote them. On another note to allow more insight, another route of research that could have been taken is looking into how mould growth is controlled in the production of foods such as blue cheese.

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Graphs were drawn using Excel and Geogebra.

All pictures were self-taken with a Huawei P9 phone camera.

Magnification pictures were also self-taken with a microscope at 40x. (all done with safety precautions in mind. eg. under a fume cupboard when opening the plastic bag containing the bread).

# APPENDIX

**Table 1.1** raw data of the area of mould grown (in mm<sup>2</sup>) on bread in the dark and under white light in 14 days.

DAY	Time (hours)	Dark 1	Dark 5	White 1	White 2	White 4	White 5
1	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
2	36	0	0	0	0	0	0
	48	0	0	0	0	0	0
3	60	0	0	0	0	0	0
	72	0	0	0	0	0	0
4	84	0	0	0	0	0	0
	96	0	0	0	0	0	0
5	108	9	0	0	0	0	0
	120	12	0	17	0	0	0
6	132	25	10	50	0	0	0
	144	36	50	75	0	0	0
7	156	64	100	90	0	0	0
	168	90	150	100	0	0	0
8	180	120	210	110	0	20	0
	192	200	320	150	0	30	0
9	204	350	450	550	0	50	0
	216	700	700	1800	6	70	0
10	228	1300	1300	2000	10	110	15
	240	2100	2500	2500	30	700	100
11	252	2600	3000	3000	400	900	260
	264	4000	3600	3700	420	1000	400
12	276	4600	4200	3800	450	3000	700
	288	4850	4500	4000	700	3200	1200
13	300	5000	4800	4800	1000	4000	1500
	312	5500	5000	5200	1600	4200	1900
14	324	5500	5500	5400	2000	4500	2300
	336	5500	5500	5500	2200	4600	2500

**Notice:** Breads were labelled from 1 to 5 and mould growth was not present on all breads. At 5500mm<sup>2</sup>, maximum mould growth has been reached and the entire surface has been covered.

**Table 1.2** raw data of the area of mould grown (in mm<sup>2</sup>) under blue, red and yellow light in 14 days.

DAY	Time (hours)	Blue 4	Red 2	Red 3	Red 4	Yellow 3	Yellow 4
1	12	0	0	0	0	0	0
	24	0	0	0	0	0	0
2	36	0	0	0	0	0	0
	48	0	0	0	0	0	0
3	60	0	0	0	0	0	0
	72	0	0	0	0	0	0
4	84	0	0	0	0	0	0
	96	0	0	0	0	0	0
5	108	0	0	0	0	0	0
	120	0	0	0	0	0	0
6	132	0	0	0	0	0	0
	144	0	0	0	0	0	0
7	156	0	0	0	0	0	0
	168	2	0	90	0	0	0
8	180	3	0	150	0	0	0
	192	3	0	200	0	0	0
9	204	4	0	225	0	3	0
	216	5	0	235	0	15	0
10	228	5	0	300	0	50	0
	240	6	0	400	5	75	12
11	252	6	0	500	50	200	25
	264	6	5	600	170	250	30
12	276	6	50	630	210	300	40
	288	8	70	900	300	500	50
13	300	8	100	1100	350	900	75
	312	9	150	1800	400	1300	100
14	324	20	200	2500	450	1600	300
	336	25	800	3500	800	2400	700

**Notice:** Breads were labelled from 1 to 5 and mould growth was not present on all breads. At 5500mm<sup>2</sup>, maximum mould growth has been reached and the entire surface has been covered.