The Effectiveness of Natural Preservatives in Preventing Microbial Growth on Plain Bread

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Abstract

Preservatives are an important component in various products, helping to prolong the shelf-life of foods and postpone spoilage. However, adverse health impacts associated with artificial preservatives have been reported. With more than half of food waste resulting from household food spoilage, it is important to address global food security concerns and explore safer preservative alternatives. Therefore, this study investigates readily available natural preservatives such as lemon, garlic, and salt, to mitigate household food waste. Focusing on the antimicrobial properties, plain bread was baked with the preservatives mixed in, and mold growth was monitored using acetate grids. Observations under a microscope revealed varied coloured molds and textures on the bread surfaces. Statistical analyses, including one-way ANOVA, were conducted on the data collected. The overall p-value was determined to be 0.0038, revealing significant differences between the treatments Furthermore, Tukey's test results reveal salt as the most effective preservative, with significantly slower mold growth compared to the control and the other treatments. In contrast, garlic and lemon show no significant preservation effects. This could be attributed to heat-induced loss of antibacterial activity of lemon and garlic, or systematic errors in the methodology of collecting the data. The findings are representative of the potential of natural preservatives, particularly salt, in promoting healthier food practices and reducing dependency on artificial additives.

Introduction

While food is a fundamental necessity for all humans, 800 million individuals still lack access to nutritious foods (Skaf et al., 2021). When discussing global food security, the ethical concern of food waste is brought to light. From 2001 to 2010, one-third of edible food was wasted each year (Gustavsson, 2011). Notably, 60% of the food loss was a consequence of household food spoilage, 50% of which was avoidable (Tomaszeska et al., 2022; Heng & House, 2021). Food waste is also increasingly recognized as an environmental problem (Papargyropoulou, 2014). As a result of the CO_2 produced by transportation, storage, and food preservation, Canada's annual contribution of food waste to climate change is 16.3 million tonnes of CO_2 per capita (Skaf et al., 2021). This is equivalent to 3.6 million gasoline-powered vehicles (United States Environmental Protection Agency, 2023). This problem is even further amplified due to the rising global population, meaning the demand for food is gradually increasing (Papargyropoulou, 2014). To help mitigate household food waste, this study researches the antimicrobial properties of natural preservatives. Minimizing the use of artificial preservatives is crucial as they have been found to have adverse health effects including increased irritability, restlessness, reduced concentration, exacerbated asthma and allergies, and increased risk of cancer (Dengate & Ruben, 2002; Anand & Sati, 2013). Alternatively, natural preservatives can offer greater benefits due to their less toxic nature and spectrum of health advantages (Anand & Sati, 2013).

Past literature discovered that citrus-based essential oils were found to reduce or completely inhibit fungal growth, particularly discovering that lemon was the second greatest inhibitor against the fungi *A. niger*, *A. flavus, P. chrysogenum* and *P. verrucosum* (Viuda-Martos et al., 2008). Pastes with added garlic had an extended shelf-life of 2.5 additional weeks, specifically inhibiting the growth of the fungi *Candida utilis*, *Candida mycoderma, Candida tropicalis, Candida krusei,* and *Rhodotorula glutinis* (Olaniran et al., 2020). Lastly, salt has been used to prevent food spoilage for thousands of years, but its excess consumption has been associated with high blood pressure (Sofos, 1983). Lemon (zest), garlic, and salt are three low-cost, easily accessible, and natural preservatives used in this study. It is hypothesized that there will be a difference in the mold growth curve for the bread baked with different preservative compounds (garlic, lemon zest, and salt) when compared to no-treatment control bread. Due to their antimicrobial properties, it is predicted that using these preservative compounds will prolong the shelf life of the bread, represented through a slower growth curve of mold compared to a no-treatment control bread. A secondary objective is to compare salt with the other preservatives to determine if they have similar preservation effects while being safer.

Methods

The experimental design was to record the growth of mold on bread that was baked with three different natural preservatives. The chosen treatments were garlic, lemon zest, and salt (Fig 1). For monitoring mold growth, an acetate grid was used to see how much of the bread was covered in mold every 1-2 days. Simultaneously, pictures of the bread were taken to record any qualitative changes. To obtain a more accurate measurement, we had three replicates for each type of preservative and three replicates for control, resulting in 12 pieces of bread needed for our experiment.

Figure 1: (from left to right) Lemons, minced garlic, salt.

The bread was prepared using a recipe found online (Allen, 2022) that was adapted for the needs of this experiment. The recipe started with 475 mL of warm water, with 9 g of active dry yeast mixed into it along with a pinch of sugar to activate the yeast and determine if it was viable. After the yeast and water mixture started foaming, it was mixed with 375 g of all-purpose flour and 26 g of canola oil. It was then mixed to combine. Another 215 g of all-purpose flour was gradually added while kneading for 10 – 12 minutes until the dough became smooth, elastic, and tacky.

The dough was then divided into 4 equal balls, each weighing 283 g. According to past literature, the most efficient ratio of garlic baked in bread was 3.3% of the weight of the dough

(Bandalan & Lauzon, 2018). Using this as a guide, 3.3% of the weight of a single dough ball (9 g) of each preservative was prepared. The garlic was minced using a kitchen knife, with an effort to ensure even size among all the pieces. The lemon zest was obtained from 2.5 large lemons that were thoroughly washed and dried, then zested using a steel zester. For the salt, Windsor brand table salt was used. Each dough ball was then kneaded with a single preservative until it was evenly distributed, except for the control. They were left to rise at room temperature for 90 minutes on a baking sheet covered with a clean dish towel. Since 4 half-loaves were being made, two 22. 8cm X 12.7 cm non-stick loaf pans were prepared. The loaf pans were split in half using aluminum foil, ensuring that the two dough balls would not touch each other in the baking process (Fig 2).

Figure 2: Dough ball before the second rise in the partitioned loaf pan.

After the dough had risen, it was punched down to remove air bubbles and placed into the loaf pans. It was left to rise for another 60 minutes at room temperature. The loaf pans were then placed into a 350 ºC oven for 32 minutes, until they were golden brown on the top and a toothpick inserted into the loaf came out clean. The loaves were left to cool on a baking sheet and once they cooled down (Fig 3). They were sliced into 12.5cm x 7.5cm slices with a thickness of 1cm.

Figure 3: Baked and cooled bread loaves.

To initiate mold growth, the slices of bread were each sprayed with 1 full spray of water on each side from a distance of 10cm (Fig 4). They were then placed into Ziploc bags, sealed, labeled, and put into a lidded box (Fig 5). The box was then kept in a room with minimal sunlight and low heating. The bread was checked 3 times a week on Monday, Wednesday, and Friday until mold growth was apparent, after which, it was monitored and measured daily. Once any sample of the bread had reached 100% coverage on a single side, the experiment was concluded.

Figure 4: Bread being sprayed with sterilized water.

Figure 5: Day 1 (Nov 1). Bread after spray, in ziplock bags and in the box.

To determine the amount of mold growth, an acetate grid was used to measure the number of squares on the bread occupied by mold (Fig 6). The acetate grid was lined up with the left corner of each slice to ensure precise measurements across all samples. If 50% or more of a square was covered with mold, then it was counted as one square. Using the data collected, a growth curve will be plotted to measure any difference between the 4 loaves in regard to mold growth. Qualitative observations were obtained by the naked eye as well as with a dissecting microscope at the end of the experiment.

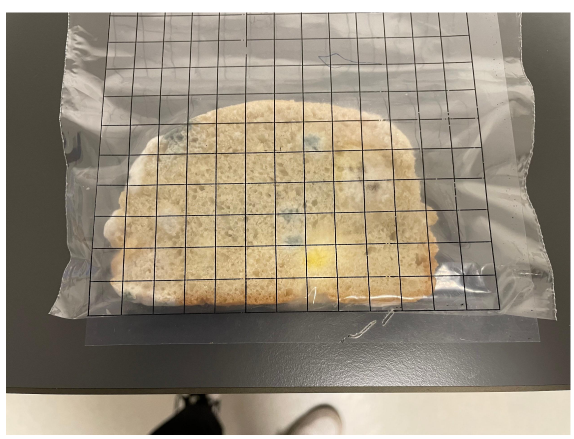

Figure 6: Acetic grid used to measure bread.

To analyze the results, a growth curve was plotted for each replicate, and a one-way Analysis of Variance (ANOVA) test was done. Tukey's test was also conducted to obtain multiple comparisons between treatments. Significance testing will be conducted on the growth curve obtained from day 6 to day 10 as it is under continuous observation and represents the phase of maximum growth rate.

Results

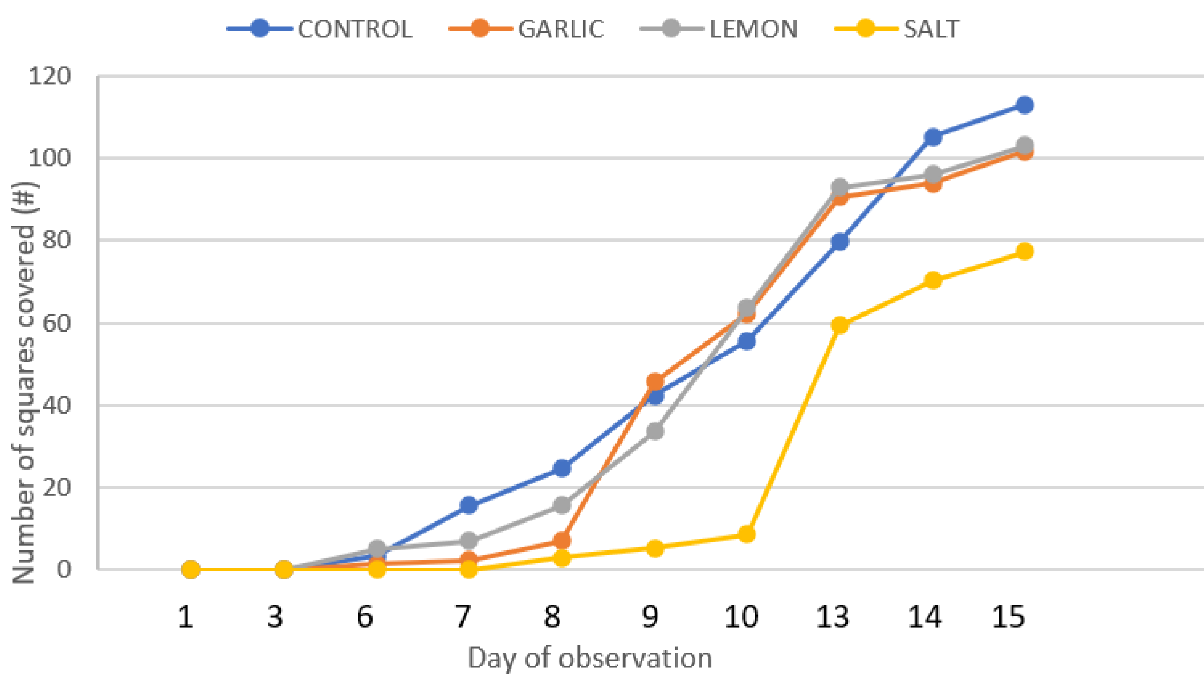

Figure 7: Number of squares covered by mold by each treatment group over time. Data points are presented as the mean of 3 replicates. Data collected over 15 days from November 1, 2023 to November 15, 2023 at 2 pm daily.

The first incidence of mold was observed on November 6. A logarithmic-like growth curve is observed across all treatments. From day 13 to 15, the growth appears to slow down and plateau. The initial days from day 1 to day 3 also depicted no observable growth. Since data was not collected on the weekends, days 4-5 and 11-12 are not accounted for. Each treatment from day 6 to day 10 depicted varying slopes of growth and a phase of maximum growth rate for most treatments, with the control having the steepest slope of growth and salt having the smallest slope. While salt has the smallest overall slope, the slope from day 10-13 is observed to be very steep. Overall, the number of squares covered by the mold shows an increasing trend over time for all treatments. Control replicate 3 and salt replicate 1 were the first to achieve complete mold coverage on day 15, leading to the end of the study.

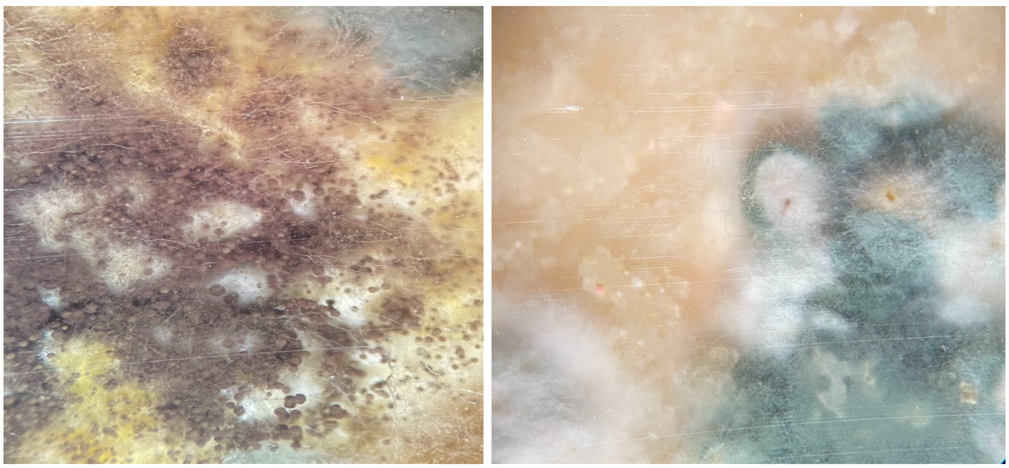

Figure 8: Observation of randomly selected mold spots under a dissecting microscope, photo was taken on November 9, 2023.

Periodically the mold was observed under a microscope and a variety of coloured molds were seen, consisting of brown, black, yellow, orange and white. Under the microscope, they appear like a cluster of tiny dots as well as a network of many threadlike structures. The areas mold grew on the bread appear to be variable. Mold growth was observed on all surfaces of the bread.

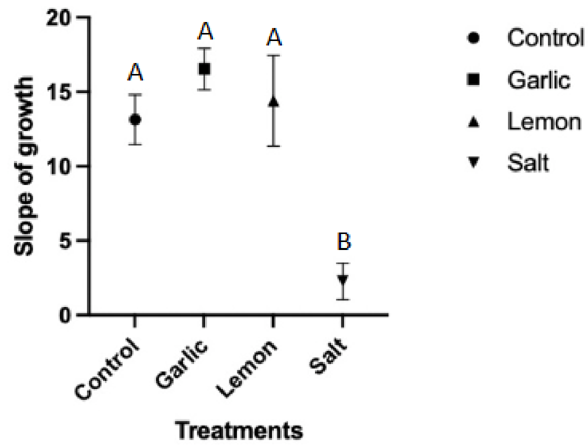

Figure 9: The slopes of growth for the various treatments between November 6 (Day 6) to November 10 (Day 10) collected at 2 pm. Data is presented as mean±S.E.M. The means denoted by a different letter indicate significant differences between treatments

The slope of the growth curves of each replicate of each treatment was evaluated from day 6 to day 10 using linear regression via Microsoft Excel. A one-way ANOVA was conducted using the slopes in Table 1 to obtain p-values in GraphPad Prism. A p-value of 0.0038 was obtained under an alpha value of 0.05. Tukey's range tests were conducted to perform multiple comparisons between treatments. Tukey's range test obtained an adjusted p-value of 0.6307 between the treatment of garlic and control, an adjusted p-value of 0.9666 between the treatment of lemon and control, and an adjusted p-value of 0.0188 between the treatment of salt and control. Between treatments of garlic and lemon, an adjusted p-value of 0.8669 was obtained. An adjusted p-value of 0.0039 was obtained between treatments of garlic and salt, as well as an adjusted p-value of 0.0103 between lemon and salt.

Discussion

Food waste is a major global concern that can be mitigated by employing preservatives to extend the shelf life of many perishable food items. However, artificial preservatives have been found to have adverse health effects like increased risk of cancer, which is why it is important to transition towards natural preservatives (Dengate & Ruben, 2002; Anand & Sati, 2013). This study was conducted to research the antimicrobial properties of natural preservatives such as lemon zest, salt, and garlic, which are all easily accessible to most people. According to Figure 7, salt seems to be the best preservative represented by its slower growth curve, in comparison to the control and other treatments. Meanwhile, garlic and lemon exhibit a growth curve similar to the control, which entails that they are not effective as a preservative.

Regardless, the results of the study are significant based on the overall p-value of 0.0038 of the single-factor ANOVA test, which is smaller than 0.05. Therefore, the null hypothesis is rejected. This means that natural preservatives do have an effect on inhibiting the development of microbial growth on plain bread. Simultaneously, Tukey's Range Test can be used to assess the effectiveness of each treatment and verify whether garlic and lemon are truly ineffective as seen in Figure 7. Based on the results of Tukey's Range Test, it can be said that salt is the strongest preservative out of the three due to the adjusted p-value of 0.0188 between the treatment of salt and control, the adjusted p-value of 0.0039 between treatments of garlic and salt, as well as the adjusted p-value of 0.0103 between lemon and salt, all of which are less than 0.05. However, it is difficult to rank the other natural preservatives (lemon and garlic) in terms of their effectiveness due to an insignificant result (>0.05) from Tukey's Test for their comparison between treatments, including the control.

Overall, the findings of this experiment present salt as a strong natural preservative. This is consistent with past research, which states that salt has been used to prevent food spoilage for thousands of years (Sofos, 1983). In addition, salt has a very high melting point (801ºC), which preserves its antimicrobial activity even when heated (PubChem). Furthermore, salt's antimicrobial activity functions based on reducing the water activity in food, and heat would also contribute to the same. However, the secondary objective of the research, which was to compare the effectiveness of other, less harmful preservatives like garlic and lemon zest, has no significant results.

It appears that lemon zest and garlic do not have any effect on the mold growth, when compared to the control. This is not consistent with previous findings which state that garlic extended the shelf-life of pastes by 2.5 weeks (Olaniran et al., 2020). Additionally, it contradicts past research about citrus-based essential oils reducing or completely inhibiting fungal growth, particularly lemon being the second greatest inhibitor against certain fungi (Viuda-Martos et al., 2008). Such a difference may be due to this study's use of heat, as previous experiments did not apply heat to the preservative itself and used it raw instead. Lemon zest, which is composed of naringin, has a much lower melting point (83ºC) than the temperature at which the bread was baked (Diehl, B., 2008) (PubChem). Another research states that most of garlic's antimicrobial activity is derived from one of its compounds called allicin, which also has a low melting point (<25ºC) and loses effectiveness as it is exposed to higher temperatures (Kim et al., 2002). Hence, while baking the bread with garlic and lemon zest for this experiment, both the natural preservatives might have lost their antibacterial activity.

Other factors that could have had an impact on the results include systematic human error. The bread was attempted to be cut evenly for each sample, as well as sprayed with water evenly. However, it is likely that not all parts of the bread were exactly the same size, or sprayed evenly across its area. This error is due to the methodology implemented, where although the bread was sliced into 12.5cm x 7.5cm slices with a thickness of 1 cm, the shape or weight of each slice was not accounted for, which caused differences in size. To improve it in the future, the bread could be cut into even rectangular-shaped pieces, each measured by a ruler and a weighing scale. In terms of spraying error, although the distance between the spray bottle and the bread is consistent, holding it out by hand can concentrate the spray on one side of the bread. Hence, the spraying could be done by placing the bread in a box rather than holding it out by hand, to ensure it is evenly moist. Another error that could have taken place is the incorrect counting of the coverage of mold, due to four different people taking counts on different days. To prevent this in the future, only one person should be assigned to record the mold coverage. This will ensure that even if an error occurs, it is consistent with all the samples, maintaining the integrity of the study.

Conclusion

Ultimately, a significant difference was found between salt and the other treatments, corresponding with our prediction. This newfound information could potentially lead to further research regarding natural preservatives, culminating with the adoption of natural preservatives in place of artificial ones, reducing the negative health effects associated with them and providing a healthier, better life for consumers.

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Appendix

Table 1: Raw data of mold counts for each bread slice, front and back cumulative.

	day 1	**day 3**	**day 6**	**day 7**	**day 8**	**day 9**	**day 10**	**day 13**	**day 14**	**day 15**
c1	0	0	8	25	35	57	64	99	110	117
c2	0	0	0	16	26	36	39	41	81	92
c3	0	0	2	6	13	34	64	99	125	130
g1	0	0	1	1	8	54	71	87	89	95
g2	0	0	1	2	7	38	59	84	89	94
g3	0	0	2	4	6	45	57	101	104	116
l1	0	0	4	4	8	22	46	77	82	84
l2	0	0	6	13	25	38	57	82	87	102
l3	0	0	5	4	14	41	88	120	119	123
s1	0	0	0	0	4	11	17	89	107	109
s2	0	0	0	0	4	4	8	46	53	62
s3	0	0	0	0	1	1	1	43	51	61

Table 2: Average of all three slices of bread (front & back) of each type.

		Average number of squares in the grid with mold (cumulative)			
		Control	**Bread with garlic**	**Bread with lemon zest**	**Bread with salt**
Day 1	**Weds Nov 1**	0	0	0	0
Day 2	**-**	**-**	**-**	**-**	**-**
Day 3	**Fri Nov 3**	0	0	0	0
Day 4	**-**	**-**	**-**	**-**	**-**
Day 5	**-**	**-**	**-**	**-**	**-**
Day 6	**Mon Nov 6**	3.3	1.3	5	0
Day 7	**Tues Nov 7**	15.7	2.3	7	0
Day 8	**Weds Nov 8**	24.7	7	15.7	3
Day 9	**Thurs Nov 9**	42.4	48	33.4	5.3
Day 10	**Fri Nov 10**	55.6	62.3	63.7	8.7
Day 11	**-**	**-**	**-**	**-**	**-**
Day 12	**-**	**-**	**-**	**-**	**-**

Day 13	**Mon Nov 13**	79.7	90.7	93	59.3
Day 14	**Tues Nov 14**	105.3	94	96	70.3
Day 15	**Weds Nov 15**	113	101.7	103	77.3

Table 3: Slopes of growth curves of each treatment and its replicates obtained from linear regression of growth from day 6 to day 10

	Control	**Garlic**	**Lemon**	**Salt**
Replicate 1	14.4	19.3	10.2	4.5
Replicate 2	9.8	15.2	12.7	2
Replicate 3	15.2	15.1	20.3	0.3