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Research Article

Sodium Sulfite and Sodium Nitrite: Helpful in Promotion of Antibiotics Sensitivity and Overgrowth of Food Poisoning Bacteria by Stress

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A B S T R A C T

The purpose of this research is to examine the effect of food additives bacteria B. cereus, E. coli, and S. gallinarum, which were exposed to three different environments containing food additives: first, exposed to the standard chemical of sodium nitrite, 0.2%, and sodium sulfite, 0.0625% before agar diffusion test to observe difference in antibiotics resistance; second, the same as the first except replacing the standard chemical chemicals with food additives; third, changing the concentration of the food additives to observe how the food additives affect the antibiotics resistance; fourth, food additives, meat and C. perfringens mixed and cultured to observe the number of resulting bacteria; lastly, the three bacteria treated with heat and UV light in the same concentration to observe general vulnerability change in bacteria. The results reveal that food additives decrease the antibiotics resistance of bacteria, but that bacteria tend to grow more rapidly when exposed to antibiotics than under normal condition. It has also been found that food additives weaken the activity of bacteria, but bacteria react to enhance their reproduction to compensate for downsizing colony.

Keywords: Bacteria, Antibiotics Resistance, Food Additives

Introduction

Antibiotics resistance is well known to appear when bacteria can survive and reproduce even when therapeutic level of antibiotics is present.[1] Bacteria can acquire aforementioned resistance by two means. One is genetic mutation, which will transform bacteria in certain way to resist antibiotics. The other is conjugation by which genetic materials of the resistance are transferred to one another. A research on the relationship between the concentration of antibiotics and the acquisition of antibiotics resistance revealed that an exposure to lincomycin can strengthen the resistance of certain bacteria to cefazedone, and that an exposure to a chemical can trigger resistance in bacteria to other chemicals. This research extended those researches to food additives with which bacteria are more likely to get in contact. There exist two major categories in food additives.[2] One is for enrichment such as the iodine added in salt to prevent iodine deficiency and goiter and the other is for technological parts whose functions are preserving, adding taste,[3] and maintaining consistency: preserving additives lengthen life of food like raw meat or fish paste; taste adding additives alter the taste in natural products like

vegetables or create flavor for products without pleasant taste; consistency maintaining additives modify certain property in the food while modifying other properties. In lieu of this, this research chose sodium nitrite and sodium sulfite as basic food additives, which are used to retain color and freshness of meat, because more deadly bacteria grow in meat products.[4] Sodium nitrite may disable red blood cells to transport oxygen, and cause gastrointestinal and brain cancer, or higher childhood type 1 diabetes risk during pregnancy when consumed.[5] Although most side effects of sulfites were observed in asthmatics, sulfites may cause wheezing, nausea, diarrhea or narrow airways.[6]

In this research, five experiments were conducted. The first was exposing bacteria to official standard of food additives and done agar diffusion test to observe how food additives can affect bacteria's antibiotics resistance. The second was exposing bacteria to both food additives and antibiotics and done agar diffusion test to whether antibiotics can have synergic effect on resistance change. The third was exposing bacteria to different concentrations of food additives and done agar diffusion test to observe how different concentrations can affect the resistance. The fourth was culturing C. perfringens with meat and food additives and counting resulting bacteria after incubation to observe food additives effect on new bacteria growth. The last was exposing bacteria to food additives and treating with heat and UV light to observe general vulnerability of bacteria exposed to food additives.

Materials and Methods

Preparing Nutrient Broth (NB)

8 grams of nutrient broth powder (Difco, France) were mixed with 1 litter of distilled water. The mixture was sterilized in autoclave (Tomy, Japan) at 121 degree Celsius and 1.2 atm. for 15 minutes. The NB media was then cooled down to around 50 degree Celsius. The NB media was moved to a clean bench. Media was divided 40 milliliters each in conical tubes. Media was stored in 4 degree Celsius before use.

Preparing 6.25% Sodium Nitrite Solution (SN)

0.625 grams of sodium nitrite (Daejung, Korea) was dissolved in 10 milliliters of nutrient broth. Therefore, 6.25% sodium nitrite solution was made.

Preparing 2% Sodium Sulfite Solution (SS)

0.2 grams of sodium sulfite, anhydrous, 95.0% (Samchun Chemicals, Korea) was dissolved in10 milliliters of nutrient broth. Therefore, 2% sodium sulfite solution was made.

Making Tryptose Sulphite Cycloserine Agar (TSC agar)

23.5 grams of TSC agar base were suspended in 475 milliliters of distilled water. The mixture was stirred until they were dissolved. The solution was sterilized in 121 degree Celsius for 15 minutes. The solution was cooled to 50 degree Celsius and 25 milliliters of Egg Yolk Emulsion (MB-E1864) and contents of 1 vial of Perfringens.

Selective supplement, TSC (MB-P2551) or 1 vial of Perfringens Selective supplement, SFP (MB-P2550) were mixed. The solution was poured on petri dishes.

Clostridium Perfringens Aerobic and Anaerobic Culture

TSC agar and Clostridium perfringens (KCTC 3269) were used. C. perfringens was freeze-dried in inactive form. It was kept in freezer, and the tip of the glass vial was cut 5 millimeters inward with an ampule cutter. The tip was pulled and broke. The cut side was sterilized with alcohol lamp. The Clostridium perfringenspowder in vial was mixed with 10 % skin milk.[10] microliters of resulting solution was dropped on four TSC agars, respectively. Two TSC agar was put in anaerobic culture and were incubated in 30 degree Celsius for 24 hours. Other two were put in 30 degree Celsius with aerobic environment.

Experiment 1: Antibiotics Resistance Change due to Exposure to Food Additives

NB media (control group), Sodium nitrite media, and Sodium sulfite media were prepared. Control group was simply 10 milliliters of nutrient broth. 0.0625% sodium nitrite media was made by mixing 9.9 milliliters of nutrient broth and 100 microliters of 6.25% sodium nitrite solution.0.2% sodium sulfite media was made by mixing 9 milliliters of nutrient broth and 1 milliliter of 2% sodium sulfite solution. 1 milliliter of each media was poured into three microtubes respectively, yielding total 9 microtubes.10 microliters of each bacterial solution, Bacillus cereus, Escherichia coli, and Staphylococcusgallinarum, were inoculated into three groups of solution.

All the solutions were incubated in 37 degree Celsius for 24 hours before agar diffusion tests. 20 microliters of bacterial solutions were dropped on nutrient agar, smeared by spreader. Four and three equally distanced holes were poked with a micropipet white tip picked with inoculate tweezers Seven antibiotics, kanamycin, gentamycin, ampicillin, penicillin, 0.001X lincomycin, cefazedone, and chloramphenicol, were put in different holes. The dishes were put in 37 degree Celsius incubator for 24 hours. Finally, the size of clear zone in each agar plate was measured.

Experiment 2: Antibiotics Change due to Exposure to both Food Additives and Antibiotics

Total 18 media were made, with three mediums in one of six groups. 10 microliters of three bacteria, B. cereus, E. coli, and S. gallinarum, were put in every group, thus yielding three media in one group: control, cefazedone,

cefazedone with sodium nitrite, cefazedone with sodium sulfite, sodium nitrite, and sodium sulfite. Control was made by adding 10 microliters of bacterial solution into 10 milliliters of nutrient broth. Cefazedone group was prepared by mixing 9.9 milliliters of nutrient broth and 100 microliters of cefazedone 0.01x. Cefazedone with sodium nitrite group was ready by mixing 9.8 milliliters of nutrient broth, 100 microliters of 6.25% sodium nitrite solution, and 100 microliters of cefazedone 0.01x. Cefazedone with sodium sulfite group was made by mixing 8.9 milliliters of nutrient broth, 1 milliliter of 2% sodium sulfite solution, and 100 microliters of cefazedone 0.01x. Sodium nitrite group was made by mixing 9.9 milliliters of nutrient broth and 100 microliters of 6.25% sodium nitrite solution. Sodium sulfite group was made by mixing 9 milliliters of nutrient broth and 1 milliliter of 2% sodium sulfite solution. Next, 1 milliliter of each media was poured into six microtubes, yielding all 18 microtubes. These solutions were stored in an incubator of 37 degree Celsius for 24 hours. After incubation, agar diffusion test was done by vortexing, dropping 20 microliters of all solution on nutrient agar, and spreading 30 times with a spreader. A tweezers heated with alcohol lamp was used to pick up a white micropipette tip, and the tip was used to poke a hole in the middle. 10 microliters of cefazedone were dropping in the hole. All agars were incubated in 37 degree Celsius for 24 hours. Finally, the size of clear zone in each agar plate was measured.

Experiment 3: Antibiotics Resistance Change due to Exposure to Different Concentrations of Food Additives

Five groups of mediums were made. One was control group. The other four were SS or SN with different concentrations of 0.01x, 0.05x, 0.1x, 0.5x, and 1x , all made by mixing one another in NB. Each of three bacteria, E. coli, B. cereus, and S. gallinarum, was inoculated with five mediums. 10 microliters of bacteria were out in all five mediums. The entire medium was incubated in 37 degree Celsius for 24 hours, before agar diffusion test with 10 microliters of cefzaedone.

Experiment 4: Overall Bacterial Growth in Meat with C. Perfringens and Food Additives

C. perfringens was cultured in grinded pork and spam with different conditions. 25 grams of grinded pork were put in six 45 ml conical tubes, and 25 grams of spam were put in six 45 ml conical tubes. Two from each group were control. Four remaining from each group were divided into two groups with two tubes in each group. Two groups were mixed with 5 grams of sodium sulfite, and the other two groups were mixed with 1.56 grams of sodium nitrite by streaking loop. This yields four groups of meat with nothing, sodium nitrite, and sodium sulfite respectively. C. perfringens was put in one group of meat with nothing, meat with sodium nitrite, and meat with sodium sulfite. The tubes were then incubated in room temperature for 24 hours. Streaking loops were put into each tube and were used to streak on TSC agar. TSC agars were incubated in 37 degree Celsius for 24 hours in anaerobic culture.

Experiment 5: Vulnerability Change due to Exposure to Food Additives

Four groups of four solutions were made. The first group was made by mixing 1.8 milliliters of NB and 200 microliters of SN 6.25%. 10 microliters of one of three bacteria or 2.5 grams of grinded pork were put into one solution.

The second group was made by mixing 10 microliters of one of three bacteria or 2.5 grams of grinded pork with 2 milliliters of NB. Third and fourth groups were made by duplicating first two respectively. First two groups were heated in microwave for ten seconds. Last two groups were exposed to UV light for 15 minutes, after which the solutions were incubated in 37 degree Celsius for 24 hours and the absorbance with UV spectrophotometer with NB having 0 absorbance in 630 nm wavelength was measured.

Result

In Fig. 1, antibiotics resistance change due to exposure to food additives, a clear trend is observed in the graphs that antibiotics resistance is slightly increased, even if there is a kink for E. coli's SS., even if the source of the irregularity is either experimental error or link between E. coli' resistance or SS.

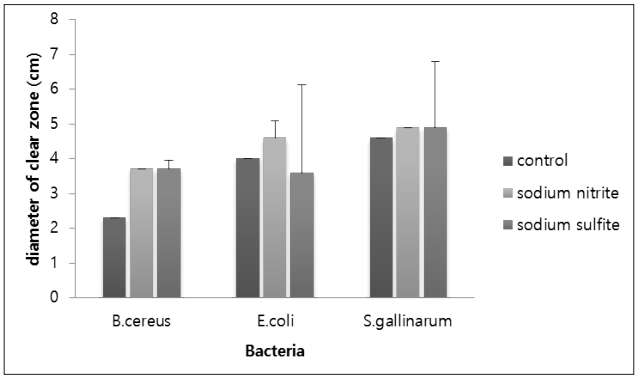


Figure 1.Diameters of clear zone of bacteria exposed to food additives and cefazedone

Figure 2, shows the result of the antibiotics change due to exposure to both food additives and antibiotics. Except the E. coli's null value of diameter for SN, which is a kink assumed to be a failure of the growth, there is a tendency that both SS and SN with cefazedone increase the zone diameter, a clear fact that strongly suggests the decrease of antibiotics resistance of the bacteria.

In Fig. 3, it is statistically uncertain that the exposure to food additives and gentamycin decrease bacteria's antibiotics

resistance, even if there is an increasing trend in diameter for B. cereus.

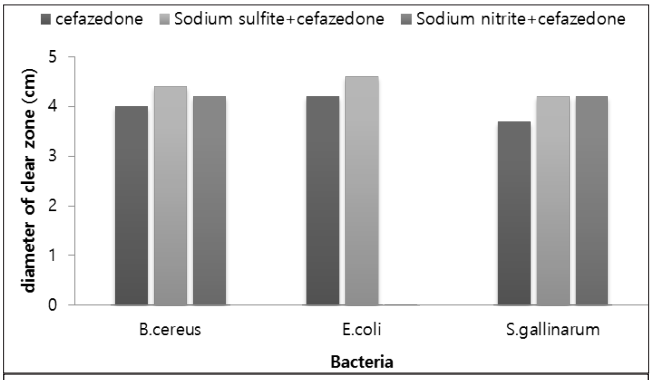


Figure 2.Diameters of clear zone of bacteria exposed to food additives and cefazedone

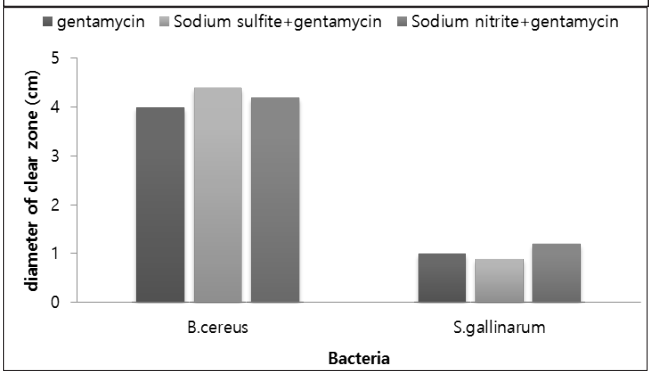


Figure 3.Diameters of clear zone of bacteria exposed to food additives and gentamycin

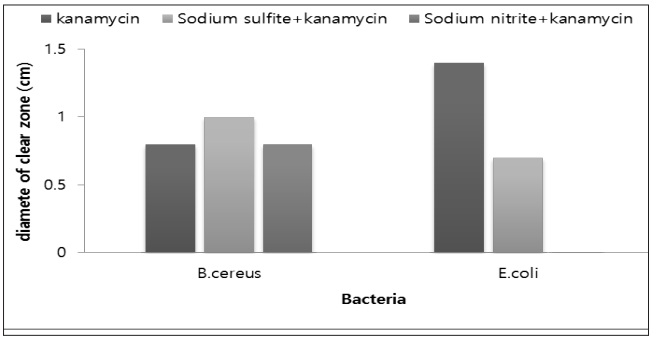


Figure 4.Diameters of clear zone of bacteria exposed to food additives and kanamycin

In Fig. 4, S. gallinarum failed to grow and was excluded in the graphs. For the other two bacteria, each shows different dependence on kanamycin, suggesting each bacterium reacts oppositely to kanamycin.

Figure 5, shows the antibiotics resistance change due to exposure to different concentrations of food additives for E. coli. Here, noticeable is the fact that the antibiotics resistance revealed high contrast at a certain concentration of two food additives (here at 0.01x), while it does not remarkably change at the other concentrations.

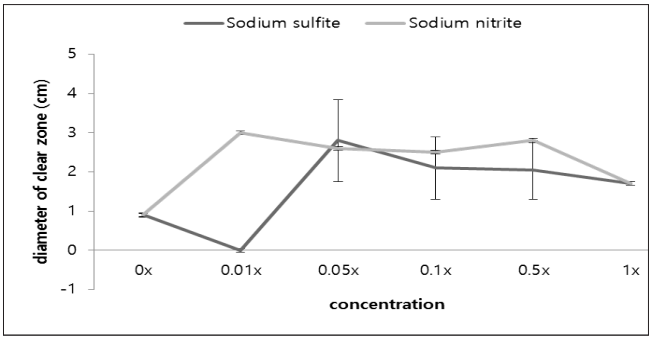


Figure 5.E. coli's diameters of clear zone vs. the concentration of food additives

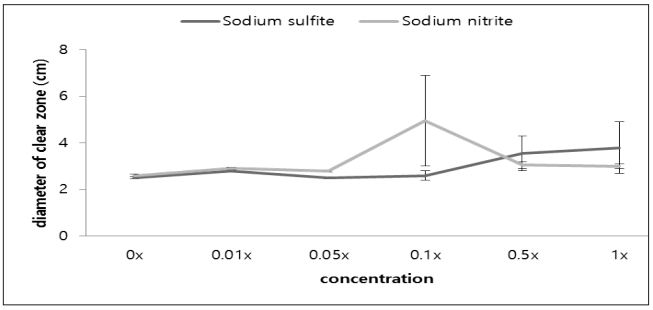


Figure 6.B. cereus's diameters of clear zone vs. the concentration of food additives

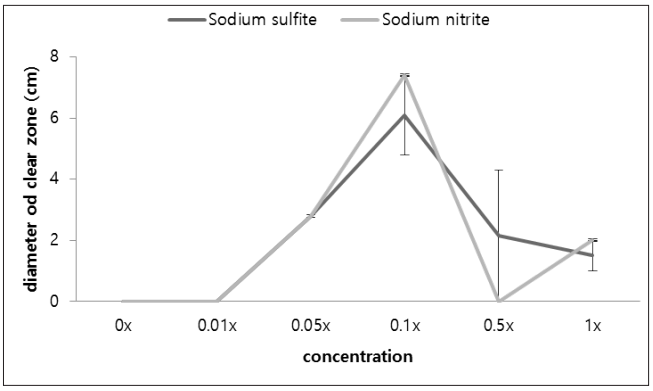


Figure 7.S. gallinarum's diameters of clear zone vs. the concentration of food additives

In Fig. 6, the same phenomenon as observed in Fig. 5 appeared for B. cereus. Here, it is also conspicuous that the difference of antibiotics resistance shows abrupt change at a certain concentration of the two food additives (0.1x), while it does not remarkably change at the other concentrations.

In Fig. 7, as with S. gallinarum, the fluctuation higher than the other two bacteria is obvious, while still the greatest difference of the resistance appears at certain concentrations.

It is clear in Fig. 8 that the existence of inoculated C. perfringens suppresses bacterium colony formation in pork.Figure 9 indicates that unlike the case of the colony on pork, C. perfringens does not have significant effect on the bacterium colony formation in spam.

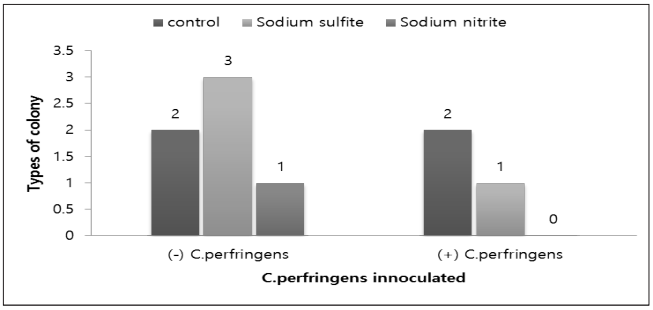


Figure 8.Dependence of the types of colony on pork on C. perfringens inoculated

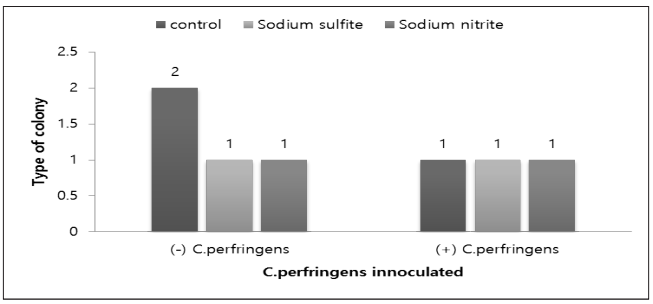
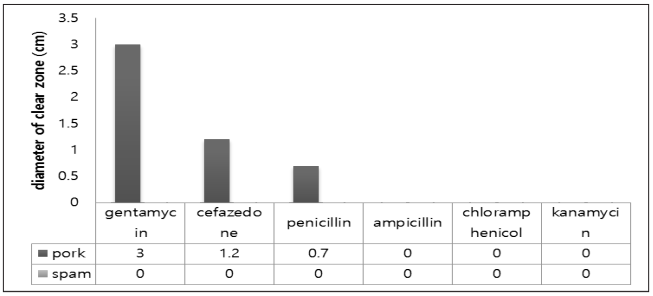


Figure 9.Dependence of the types of colony on spam on C. perfringens inoculated



	gentamycin	cefazedone	penicillin	ampicillin	chloramphenicol	kanamycin
pork	3	1.2	0.7	0	0	0
spam	0	0	0	0	0	0

Figure 10.Antibiotics resistance of pork and spam bacteria

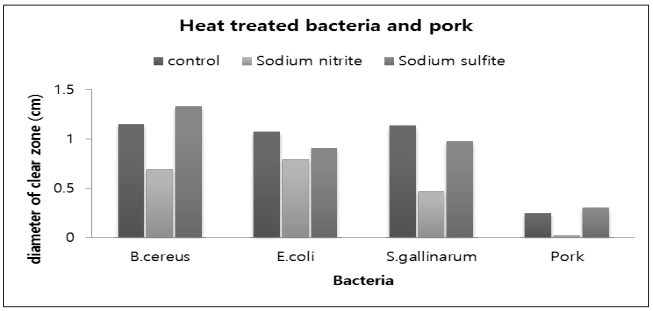


Figure 11.Absorbance of heat-treated bacteria in NB or NB with SN

It is seen in Fig. 10 that while bacteria in spam culture had no resistance for all antibiotics, bacteria in pork culture had resistance to gentamycin, cefazedone, and penicillin, a clear indication that spam bacteria had more resistance to antibiotics than pork bacteria.

As seen in Fig. 11, the vulnerability change due to exposure to food additives shows that the increase of the resistance depends on bacterium, and that heat-treated SS has less resistance than SS. High resistance for SN is conspicuous.

As observed in Fig. 12, the same is true of the UV light treated bacteria in NB or NB with SN as in the case of the absorbance of heat-treated bacteria in NB or NB with SN.

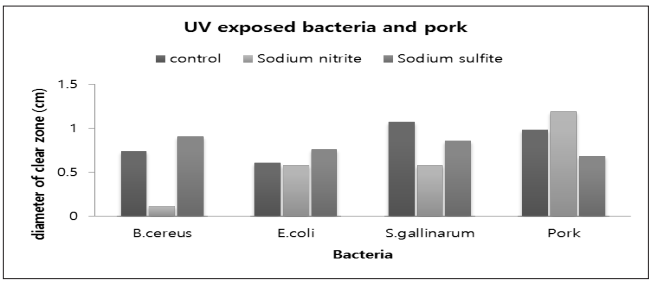


Figure 12.Absorbance of UV light treated bacteria in NB or NB with SN

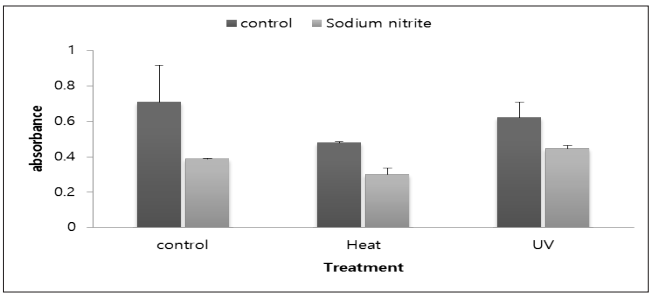


Figure 13.Average absorbance of bacteria in control, heat-treated, UV light treated with NB or NB with SN

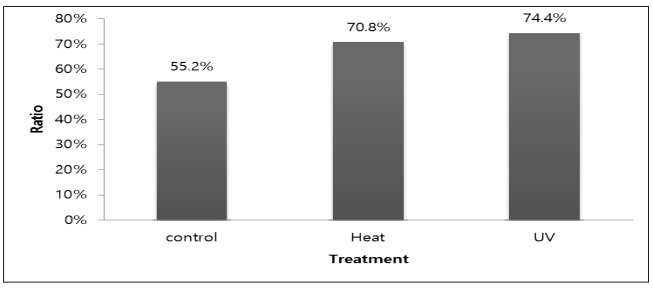


Figure 14.Ratio of SN with NB to NB

To observe comprehensive absorbance difference due to exposure to food additives, the absorbance of each control group and sodium nitrite exposed group was averaged in Fig. 13. It is evident that exposure to sodium nitrite generally increase bacteria's vulnerability to heat and UV light.

To observe comparative absorbance difference due to exposure to food additives, in Fig. 14, the absorbance of sodium nitrite exposed group was divided by absorbance of control group in control, heat, and UV light group. It is obvious that the exposure to sodium nitrite generally increase bacteria's comparative growth under heat or UV light

Discussion and Conclusion

Experiment 1 and experiment 2 reveal that the bacteria

seemed to have gained increased sensitivity after exposure to food additives, given the general trend of increasing clear zone diameter. However, it is believed that because of increased sensitivity, the food additives did not affect the antibiotics resistance of the bacteria. Except E. coli exposed to SN and antibiotics with the resistance to cefazedone and kanamycin, the reason of which is not known yet, all bacteria had increased sensitivity after exposure to mixture of antibiotics and food additives. Also due to increased sensitivity, the mixture of food additives and antibiotics is believed not to have affected antibiotics resistance. In experiment 3 E. coli and B. cereus showed the greatest difference at certain concentration while having similar behaviors in the other concentrations.

This means different species of bacteria show uniquely different anti-biotics resistance at specific concentrations, which need further research. Experiment 4 could not result in coherent data. While most showed decrease in number of colonies due to exposure to food additives, the existence of inoculated C. perfringens deterred bacterium colony formation in pork. Yet, unlike the case of pork, C. perfringens did not have significant effect on the bacterium colony formation in spam. Agar diffusion test with C. perfringens showed that it had resistance except for gentamycin, cefazedone, and penicillin.

Experiment 5 showed that food additives can have different effects on different bacteria. While SN slowed the growth of B. cereus and S. gallinarum, it did not have effect on E. coli and pork. While SS decreased growth of S. gallianrum and pork, it increased growth of B. cereus and E. coli.

Finally, this research has found that food additives, SS and SN, do not affect antibiotic resistance, but that at certain concentration, food additives increase sensitivity of bacteria. Food additives do increase bacteria's sensitivity, which in turn causes bacteria to reproduce in greater numbers rapidly when stress like UV light or heat is applied to compensate for the increased sensitivity.

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Conflict of Interest: None

References

1. Harrington C. Sodium Sulphite: A Dangerous Food Preservative. Oxford University Press, 1904, Mar 19. Available from: http://www.jstor.org/stable/3007 1817?seq=2#page_scan_tab_contents.
2. European Commission. Reports of Scientific Committee for Food. European Commission, 1997. Available from: http://ec.europa.eu/food/fs/sc/scf/repo rts/ scf_reports_38.pdf.
3. Alliance for the Prudent Use of Antibiotics. Background: About Antibiotic Resistance. APUA. Available from: http://www.tufts.edu/med/apua/about _issue/about_ antibioticres. shtml.
4. Ilbäck N, Leif B. Food Additives. Use, Intake and Safety. Scandinavian Journal of Nutrition 2000. Available from: http://www.foodandnutritionresearch.net/index.php/ fnr/article/viewFile/1778/ 1685.
5. Science of Cooking. What is Flavor? Available from: http://www.scienceof cooking.com/what_is_flavor. htm.
6. Brady A. Side Effects of Sodium Sulfite. Livestrong, 2013, Aug 16. Available from: http://www.livestrong. com/article/485372-side-effects-of-sodium-sulfite/.
7. Ipatenco S. The Harmful Effect of Sodium Nitrite in Food. Livestrong, 2014, Mar 13. Available from: http:// www.livestrong. com/article/416466-the-harmful-effect-of-sodium -nitrite-in-food/.
8. Fong B. List of Foods for Sulfite Allergies. Livestrong, 2013, Aug 16. Available from: http://www.livestrong. com/article/108427-list-foods-sulfite-allergies/.