Examiner's commentary

This essay has a strong biological context and significance and a clear and sustained line of argument. The topic statement and research question provide a clear and precise indication of what this essay is about, including the organisms investigated, the variables and the method used. The background material accessed is relevant to the topic under investigation and is sourced from peer reviewed journal articles for the main part. Ideas from these sources are integrated into an informative background and are used effectively to support the interpretation of the results in the discussion. The method used to inoculate and incubate the samples is described in detail and the important steps are justified by reference to published material. The approach to data processing and statistical analysis (ANOVA and correlation coefficient) is presented in detail. An ANOVA may not be the most appropriate statistical approach for these data, but the correlation coefficient supports the interpretation of the observed trend. The processed data and statistical results are interpreted correctly and broadly valid conclusions about the effect of heating time on the effectiveness of Allium are made. The essay has a clear and appropriate layout and structure, supported by detailed tables of data and a precise graph of processed data (figure 2) that addresses the research question directly. Other illustrative material is used judiciously and supports understanding of the text. Overall the essay represents a coherent, well-argued and convincing piece of research.

Extended Essay
Biology HL

Investigation of the effect of heating time on antibacterial activity of garlic, *Allium sativum*

How and to what extent different heating times (5 – 30 minutes, with 5 minutes increments) affect garlic, *Allium sativum*, in terms of its antibacterial activity against *Micrococcus luteus* bacteria, measured as radii of zones of inhibited bacterial growth using agar well diffusion assay?

Subject: Biology

Word count: 3996

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Introduction

Research question

How and to what extent different heating times (5 – 30 minutes, with 5 minutes increments) affect garlic, Allium sativum, in terms of its antibacterial activity against Micrococcus luteus bacteria, measured as radii of inhibited bacterial growth using agar well diffusion assay?

Background information

The history of garlic*, Allium sativum*, usage as an antimicrobial agent dates back to the ancient times. Ancient Egyptian people were familiar with its great potential and used it as a crucial herbal remedy for treating various diseases and infections, including heart problems, dysentery, headaches and asthma. (1) Apart from the Egyptians, other nations have also recognized the importance of garlic, such as Babylonians and the Hebrews, as well as Greek and Roman armies, who used it to prevent different illnesses. (1,2,3,4) Nowadays garlic is still used as an antioxidant and antithrombotic herb against heart diseases, atherosclerosis and in cholesterol and blood pressure regulation. (3,5)

Garlic, *Allium sativum*, the bulb-shaped vegetable, belongs to genus *Allium*, together with various species of onion, leek, etc. It is known for its characteristic odor and flavor, which are due to its high sulfur content. While many find that unpleasant, others cherish and value garlic and extensively use it in medicine and cooking. (6)

Given its importance, garlic has been widely scientifically investigated over time. Previous research has considered its antibacterial, antioxidant and anticancer properties, antiplatelet activity, some have compared it to antibiotics and very importantly, in curing heart diseases, which all has shown positive results. (3,7-9)

However, the most interesting and investigated property of garlic is its antibacterial activity. The most effective and abundant antibacterial compound in garlic, allicin, was first described and isolated by Cavallito and Bailey in 1944. (7) Allicin is a volatile sulfur compound, precisely a thiosulfinate, which gives garlic its recognizable odor and taste and can degrade to form other sulfur compounds, without completely losing its antibacterial activity. It is usually stored in the garlic flower, rather than the bulbs, but the bulbs contain a chemical substance called alliin. When the tissue is damaged, alliin, with the help of an enzyme called alliinase, forms allicin, giving the antibacterial properties. (1)

Among all the extensive research on garlic, the effect of temperature and heating on its properties has also been tested, concluding that temperature negatively impacts garlic's beneficial properties, including antibacterial activity. (8,10,11) Therefore, the initial idea of mine when preparing for this study, the effect of solely temperature on antibacterial properties of garlic, was dismissed since it had already been well established through many investigations and would have been of less interest. However, only few papers I managed to find have considered the effect of an increase in heating time on garlic's antibacterial properties, which to me, seemed as an interesting area to investigate further. In addition, despite its health benefits as raw, people tend to extensively use garlic in cooking, instead of consuming it raw. Having personally refused to eat raw garlic many times, due to its unappetizing taste and smell, even when I was told it would prevent sickness and having participated in discussions about that topic, it served as an inspiration for this investigation. I do, however, find many dishes containing garlic tasty and have no problems eating it after it has been cooked. Therefore, this area is of a special interest for me and I decided to investigate how the antibacterial properties of garlic would be affected after different periods of heating time, and whether the antibacterial activity would remain as effective. Moreover, since the study investigates the effect of heating

times on the antibacterial activity, it is representative of the real life and will show whether the cooked, usually tastier garlic could be used as an effective health remedy. Previous research on this specific topic has generally reported the reduction of antibacterial activity after different heating times. (8,12)

The method chosen after conducting a pilot study was agar well diffusion assay. Moreover, both gram-positive and gram-negative bacterial strains were taken into consideration due to differences in their cell wall structure. (7) However, after the pilot study, gram-positive species, *Micrococcus luteus*, seemed the most fitting since gram-negative, *Escherichia coli*, showed no determinable results.

The aim of this investigation was to determine how and to what extent heating time affects antibacterial activity of aqueous garlic extract, in terms of inhibiting *M. luteus* bacteria, using agar well diffusion assay.

Hypotheses

Alternative hypothesis (H1)

There will be a difference in the radii of zones of inhibited bacterial growth between heated and non-heated garlic, using the agar well diffusion assay. The expected difference is a general decrease in the size of inhibitory zones as the time of heating increases.

Null hypothesis (H0)

There is no significant difference in the radii of zones of inhibited bacterial growth between heated and non-heated garlic and thus no correlation between heating time and inhibition.

Materials and methods

Methodological considerations

Various methods have been used to study antibacterial activity of garlic in the past decades, including agar dilution method, agar well diffusion, disc diffusion method, etc... (7,13, 15-17) After conducting a pilot study and researching about methods, agar well diffusion assay was chosen as the most appropriate method for this investigation. In the pilot study agar disc diffusion method was firstly tested and generated indeterminable results when tested against gram-negative bacteria, *E. coli*, as, surprisingly, no zone of inhibition appeared. The cause could have been the garlic itself that might have been aged, as supported with a study done by Urios, Grigorova-Borsos and Stenberg in 2007, reporting that storage time could reduce the antimicrobial activity. (18) After some research, gram-positive bacteria *M. luteus*, was selected for the investigation, as the results from a study (7) showed its greater susceptibility to garlic compared to gram-negative strains, fresher garlic was purchased for the following experiments and agar well diffusion method was tested. Finally, after conducting the second experiment which showed conclusive results, agar well diffusion method was chosen for the final experiment of the investigation, along with *M. luteus* as the bacterial strain. However, spectrophotometry was a method of interest, but it was not feasible. In some investigations (19,20), *M. luteus* bacteria was handled using either Fourier-transform infrared spectroscopy or Ultraviolet-visible spectrophotometry but neither were achievable at the school's laboratory. Lastly, five replicates, Petri dishes, needed for each condition (0 to 30 minutes heating time, with 5 minutes increments) with five repeats, wells, in each was chosen as optimal amount. That way the investigation was more easily attainable in terms of cost and supply of materials, while still obtaining sufficient data to avoid the negative impact of random errors. The heating

times between 0 and 30 minutes were chosen as those are the most usual cooking times in garlic meal preparation.

Materials

- Merck; Standard nutrient agar 1
- Sterile Petri dishes, x35
- Cork borer (9mm diameter)
- 200µl micropipette (±1µl)
- Sterile tips
- Ethanol, 95%
- *Micrococcus luteus* bacteria
- Bacterial spreader
- Alcohol burner
- Glass beakers (25ml), x3
- Measuring cylinder (10ml ±0,5ml), x5
- Sterile distilled water
- Fresh garlic cloves
- Digital scale (±0.001g)
- Kitchen mixer
- Cooking plate
- Sterile boiling tubes, x7
- Pot with boiling water
- Stands and clamps, x2
- Ice bath
- Timer (±1s)

- Knife
- Spoon
- Matches
- Autoclave
- Incubator (±1˚C)
- Ruler (±1mm)
- Marker
- Tape

Experimental design

The effect of heating time on garlic's antibacterial properties was tested using agar well diffusion method. All the experiments were performed in the school's laboratory in May 2018.

For safety precautions, all work surfaces were cleaned using ethanol before and after the procedure to avoid spreading of the bacteria and contamination of the discs. Hands had been washed prior to the procedure and bacteria was grown in incubators at 30 degrees, avoiding possible growth of any human pathogenic bacteria. Lab coat was worn during the whole procedure, avoiding contamination of clothes. Furthermore, all the equipment was sterilized in the autoclave and the mixer was washed using hydrogen peroxide. After the experiment, the waste was carefully disposed, the equipment sterilized, the old agar plates autoclaved and thrown away. The experiment was conducted at room temperature (22˚C) unless stated otherwise.

M. luteus bacteria was cultured in nutrient broth overnight, prior to the experiment, as well as sterilized in the autoclave afterwards. To make agar plates, nutrient agar was autoclaved for 30

minutes, poured into sterile Petri dishes and left to solidify. 200µl of bacteria was placed in each dish using a micropipette and spread out evenly using a flamed spreader. Using a cork borer (9mm), five equal wells were made in each agar plate. To prepare 40% garlic extract, 80g of garlic was mixed with 120ml of water using a kitchen mixer. The extract was then transferred into 6 sterile boiling tubes attached to stands and cooked in the boiling water, each for different time periods (5 to 30 minutes, with 5 minutes increment). Heating was timed using a mobile phone timer. There was also a control group with non-heated garlic juice. After cooking, 150µl of the extract was transferred into each well using a micropipette, while avoiding the overflowing of the extract on the nutrient agar. This process was repeated to create 35 replicate plates, 5 per each increment including the control group, and 5 repeats in each plate. The plates were then incubated in incubators for 24 hours at 32°C, after which the radii of zone of inhibition (mm ±1mm) were measured using a ruler and recorded.

Calculations and statistical analysis

Mean

The mean was calculated for easier comparison between results of each conditions. The mean zone of inhibition was calculated for each replicate (mean of 5 repeats) followed by calculating the mean of 5 replicates' mean values for each condition.

The mean was calculated using Formula 1 in Microsoft Excel.

Formula 1. The mean

$$\bar{x} = \frac{\sum x_i}{n}$$

Where:

$\bar{x}$ - the mean

$\sum x_i$ – sum of individual values in a data set

n – number of values

Example:

- Calculating the mean of 5 repeats for replicate 2 for 10 minutes heating time (Table 1):

$$\frac{(16 + 15 + 16 + 16 + 16)}{5} = 15.8\ mm$$

Standard deviation

The standard deviation (SD) was calculated to determine the dispersion of data around the mean, hence the precision and accuracy of data. SD was calculated for each total mean value, using Formula 2 in Microsoft Excel:

Formula 2. Standard deviation

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

Where:

$\boldsymbol{\sigma}$ = standard deviation

$\sum$ = sum of

$\boldsymbol{x_i}$ = each value in a data set

$\boldsymbol{\bar{x}}$ = the mean

n = number of values in a data set

ANOVA test

Analysis of variance (ANOVA) test was carried out to determine whether the difference between the radii of zones of inhibition between each condition is statistically significant, with the significance level of $p<0.05$. The test was performed using an online calculation tool (21) and the result of $p<0.0001$ obtained.

Results

The effect of heating time on antibacterial properties of aqueous garlic extracts has been established using agar well diffusion assay and by measuring the radii (mm ± 1mm) of inhibitory zones, as shown in Figure 1 below.

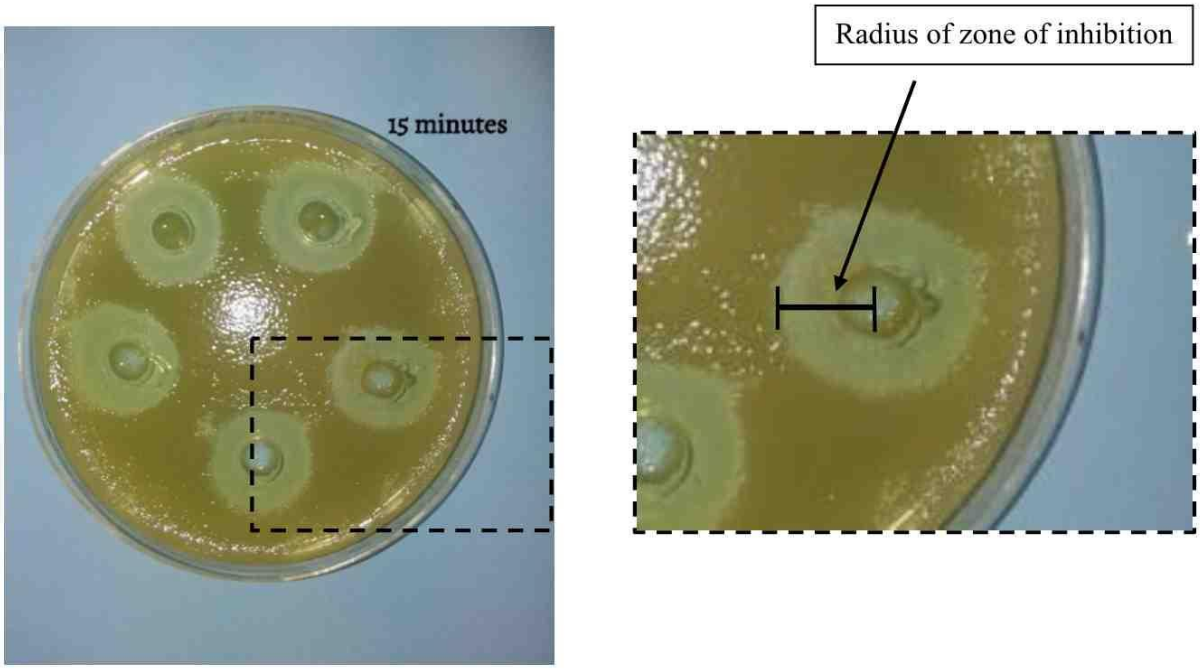

Figure 1. An example of a radius of zone of inhibition from one well with aqueous garlic extract, presented to illustrate how the zones of inhibition were measured.

Zones of inhibition were measured for each condition in five replicate dishes, with five repeats in each. The raw data of radii of inhibitory zones for each condition is presented in Table 1 below.

Table 1. The radii (mm) of inhibited growth of *M. luteus* after different heating periods (0-30 minutes, with an increment of 5 minutes) of 40% aqueous garlic extract, including five replicates (Petri dishes) per each condition and five repeats (wells) per each replicate.

Diameter of inhibited growth of *M. luteus* /mm (± 1 mm)								
		Heating time (minutes ±5s)						
	Repeats	**Control (0)**	**5**	**10**	**15**	**20**	**25**	**30**
Replicate 1.	1	24	20	17	11	12	7	5
	2	25	19	17	12	12	6	5
	3	24	20	16	11	13	5	6
	4	22	17	16	12	11	7	5
	5	23	18	18	11	12	8	5
Replicate 2.	1	22	16	16	12	10	7	7
	2	24	17	15	11	14	8	5
	3	18	18	16	11	11	7	5
	4	20	17	16	11	9	7	5
	5	21	16	16	11	9	8	5
Replicate 3.	1	22	18	17	11	10	8	5
	2	23	17	17	11	10	6	7
	3	23	19	15	11	13	6	5
	4	24	18	16	10	12	5	5
	5	21	15	18	11	11	7	5
Replicate 4.	1	22	18	16	11	11	8	6
	2	22	18	15	12	12	6	5
	3	23	18	17	11	12	8	6
	4	22	17	16	12	11	6	6
	5	22	18	15	11	10	6	5
Replicate 5.	1	19	20	15	10	9	5	5
	2	25	19	17	10	9	5	5
	3	23	19	16	10	10	6	5
	4	22	19	17	10	9	5	5
	5	21	19	16	11	9	6	6

For clearer comparison between conditions, mean values for each condition were calculated (total mean) as the total mean of five mean values of the replicates and rounded to one decimal place, for a clearer distinction due to very close values in some conditions. Table 2 shows the mean values for each replicate in each condition as well as the total mean of each condition and standard deviations (SD). Standard deviations are rounded to one decimal place for the same reason as the total mean values, an easier clarification due to values being very close.

Table 2. Calculated mean values (mm) of radii of zones of inhibited *M. luteus* growth for each of the five replicates (Petri dishes) per each heating time (0-30 minutes with an increment of 5 minutes) of 40% aqueous garlic extract. The mean value of each replicate is calculated as the mean of five repeats (wells) per replicate. The total mean of each condition is also presented, along with the standard deviations (SD).

Mean radii of zone of inhibition in *M. luteus* /mm							
Heating time (minutes ±5s)	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	**Total Mean**	SD
Control	23.6	21.0	22.6	22.2	22.0	**22.3**	1.4
5	18.8	16.8	17.4	17.8	19.2	**18.0**	0.9
10	16.8	15.8	16.6	15.8	16.2	**16.2**	0.8
15	11.4	11.2	10.8	11.4	10.2	**11.0**	0.5
20	12.0	10.6	11.2	11.2	9.2	**10.8**	1.1
25	6.6	7.4	6.4	6.8	5.4	**6.5**	0.9
30	5.2	5.4	5.4	5.6	5.2	**5.4**	0.6

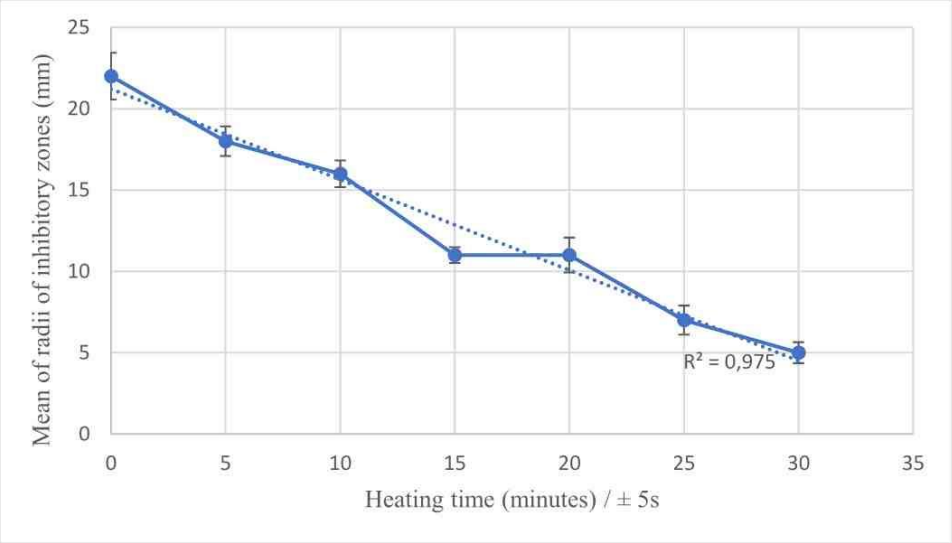

Figure 2. Calculated mean zone of inhibited bacterial growth of *M. luteus* after different periods of heating 40% aqueous garlic extracts, displayed on the scatter plot graph. Standard deviations are represented in error bars and the calculated coefficient of determination (R^2) value is given.

The general trend in the data demonstrates a gradual decrease in the size of zones of inhibited bacterial growth as the heating time increases. The control condition (no heating) shows the greatest antibacterial effect against the bacteria, whereas the condition with 30 minutes heating time shows much weaker effect. In addition, generally low values of standard deviation (SD)

indicate the accuracy and reliability of the results. Furthermore, the results from the one-way ANOVA test showed that the difference between the results is statistically significant ($p<0.0001$).

Qualitative observations

Initially white, garlic juice changed color after heating. Firstly, it turned bright yellow, then darker yellow and finally dusty orange color as the time of heating increased to 30 minutes, as shown in Figure 3 below, indicating that garlic extracts have undergone changes in the heating process.

Figure 3. Aqueous garlic solution (40%) in measuring cylinders before heating (left) and after 30 minutes heating time (right).

Inhibitory zones, formed in the plates after 24 hours of incubation, could easily be observed with naked eye, as shown in Figure 4 below.

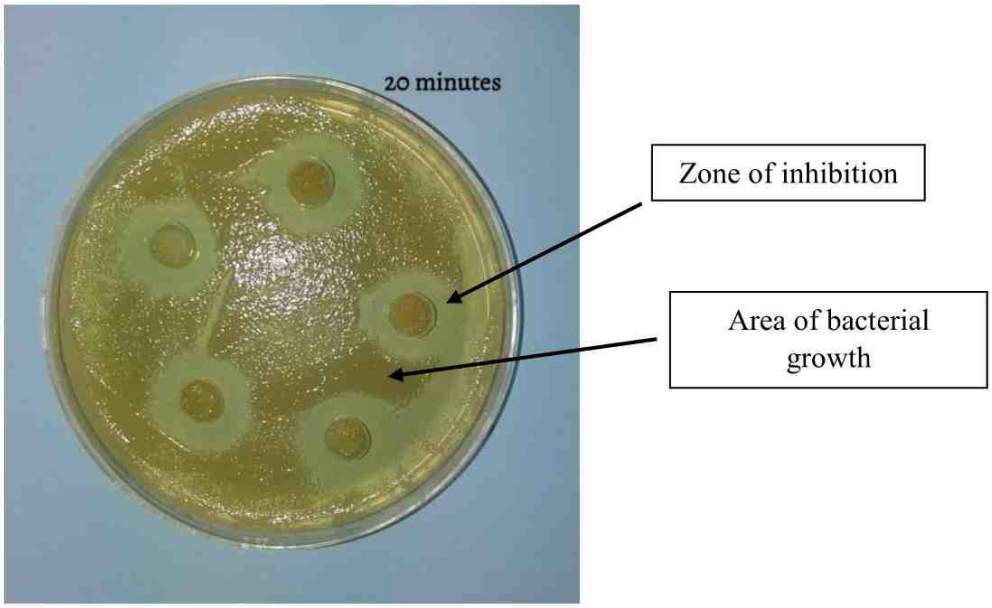

Figure 4. The identified zone of inhibition and the area of bacterial growth, presented with arrows on the picture of an agar plate to illustrate the identification of the zones right after the incubation.

Moreover, the gradual decrease in size corresponding to the increase in heating time was clearly noticeable, with the largest inhibitory zones in the control condition and smallest in 30 minutes condition, as shown in Figure 5.

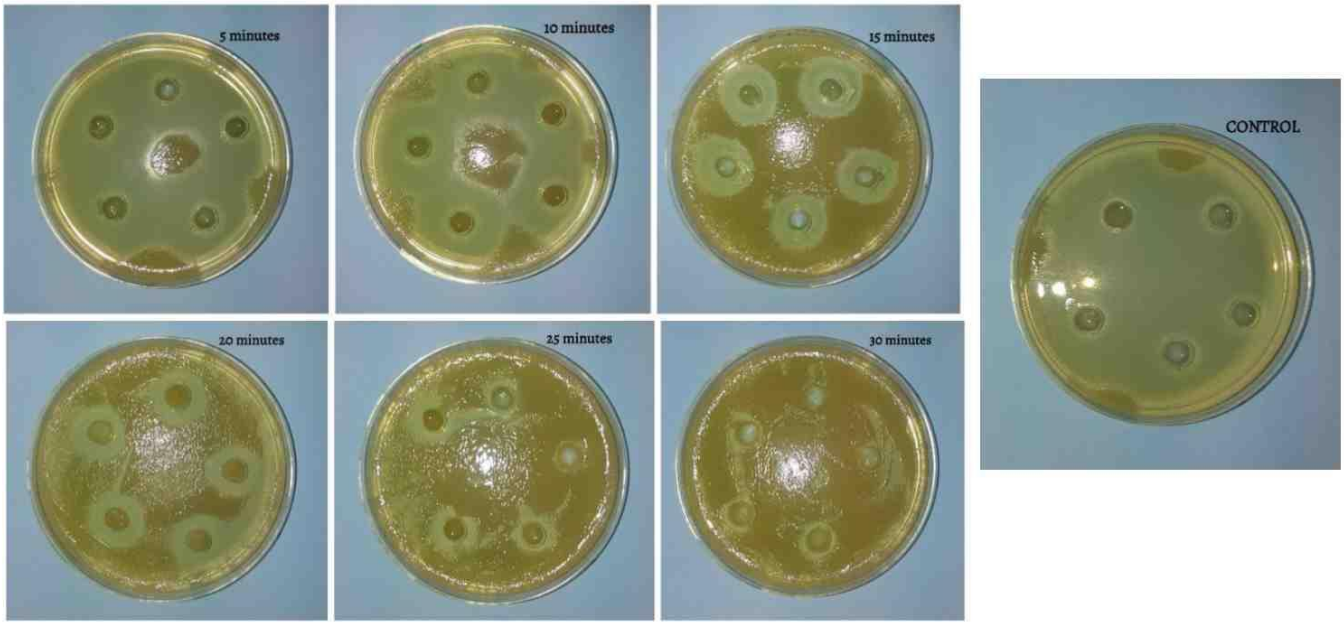

Figure 5. A side by side arrangement of 7 agar plates (1 from each heating condition), for a clearer view of the general trend, reduction of antibacterial activity as time of heating increases.

Discussion

This investigation aimed to determine the effect of heating time on antibacterial activity of aqueous extracts of garlic, in terms of inhibiting *M. luteus* bacteria, using agar well diffusion assay.

Overall, the experiment was successfully conducted as supported by the consistent results between the repeats in each condition and quite low standard deviations (range from 0.5-1.4 mm). Furthermore, one-way ANOVA test results showed that the difference between the means is statistically significant with the probability value, assuming the H_0, of $p<0.001$. This suggests that the H_0, should be rejected and the H_1 is to be accepted, stating that heating time would negatively impact garlic in terms of its antibacterial activity and the difference in zones of inhibition would be observable.

Analysis and interpretation of results

This investigation was intended to address the effect of heating time on antibacterial activity of garlic and to deduce the trend of an expected decrease in the antibacterial activity as the heating time increases. As demonstrated in Figure 2, the antibacterial properties of garlic have been significantly reduced after heating. The graph shows a clear trend of a reduction of antibacterial activity as the time of heating increased. The coefficient of determination (R^2) (Figure 2), representing the closeness of the trend line to the data points (22), is 0.975. With the value of 1 being the perfect fit, 0.975 indicates a very strong, negative correlation between heating time and antibacterial properties of the aqueous garlic extracts.

Firstly, the gradual trend of reduction of antibacterial activity in garlic is observable from the qualitative data analysis. In Figure 4 all the discs are displayed next to each other for a better

comparison. The inhibitory zones for the control condition and for 5 minutes heating time were noticeably much larger compared to 30 minutes heating time, when the zones around some wells were almost unnoticeable and hardly measurable. Furthermore, it is very clear how circular zones of inhibition gradually decrease in size as the heating time increases and more bacteria (darker area) is present. However, the biggest difference in size between two subsequent conditions could be observed between 10 and 15 minutes, as reflected in the quantitative data discussed later.

Moreover, the trend was confirmed by quantitative data analysis. Among all heating conditions, 5 minute one had the greatest antibacterial effect against *M. luteus* with the total mean value of the radii of inhibitory zones of 18 mm. Comparing this with the control condition when raw garlic inhibited the bacteria most effectively giving the total mean value of 22 mm, it could be noticed that 5 minute heating time has negatively affected garlic's antibacterial activity to certain extent but the effect was still prominent. Furthermore, as presented in Figure 2 and Table 2, zones of inhibition drastically reduced in size after 30 minutes of heating, compared to the control group, with only 5.4 mm mean radius size, indicating that the antibacterial properties have significantly weakened. Moreover, 15 minutes conditions showed bigger deviation from the general trend compared to other conditions when the value dropped to 11 mm, proposing that the antibacterial activity starts to significantly weaken after 10 minutes heating time.

As the results indicate a reduction of antibacterial activity of aqueous garlic extracts with the increase in heating time, this investigation proposes that the well-known healing properties of garlic are negatively affected during thermal processing in cooking and meal preparation.

Previous findings and explanations

Numerous papers have confirmed garlic's strong antifungal, antioxidant, anticancer, antiplatelet and antibacterial activity (either aqueous extracts or oil) against various pathogens and also focused on the effect of heat treatments on garlic, mainly reporting the negative effect of temperature on its beneficial properties. (7,8,10-12,23) Yin and Cheng (1998) found that garlic had weaker inhibitory effect against fungi after temperature was increased from 60 to 100°C and Ali (1995) reported a reduction of inhibition of cyclooxygenase activity of boiled garlic compared to raw, in rat tissue. (8) A study from 2007 has also reported that heating, UV exposure and storage inhibits garlic's antimicrobial activity, possibly explaining why the pilot study with aged garlic was unsuccessful. (18) Those conclusions agree with the ones in this investigation, temperature inhibits the antibacterial activity of garlic. However, there has not been much research specifically focusing on how the antibacterial activity in garlic is affected by increasing the heating time. Nevertheless, Chen *et al*. (1985) reported that antibacterial activity of garlic against various bacterial strains was completely suppressed after 20 minutes heating at 100°C, which doesn't fully correspond to my findings. In my investigation, although reduced, there was still perceptible inhibitory effect after heating for 20 minutes. As well as that, heating garlic at 100°C for 20, 40 and 60 minutes reduced its antioxidant activity, as reported by Prasad *et al.* (1996) (8). Moreover, it was reported that 60s microwaving time, significantly weakened garlic's ability to reduce the *in vivo* DMBA bioactivation in rats. (24)

The strong antimicrobial effect of garlic is mainly due to allicin, the substance which is at the highest concentration present in fresh garlic. However, allicin does not form until garlic is crushed and the tissue disrupted, being a form of self-defense mechanism. When that happens, a chemical substance present in garlic, alliin, turns into allicin with the help of alliinase enzyme,

hence it is only used when necessary (1). As it belongs to thiosulfinates, volatile and unstable sulfur compounds responsible for garlic's resistance against microbes, allicin can undergo many transformations depending on the temperature, pH and form more stable compounds such as sulfoxides and sulfides, while partially retaining its antimicrobial properties (3,8,18,25). A review paper by Singh Papu *et al.* (2014) mentions that under room temperature it takes hours for alliinase to function, whereas it only takes minutes during heating (3). Moreover, 6 minutes heating time significantly reduced garlic's antiplatelet activity, which allicin is also responsible for, whilst heating for 3 minutes did not have any effect (11). However, as mentioned above, those properties, including antibacterial activity, are largely affected by higher temperatures and longer heating times.

Furthermore, keeping in mind that enzymes generally have an optimum temperature at which their activity is the greatest, and that by increasing temperature above optimum causes the enzymes to denature and lose their activity (26), it could be assumed that alliinase functions the same way. Therefore, high temperatures and long periods of heating diminish the ability of alliinase to catalyze the production of allicin and thus suppress garlic's antibacterial activity, while moderate temperatures allow alliinase to work more efficiently. Further studies could therefore look at the difference of the extent of garlic's antibacterial activity relative to different time periods it spends in room temperature, and thus deduce how long it takes for alliinase to work under that condition. That could be compared with a shorter heating time condition, allowing the alliinase to work before denaturation and reduction of antibacterial activity, and then deducing the optimum heating time at which alliinase would work most effectively, converting alliin to allicin, hence allowing garlic to act as an antibacterial agent.

Moreover, a curious observation in certain papers was that the antibacterial properties are affected differently depending on whether garlic was crushed before or after heating. Kim,

Jeon-Youn (2002) showed that garlic when firstly crushed and then steamed retained some antibacterial activity, while when firstly steamed and then crushed, the activity was gone (10). This supports that allicin and other antibacterial compounds in garlic are only formed when crushed as well as that the temperature dependent enzyme, alliinase, becomes inactive and unable to catalyze the formation reaction of allicin when garlic is firstly steamed. Similar conclusions were reported for the antiplatelet activity of garlic (11). Interestingly, in my investigation, even though the garlic was firstly crushed to prepare the aqueous extracts and heated afterwards, the antibacterial activity was significantly reduced after the heat treatment. These discrepancies and lack of research on this specific topic indicate that mechanisms behind garlic's strong antibacterial properties are very complex and there is still room for new discoveries. Therefore, further investigations could focus more on examining how the antimicrobial properties are affected depending on when the garlic was crushed and heated.

Moreover, there are studies reporting antibacterial activity of other, non-sulfur and thus non-volatile, agents in garlic such as allyl alcohol (AA) that are altered by pH and not temperature (12). This demonstrates the complexity of garlic's structure and activity suggesting that more research is needed to gain a deeper understanding of it.

Evaluation and improvements

The investigation was carried out successfully and judging by the quite consistent results within conditions, with reasonable variations reflected in the quite small standard deviations, it could be concluded that the obtained results are adequately precise and the agar well diffusion method was appropriate, reliable and cost-effective. However, there are minor issues to be considered. Though the experiment was carefully conducted, the boiling water in the pot wasn't mixed throughout the process and therefore the temperature was possibly higher at the bottom, closer

to the cooking plate. This might have resulted in some boiling tubes being exposed to higher temperatures than others, depending on how close they were to the bottom, leading to discrepancies of the results. This could possibly explain the higher SD (1.1mm) for 20 minutes heating condition that stands out. Despite the minor effect on the results, it could be improved by mixing water throughout the process and keeping all test tube at the same height in the water bath. However, high SD value (1.4) of the control condition indicates other sources of errors as well. Having some solid particles in the garlic extract might have interfered with the results, however, it could be improved by filtrating the extract by vacuum filtration. A random error could be accidentally spilling the extracts outside of the wells, causing discrepancies in the size of the zones of inhibition. To reduce such random errors and improve reliability of the results, the range of number of replicates and repeats should be even wider. Furthermore, for a more extensive and detailed investigation, both gram-positive and gram-negative bacterial species should be considered, as well as different kinds of garlic plants from various geographical locations, to investigate if there would be any difference between them and thus in the efficiency of garlic's antibacterial properties in different cuisines around the globe.

Final conclusions

This investigation showed a negative effect of heating time on antibacterial properties of aqueous garlic extract against *M. luteus* bacteria, as demonstrated in the gradual decrease in the size of radii of inhibitory zones as the time increased, leading to almost complete suppression after 30 minutes.

Comparison of these findings with previous ones leads to the conclusion that there is a lot more to discover about the mechanisms behind the antibacterial activity and its dependency on other factors such as temperature, heating time or pH levels. Finally, according to the findings, heating time should be considered in everyday cooking if one wants to retain the antibacterial

effect of garlic and use it as a health remedy. 5-10 minutes seem to be reasonable cooking time for partially retaining the antibacterial activity and having beneficial effects, while avoiding the unpleasant odor and flavor of garlic.

Acknowledgements

I would like to express my profound gratitude and appreciation to my teacher, Annette Carlström, for her excellent supervision, persistent help and guidance, excellent feedback as well as devotion all throughout this investigation.

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Extended essay - Reflections on planning and progress form

Candidate: *This form is to be completed by the candidate during the course and completion of their EE. This document records reflections on your planning and progress, and the nature of your discussions with your supervisor. You must undertake three formal reflection sessions with your supervisor: The first formal reflection session should focus on your initial ideas and how you plan to undertake your research; the interim reflection session is once a significant amount of your research has been completed, and the final session will be in the form of a viva voce once you have completed and handed in your EE. This document acts as a record in supporting the authenticity of your work. The three reflections combined must amount to no more than 500 words.*

The completion of this form is a mandatory requirement of the EE for first assessment May 2018. It must be submitted together with the completed EE for assessment under Criterion E.

Supervisor: *You must have three reflection sessions with each candidate, one early on in the process, an interim meeting and then the final viva voce. Other check-in sessions are permitted but do not need to be recorded on this sheet. After each reflection session candidates must record their reflections and as the supervisor you must sign and date this form.*

First reflection session

Candidate comments:

As my father always advised me to eat garlic whenever I was ill, I knew that garlic was the field I wanted to study more thoroughly and investigate if it has some health benefits. So, I decided to investigate how the time of cooking would affect antibacterial properties of garlic against a gram-positive bacteria Micrococcus. Previous studies on this topic have shown certain health benefits of raw garlic and inhibitory effects on various bacteria, but to me it seemed particularly interesting to see if it would still have the same properties after a heat treatment. I intend to use the agar well diffusion method which seemed to be the most promising and successful one after carrying out an ineffective pilot experiment with additional agar disc diffusion method. Next step is carrying out the actual experiment and starting to write the essay. The research question considered is: How and to what extent different heating times (5 – 30 minutes, with 5 minutes increments) affect garlic, Allium sativum, in terms of its antibacterial properties against Micrococcus luteus bacteria, measured as radii of inhibited bacterial growth using agar well diffusion assay?

Date: April 12, 2018

Supervisor initials:

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Interim reflection

Candidate comments:

The experiment proceeded according to plan without any major issues or changes. However, the procedure had to be redone with 40% extracts of newly purchased garlic, since the experiment with 20% extract of older garlic failed. That and the preparation of the discs and wells was more time consuming and required more patience than expected. Nonetheless, sufficient data was collected and the general trend in the results could already be identified by qualitative observations of the discs. The data has been processed and analyzed, various reference sources found and investigated, leading to the next step, discussion and finalization of the investigation. There was no need to change previously established research question.

Date: August 29, 2018

Supervisor initials:

Final reflection - Viva voce

Candidate comments:

After months of extremely hard work that went into planning, trying out different methods, carrying out several pilot experiments and writing, my EE is finally completed! I must say I am very proud of it and the hard work was worth it all. I thoroughly enjoyed the whole process, especially researching on this topic and putting everything together. I have learned that scientific work requires a lot of patience, dedication and commitment and this project made me realize that I want to pursue my future career in the field of biological science. The results were clear and showed the negative effect of heating time on the antibacterial properties of garlic, leading to complete suppression after 30 minutes, which answered the research question. It was concluded that heating time should be considered in everyday cooking if one wants to retain the antibacterial properties, but there is still room for more discoveries about garlic's complex structure and mechanisms. Through this project I have developed scientific writing skills, skills in planning and designing experiments, learned new terminology from previous research papers and applied it to my writing which made the hard work pay off.

Date: November 24, 2018

Supervisor initials: