The Germ-inator

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Background. Food is essential for life. The food that is eaten is grown all around the world. The only way that this lifestyle is possible is with preservatives.

Preservatives are either naturally occurring or synthetically produced. Synthetic preservatives are in almost every food except for those grown locally. Manufacturers add preservatives to their food so that there will be minimum bacteria growth on their product, thus lengthening its shelf life. Preservatives are also added to make a product more appealing or to keep some of its original characteristics.

Despite their effectiveness, many synthetic preservatives that are commonly used in our foods can be harmful to our health. The list of chemicals being used to preserve our food is extensive. Their effects can be shocking.

For example sodium benzoate, a preservative often added to soft drinks such as Fanta and Sprite, can cause DNA mutation in high doses [1]. Butylated hydroxyanisole (BHA) and the related compound butylated hydroxytoluene (BHT), used to prevent fats from going rancid, are known to cause cancer in rats [2]. Health Canada reports that these chemicals are safe in small doses [3]. However, these synthetic preservatives, among others, are consumed daily through various products. With this degree of consumption, some side effects are quite possible.

Purpose and Hypothesis. The purpose of this investigation was to compare the effectiveness of natural preservatives and synthetic preservatives and to identify a natural preservative that could preserve foods and other edibles effectively without posing any health risks, therefore acting as a replacement for synthetic preservatives. It was hypothesized that natural preservatives would be an effective replacement for synthetic preservatives because of their safety and convenience.

Procedure. The procedures followed for the three experiments were slightly altered after the discovery of flaws in the previous experiment. Variables were modified for each experiment to gather different data. There were two main stages in each experiment: The preparation of the agar and the preparation of the petri dishes.

The method for the creation of the agar medium included the use of chicken broth rather than water. Chicken broth provides a nutrient rich surface for the growth of bacteria. Boiling the chicken broth to kill any bacteria is the first step. The broth is then filtered through a coffee filter to remove fat. Finally, the agar powder is added to the boiled broth to create a gelatinous substance. The ratio between agar powder and broth differed in each experiment and will be explained later.

First, 20mL of the agar mixture was poured into each petri dish. This was enough to cover the surface of the dish. The dishes were then placed flat in a cold environment for 15 minutes to change into a solid, gelatinous substance. Once the agar gelled, the preservatives would be evenly inserted in a triangular pattern on the dish, which created 3 samples to gather data. Homogeneous chicken juice from frozen chicken was then coated on top of the agar. This blood-like substance is a known source of bacteria, although the type of bacteria in the experiment was not confirmed. Finally, the dishes were sealed using cellulose tape.

To determine the effectiveness of the preservatives, the amount of bacterial reduction around each preservative was examined. This area of bacteria reduction will be referred to as a ring around each preservative. The diameter of this area was then measured as was the concentration of bacteria in the ring.

Experiment 1. The initial trial consisted of garlic and ginseng, two natural preservatives, paired with alum and ascorbic acid, two synthetic preservatives. In this process, the agar method was practiced to ensure that there were no errors in the final two experiments, which were purported for the accumulation of data.

The initial trial showed that the ratio of 6g of agar powder to 250mL of chicken broth made a product that was too firm. This made it difficult for the growth of bacteria. Another flaw found in the initial trial was that the dishes were tilted slightly which created an uneven surface for the growth of bacteria. The dishes were kept flat in later trials.

Experiment 2. The flaws from the initial trial were corrected in the second experiment. A ratio of 3g of agar to 250mL of broth was used to create a softer surface. The trial included seven natural preservatives (garlic, ginger, ginseng, salt, sugar, rosemary and marjoram), two synthetic preservatives (alum and ascorbic acid) and one control (paper). The paper was used as a control because it was identified that paper did not have antimicrobial properties. An observation can then be taken to see if bacteria growth is inhibited by the presence of another object or substance.

Within 24 hours after the experiment was conducted, all dishes showed intense bacteria growth as a beige smear across the dish. This made it nearly impossible to conduct a bacterial count; however the antimicrobial rings were very definitive. To solve this problem, the data was collected through a qualitative analysis by estimating which preservatives had fewer bacteria in the antimicrobial rings. Although this analysis cannot be compared to other results from studies, the data can show the antibacterial relationship between the natural preservatives and the synthetic ones.

Each preservative was then ranked on the size of its ring and the amount of bacteria reduction. With rankings for both factors, an overall ranking was created. This was done by adding the preservative's ranks together. Based upon these two factors, the most effective natural preservative proved to be garlic. Although the average radius was small, the concentration of bacteria was minimal. Thus, in large quantities garlic could be a very effective natural preservative.

This experiment produced results however it did not create precise results for bacterial reduction, because of the smear of bacteria that was grown. It could possibly have been a consequence of using old chicken juice that likely had bacteria growth already occurring in it. This flaw was corrected

for the final experiment by using frozen chicken juice.

Experiment 3. In the third and final experiment, a total of sixteen preservatives were tested, eleven natural (garlic, shallots, ginger, celery, rosemary, mint, lemon rind, orange rind, salt, sugar and beets) four synthetic (alum, ascorbic acid, monosodium glutamate and sulfites found in meat tenderizer) and one control (paper). The bacteria grew in colonies making it possible to count them. However the colonies were too small for the naked eye to count accurately. To solve this problem, a digital photograph was taken and analyzed using a formula.

The digital picture size may vary from sample to sample. Using the physical measurement of the petri dish diameter, 85mm, and dividing it by its diameter in digital pixels (represented by the variable x), the distance in mm per pixel (px) can calculated. When the distance per px is multiplied by the diameter of the antimicrobial ring in px, represented by the variable y, the diameter in mm can determined. The equation used was: $d = y \frac{85}{x}$, where d is the diameter of the antimicrobial ring in mm.

Another problem solved by the digital photograph method was controlling the minimal size for bacterial colonies. On some dishes, smaller colonies would merge together to form larger colonies. This was identified as a potential problem because a small bacteria colony with a 0.01mm diameter would be counted as the same as one with a 1mm diameter. As a solution, a minimum colony diameter was set at about 22px or 0.15mm.

The digital photograph method also has the advantage that it collects and preserves data for analysis at a later date.

Results. The third experiment yielded the clearest results. Three natural preservatives and three synthetic preservatives showed bacterial reduction after 72 hours. The most effective was alum with 100% bacterial reduction in comparison to the control and an average antimicrobial ring diameter of 34.33mm. Ascorbic acid followed with an average of 89.44% bacteria reduction and an average antimicrobial ring diameter of 20.58mm. Salt was the third most effective averaging a 73.09% decrease

of bacteria and an average antimicrobial ring diameter of 19.15mm. Meat tenderizer showed a 61.27% decrease of bacteria and a 13.55mm diameter. Garlic had an average 44.04% decrease in bacteria and a 14.28mm diameter. Lemon rind had a 46.11% decrease in bacteria and an 11.55mm diameter.

Conclusion and Application. Although the results of the experiment have proven that synthetic preservatives such as alum perform better than even the most effective natural preservatives (garlic, lemon rind, and salt) in terms of bacterial prevention, such historically valued natural preservatives cannot be so easily forfeited for modern synthetic preservatives. Humans do not have a full understanding of the potentially harmful effects of modern man-made preservatives. There are many consequences of using synthetic preservatives such as alum, which has been cited as a contributor to the increase in Alzheimer's disease [4].

The digital photographic technique could be used for studies with massive data analysis that would not be possible to analyze otherwise. If developed further, a computer program could automatically conduct the bacterial count and the pixel measurements. The digital photographs can be stored and used to compare daily growth, or even compared to results from future experiments. The results of our study indicate that natural preservatives found around the household can be used with significant bacterial reduction.

References

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