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The medicinal powers of honey

A bacteria busting game changer

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Abstract

Honey: sweet, delicious and great on toast! After proving a hit in our kitchen cupboards, honey is now making its way into our medicine cabinets too; next time you have a sore throat you may be reaching for the honey jar. This study looks at three different types of honey and their medicinal qualities against bacteria. Standard processed honey, unprocessed honey and medicinal grade Manuka honey all killed samples of both Gram-negative and Gram-positive bacteria, proving all of these honeys to have antibacterial properties. The Manuka honey, however, had a greater antibacterial effect against both bacteria, suggesting that Manuka honey is the best for medicinal use.

Introduction

Research in 2008 by Professor Thomas Henle from the University of Dresden suggested that Manuka honey is better than other honeys in terms of its antibacterial qualities, and that this is down to the high methylglyoxal (MGO) levels in the Manuka honey.[1]

This project aims to compare three different types of honey: processed, unprocessed and Manuka. By doing this, the difference in antibacterial qualities between the three honey types can be assessed, testing how much difference there is between honeys and whether the difference is statistically significant.

Killing Bacteria

Certain substances found in all types of honey aid in their antibacterial properties. Due to honey's high sugar concentration, bacterial cells can become dehydrated and die due to osmosis between the honey and the bacterial cell cytoplasm.

Hydrogen peroxide is found in all types of honey, being formed from a reaction between glucose and oxygen, catalysed by glucose oxidase. A hydrogen peroxide molecule has the ability to form two hydroxyl radicals. These hydroxyl radicals are known as relative oxygen species (ROS) or free radicals, as they each contain an unpaired electron, making them highly reactive.[2]

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https://archive.ysjournal.com/wp-content/uploads/2015/09/Hydrogen-Peroxide-Free-Radical-Formation-ys1-300x232.png
Hydrogen Peroxide Free Radical Formation (ys)

Fig. 1: Figure 1: Diagram showing the formation of hydroxyl radicals from hydrogen peroxide

Bacterial cells contain a cell membrane, consisting of a phospholipid bilayer (this contains phosphorous heads, glycerol backbones and fatty acid tails). A hydroxyl radical (formed from the hydrogen peroxide in the honey) will 'attack' a carbon-hydrogen bond in the fatty acid tail. This will produce a lipid radical (and water), which can then react with oxygen to produce a lipid peroxyl radical. This radical can now react with other fatty acid tails in the phospholipid bilayer, producing a lipid peroxyl radical and another lipid radical. This forms a cycle called lipid peroxidation (see diagram), which alters the structure of the bacterial cell membrane, causing the bacterial cell not to function properly and thus killing the bacteria.[3]

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https://archive.ysjournal.com/wp-content/uploads/2015/09/Lipid-Peroxidation-Diagram-ys1-300x263.png
Lipid Peroxidation Diagram (ys)

Fig. 2: Figure 2: Diagram showing how lipid peroxidation of hydroxyl radicals affects bacterial cell membranes

Some bacteria contain the enzyme catalase, which breaks down hydrogen peroxide (found in honey) into water and oxygen, thus preventing the lipid peroxidation of the bacterial plasma membrane. However, due to honey's acidity (averaging at a pH of 3.9) the catalase enzyme is denatured, preventing the break down of hydrogen peroxide and allowing lipid peroxidation to continue to kill the bacteria.[4]

Manuka honey is deemed to have a stronger antibacterial effect due to the presence of methylglyoxal in the honey.[1] Manuka honey is produced with the nectar from the Leptospermum Scoparium, found in New Zealand. This nectar produces honey that contains significant levels of methylglyoxal. This methylglyoxal is deemed to be that which gives manuka honey its stronger antibacterial properties. Manuka honey is graded in UMF (Unique Manuka Factor) which commercially ranges from 5+ UMF to around 30+ UMF.[5]

Method

Three different honeys were compared: Rowse Organic Honey (processed); Norfolk Cottage Local Honey (unprocessed); and Rowse 10+ UMF Manuka Honey. The antibacterial properties of these three honeys were tested on two bacteria: escherichia coli (Gram-negative) and bacillus subtilis (Gram-positive). Agar plates were inoculated with 0.5ml of bacteria under sterile conditions and four holes were taken out of the agar plates with a sterile cork borer. Different honey samples were placed in each hole (0.09ml in each hole). Each of the honey samples were diluted with distilled water to give four different concentrations for each honey (25%, 50%, 75% and 100% honey by volume). A control was also plated, which was distilled water (in effect 0% honey by volume). Six samples of each concentration for each honey were plated. This provided enough repeats for reliable data and a good statistical analysis. The agar plates were stored in an incubator at 25°C for 42 hours.

honey agar plates png ys crest

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Fig. 3: Figure 3: Some of the agar plates used in the investigation

Sterile Conditions

To achieve accurate and reliable results, measures had to be put into place to achieve sterile conditions when plating the bacteria. The cork borer and glass spreader used on the agar plates were both sterilised before the experiment and during the experiment these pieces of equipment were dipped in ethanol and passed through a bunsen flame before coming into contact with the agar plate. The bacterial plates were plated near the bunsen flame to reduce the chance of contaminants from the air. Furthermore, once plated, the agar plates were sealed with tape to prevent any microorganisms from the air contaminating the plates.

Safety First

This study used two bacteria: escherichia coli and bacillus subtilis. Ingestion of either of these bacteria could cause harm to the body. Hence, measures were put into place to reduce the chance of any ingestion of either of these pathogens:

- When the agar plates were being inoculated with bacteria they were kept on a white tray. This meant that if any bacteriadid spill, it would spill onto the tray and not onto the workbench.
- Any equipment used that came into contact with bacteria were immediately placed in vilcron. This included pipettes for the bacteria, glass spreaders and cork borers.
- At the end of the experiment, the workbench was wiped down with disinfectant.
- The plated agar plates were stored in an incubator at 25°C. This prevented the growth of potentially harmful bacteria. The incubator was also kept in a locked room.

Recording

After the plates were kept in an incubator for 42 hours, each hole (filled with a honey sample) had produced a clear zone; a roughly circular area where the bacteria had been killed by the honey. The bigger the clear zone, the better the honey’s antibacterial properties. The diameter of each clear zone was measured three times at different angles. This accounted for if the zone wasn’t a perfect circle. A mean diameter could then be calculated for each sample.

Results

The two line graphs shown below reflect the antibacterial quality of the three honeys against both escherichia coli and bacillus subtilis. For all honeys there seems to be a positive linear correlation between concentration of honey and diameter of clear zone (or antibacterial strength). This is confirmed by the fact that calculating Pearson product-moment correlation coefficient for each honey on each bacterium gives r values all over 0.8. These positive correlations confirm that all three honeys have antibacterial properties.

honeys graphs ys

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Fig. 4: Figure 4: Graphs depicting three honeys' antibacterial properties at different concentrations against two different bacteria

Positive correlations (r>0.8) for each honey against each bacterium suggest that the three honeys have antibacterial qualities

The two bar charts shown present the data from the three honeys at 100% concentration. For each of these honeys the standard error of the mean has been calculated and error bars added on each bar plus or minus two times the standard error of the mean. Seeing if any of the bars overlap reflect whether there is a significant difference between the antibacterial properties of each honey. Hence, the error bars reflect the fact that manuka honey is significantly better at killing bacteria than the other two honeys used. In addition, although the unprocessed honey looks to be better in its antibacterial properties than processed honey, the difference between the two honeys isn't too significant. This can be seen as against escherichia coli, the error bars for processed and unprocessed honey overlap.

ys bars

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Fig. 5: Figure 5: Bar charts depicting the difference between the three honeys' antibacterial strengths at 100% concentration.

Figure 5: Bar charts depicting the significance between the three honeys' antibacterial strengths at 100% concentration.

Analysis

This study has a few limitations; for example, only three samples of honey were used. To improve this, more honey samples could be tested using the same method. For instance, different honey brands could be used for all of the three honey types (processed, unprocessed and manuka). Also, different grades of manuka honey could be used, such as 5+ UMF, 20+ UMF and 30+ UMF graded manuka honeys.

Conclusions

The study shows that all three honeys used (processed, unprocessed and manuka) have antibacterial properties. However, 10+ UMF manuka honey has greater antibacterial properties, having roughly twice the antibacterial effect as processed honey on escherichia coli and about thrice the effect on bacillus subtilise. Manuka honey 10+ UMF is shown to be significantly better at killing bacteria than processed and unprocessed honey. Comparing processed and unprocessed honey, unprocessed seems to have a greater antibacterial effect, but this difference is rather insignificant. Perhaps next time we feel a sore throat coming along we'll be reaching for that honey jar!

The results suggest manuka honey has significantly better antibacterial properties than other honeys

Applications

This study reveals many potential uses for medicinal honey and areas for further research:

- Sore throats caused by bacterial infections (such as streptococcus progenies) could be combated partly with a spoon of honey, which lines the pharynx and kills the bacterial infection.
- Many bacterial strains have developed antibiotic resistance due to overuse of antibiotics (such as MRSA). Manuka honey could be used to kill some strains of bacteria that have antibiotic resistance.
- Stomach ulcers are caused by the bacteria helicobacter pylori. Honey could potentially be used to combat these ulcers.
- Honey could be used as an active ingredient in antibacterial hand sanitisers.
- Eating manuka honey could help prevent food poisoning, aiding to kill bacteria such as escherichia coli, campylobacter and salmonella if ingested.
- Manuka honey could be coated on a wound patch when treating wounds. This could help prevent bacterial infection via an open wound, as the honey will kill any bacteria.

Acknowledgements

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