Investigating the use of plant extracts in antimicrobial and antioxidant food packaging

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Abstract

Food preservation, quality maintenance and safety are major areas of concern in the food and beverage industry, and where this is not taken care of, food spoilage occurs, necessitating research on methods to keep food fresh, and prolong its shelf-life so its quality is maintained, and outbreaks of food-borne illnesses can be avoided. Past methods of food preservation like drying, freezing and salting can prolong the shelf-life of food products, but recontamination may occur. Antimicrobial and antioxidant food packaging involves incorporating antimicrobial and antioxidant substances into food packaging material to suppress pathogens and prevent the oxidative degradation of food, prolonging the shelf-life of food. Biodegradable packaging was used in this study as it does not contribute to landfill environmental pollution. Extracts chosen to be used were cinnamon (*Cinnamomum verum*) sticks, green tea and ginkgo (*Ginkgo biloba*) nut, and bacteria used in the antibacterial test using the *in vitro* well diffusion method were *Staphylococcus epidermidis ATCC 12228, Bacillus cereus ATCC 11778, Escherichia coli ATCC 25922* and *Serratia marcescens Carolina*, all of which are pathogens that may be found in food. This study aimed to investigate the antibacterial and antioxidant properties of cinnamon stick, green tea and ginkgo nut extracts, and whether their incorporation into a gelatin film used to wrap meat could reduce the bacterial count. It was determined that green tea extract was the most ideal to be incorporated into the food packaging as it had the most significant reduction in the number of bacterial colonies in the meat that it wrapped compared to the other packaging with respect to an unwrapped control.

Introduction

In the food and beverage industry, food preservation, quality maintenance, and safety are major causes for concern. Food spoilage result from contamination of food with microbes, causing a loss in colour, texture, nutritive value, and making it inedible. Food contamination occurs with exposure to the environment during food processing and packaging. In 2018, the Agri-Food and Veterinary Authority (AVA) of Singapore recalled frozen raw pork from the Netherlands after a *Salmonella* outbreak (Tee, 2018). The bacterium *Salmonella* which is often found in raw meat can cause a disease known as salmonellosis, characterised by diarrhoea, fever, abdominal cramps and vomiting, and symptoms may last from two to seven days.

Traditional methods of food preservation such as drying, freezing, or salting may extend shelf-life of food products, but recontamination may still occur (Malhotra, Keshwani, & Kharkwal, 2015). Antimicrobial and antioxidant packaging is a new technology incorporating antimicrobial substances into food packaging, allowing for the suppression of pathogenic microbes affecting food products, increasing the shelf-life of the food (Malhotra et al., 2015).

According to Sofi et al. (2017), antimicrobial and antioxidant packaging is categorised as biodegradable or non-biodegradable packaging. Biodegradable packaging includes edible coatings and films from proteins, lipids, starch, chitosan, and polylactic acid, while non-biodegradable packaging includes low-density polyethylene (LDPE), high-density polyethylene (HDPE) and polystyrene, which do not undergo degradation by microorganisms, have good barrier properties and mechanical strength, but cause landfill depletion, environmental pollution, and have high energy consumption during their manufacturing. Antimicrobial agents like nisin, benzoic acid and lysozyme have been incorporated in food packaging such as LDPE and HDPE (Soft et al., 2017).

Nabavi et al. (2015) studied the antimicrobial activity of cinnamon (*Cinnamomum verum*) bark essential oil, reporting good antibacterial activity against Gram-positive bacteria, *Bacillus licheniformis* and *Staphylococcus aureus*, and Gram-negative bacteria, *Escherichia coli*, *Salmonella typhi*, *Salmonella paratyphi A*, and *Pseudomonas fluorescens*. There was good antioxidant activity of aqueous and ethanolic extracts of *Cinnamomum verum* and *Cinnamomum cassia*, indicated by inhibition of pyrogallol autoxidation, and could be attributed to its flavonoids, which can inhibit fatty acid oxidation and lipid peroxidation (Rao & Gan, 2014).

Chan, Soh, Tie and Law (2011) researched on antioxidant and antimicrobial activities of green tea. The hot water extracts of green tea were tested on the Gram-positive *Micrococcus luteus*, *Staphylococcus aureus* and *Bacillus cereus*, and Gram-negative *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa.* Green tea inhibited the growth of Gram-positive bacteria, with *S. aureus* being the least susceptible. Green tea was ineffective against Gram-negative bacteria. Green tea showed high antioxidant activity, attributed to its catechins, which are effective in scavenging for free radicals and reducing lipid peroxidation.

The antimicrobial effect of ginkgo (*Ginkgo biloba*) nut was investigated and its extracts derived from various solvents showed antibacterial activity against the Gram-positive *C. acnes*, *S. aureus* and *S. pyogenes* (Chassagne, Huang, Lyles & Quave, 2019). Goh and Barlow (2002) also discovered substantial heat stable antioxidant activity due to heat stable and water-soluble polyphenolic compounds.

Objectives

The objectives of this study were to investigate the antibacterial and antioxidant properties of cinnamon stick, green tea and ginkgo nut extracts, and whether their incorporation into a gelatin film used to wrap meat could reduce the bacterial colony count.

Hypotheses

The hypotheses of this study were that cinnamon stick, green tea and ginkgo nut extracts had antibacterial and antioxidant properties, and that their incorporation into a gelatin film used to wrap meat could reduce bacterial colony count.

Materials and Methods

Preparation of plant extracts

1 g of plant sample was blended and heated to 45°C in 10 ml of deionised water. The contents were then centrifuged at 7000 rpm for 10 min and the supernatant was collected and filter-sterilised through a microfilter.

Growth of precultures of bacteria

The test organisms (*Staphylococcus epidermidis ATCC 12228, Bacillus cereus ATCC 11778, Escherichia coli ATCC 25922, Serratia marcescens Carolina*) were inoculated in 10 ml of sterile LB (Luria-Bertani) broth and grown overnight at 30°C in a shaking incubator. The absorbance of the precultures at 600 nm was measured using a UV-vis spectrophotometer and standardised at 0.8.

Antibacterial test using the *in vitro* well diffusion method

The bacterial culture was spread evenly on a Mueller-Hinton agar plate with a sterile swab. A sterile pipette was used to create wells in the agar. 80 µl of plant extract was added to the well. The positive control was 10% bleach and the negative control was sterile water. The plates were incubated at 30°C overnight and the diameter of the zone of inhibition was measured the next day. Five replicates were conducted for each plant extract and bacterial species.

DPPH test for antioxidants

In the test setup, 0.1 ml of plant extract was mixed with 1.9 ml of methanol and 1.0 ml of DPPH solution. In the control setup, 0.1 ml of deionised water replaced the plant extract. For the respective blanks, 1.0 ml DPPH solution was replaced with 1.0 ml methanol. Five replicates of each test and control setup were prepared. The mixtures were then left in darkness for 20

minutes. The final absorbance readings were taken, and the radical scavenging activity (%) was calculated as shown in Fig. 1.

$$\frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

Fig. 1: Formula for calculation of radical scavenging activity (%)

Preparation of antimicrobial food wrapper and antibacterial test

40% (w/v) gelatin was mixed with 12% glycerol (v/v), stirred at 50°C for 1 hour, and autoclaved at 121°C and 15 psi for 15 minutes. The plant extract was then mixed with the gelatin mixture in a ratio of 1:1. The mixture was poured into a Petri dish and left to dry overnight. In the control setup, sterile water replaced the plant extract. The wrappers were used to wrap 1 g of meat, which was minced pork, and left at room temperature overnight. Five replicates were carried out for each setup. Another set of controls consisting of unwrapped meat was performed. The next day, bacteria was isolated from meat as follows: 1 g of meat was added to 9 ml of saline solution and mixed, forming the 10^{-1} dilution. Serial dilution was performed with saline solution until an appropriate dilution factor, and 0.1 ml of the last dilution was spread on LB agar. The plates were incubated at 30°C overnight and the colonies were counted the next day.

Results

Antibacterial test using the *in vitro* well diffusion method

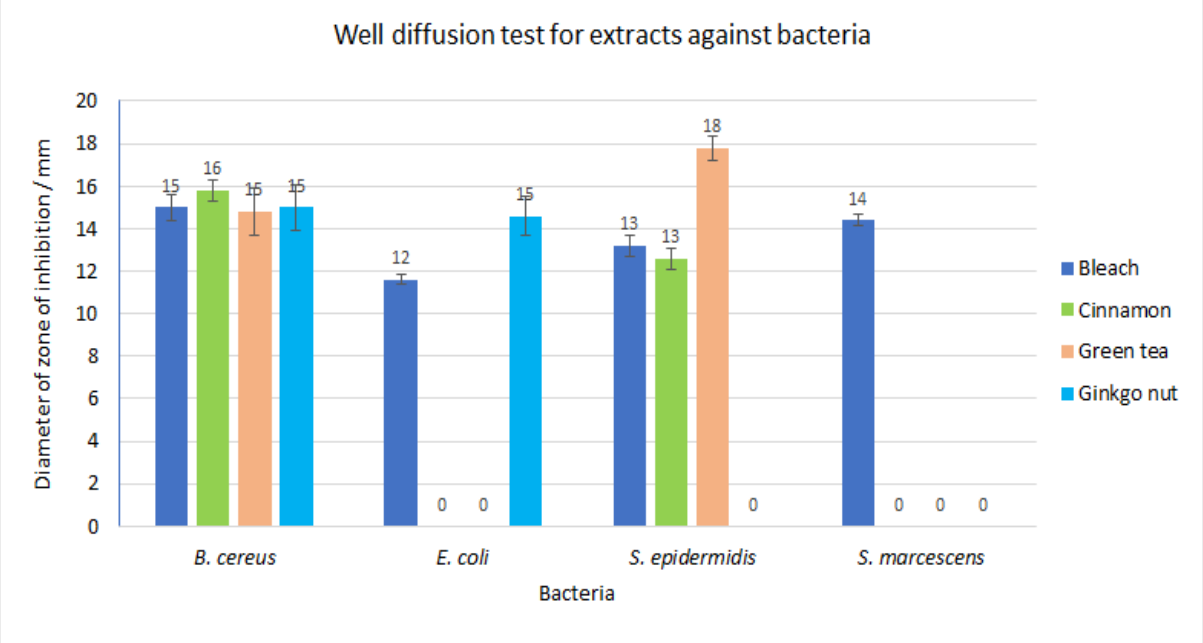

Fig. 2.1: Graph showing the diameter of the zone of inhibition observed for each of the extracts with respect to the positive control of 10% bleach for each bacterial culture

As seen from Fig. 2.1, the diameter of the zone of inhibition for each of the extracts against *B. cereus* is relatively similar, with the extracts of cinnamon, green tea and ginkgo nut displaying moderate antibacterial activities that were similar to or better than the 10% bleach control. For *E. coli*, only ginkgo nut extract had exhibited antibacterial properties against it, with a diameter of zone of inhibition greater than that of the positive control. For *S. epidermidis*, only cinnamon and green tea extracts exhibited antibacterial properties against it, with green tea extract having a much larger zone of inhibition than both cinnamon extract and the control, while cinnamon extract had a smaller zone of inhibition than the positive control. No zone of inhibition was observed for the ginkgo nut extract. For *S. marcescens*, none of the extracts exhibited antibacterial activities against it, with no zone of inhibition observed apart from around the positive control well. The Kruskal-Wallis test was conducted for each of the setups, and p-values of 0.346, 0.001, 0.001 and 1.000 were obtained for the *B. cereus*, *E. coli*, *S. epidermidis* and *S. marcescens* setups respectively, and since the p-values of the setups for *E. coli* and *S. epidermidis* is less than 0.05, there are significant differences in the antibacterial activities of the extracts against *E. coli* and *S. epidermidis*, while the p- values of the setups of *B. cereus* and *S. marcescens* is more than 0.05, so there are no significant differences in the antibacterial activities of the extracts against *B. cereus* and *S. marcescens*. It can be observed from the data that ginkgo nut extract only exhibited antimicrobial activities against the more common food pathogens *B. cereus* and *E. coli*, while cinnamon extract and green tea extracts only exhibited antibacterial activities against the Gram-positive bacteria *B. cereus* and *S. epidermidis*. None of the extracts exhibited antibacterial activity against *S. marcescens*.

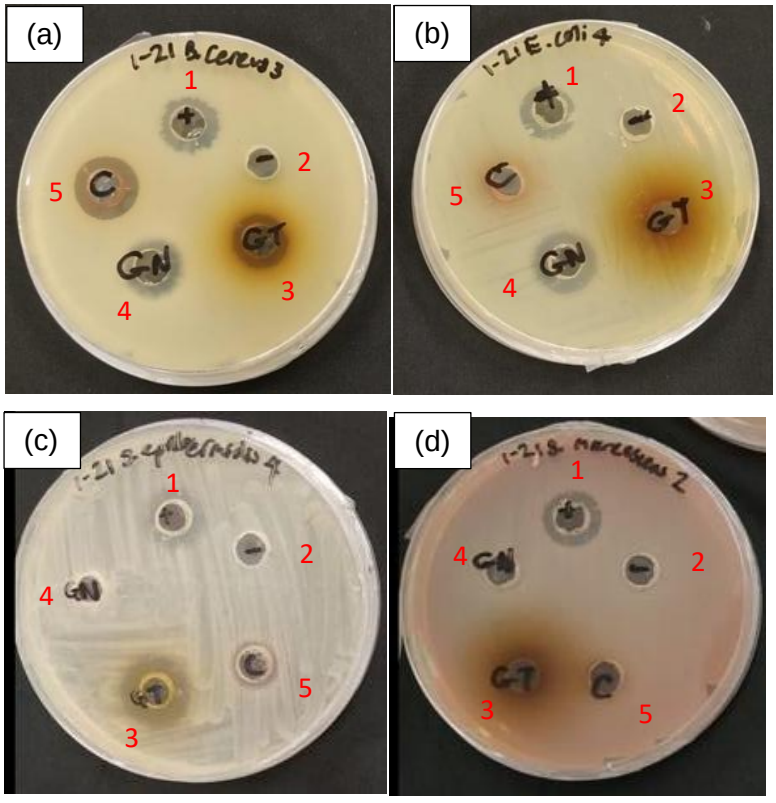

Fig. 2.2: Plates showing well diffusion test results against (a) *B. cereus*, (b) *E. coli*, (c) S. *epidermidis* and (d) *S. marcescens.* Wells were filled with (1) 10% bleach (positive control), (2) sterile deionised water (negative control), (3) green tea extract, (4) ginkgo nut extract and (5) cinnamon extract.

DPPH Test for antioxidants

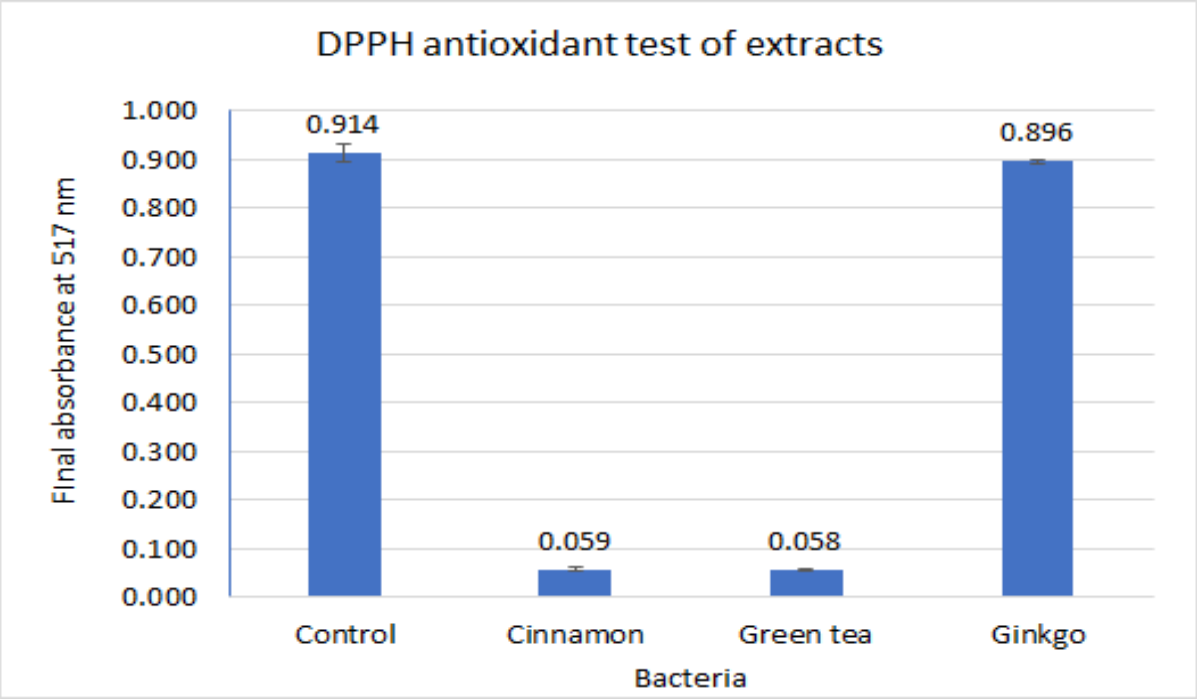

Fig. 3: Graph showing the final absorbance readings of control and extracts at 517 nm

Extract	Radical Scavenging Activity (%)
Cinnamon	93.5
Green Tea	93.6
Ginkgo Nut	1.91

Table 1: Table showing the Radical Scavenging Activity (%) of each extract

The final absorbance readings of the setups after 20 minutes in darkness are shown in Fig. 3, and the radical scavenging activity of each extract is displayed in Table 1, calculated using the formula shown in Fig. 1. As seen from Fig. 3, the control and ginkgo nut extract have similar absorbances at 517 nm, showing no reduction of DPPH, a free radical, so ginkgo nut is not shown to have significant antioxidant activity, with low radical scavenging activity of 1.91%, as shown in Table 1. Cinnamon and green tea extract displayed significant drops in absorbances at 517 nm, correlating with the reduction of DPPH, showing good antioxidant activity, with high radical scavenging activities of 93.5% for cinnamon and 93.6% for green tea. The Mann-Whitney U Test was conducted for the extracts with respect to the control, and for the ginkgo nut extract, the p-value was 0.6761, which is greater than 0.05, showing no significant difference in the absorbance of its solution from the control, so ginkgo nut extract

has no significant antioxidant activity while for cinnamon extract and green tea extract, their p-values were both 0.0122, being less than 0.05, show significant differences in the absorbance of the solutions from the control, implying significant antioxidant activity.

Antibacterial test of antimicrobial and antioxidant food wrapper

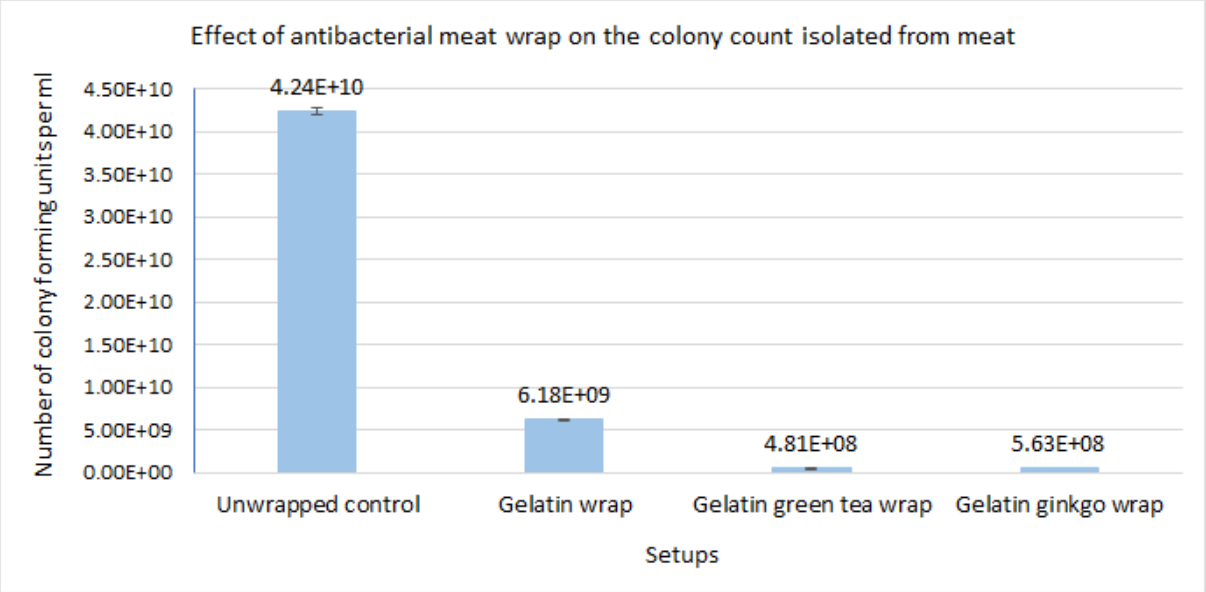

Fig. 4.1: Graph showing the effect of the antibacterial meat wrapper on the number of colonies isolated from the meat per ml

Type of food wrapper used	Percentage reduction in number of bacterial colonies (%)
Plain gelatin	85.4
Gelatin with green tea extract	98.9
Gelatin with ginkgo nut extract	98.7

Table 2: Table showing the percentage reduction in the number of bacterial colonies in the meat with respect to the unwrapped control

As shown in Fig. 4.1, the number of bacterial colony forming units in the unwrapped control is significantly higher than the plain gelatin wrap, gelatin wrap with green tea extract and gelatin wrap with ginkgo nut extract. From the data in Fig. 4.1, the percentage reduction in the number of bacterial colonies formed was determined with respect to the unwrapped control as shown in Table 2. It can be seen that the plain gelatin wrap on its own could reduce the number of bacterial colonies in the meat quite significantly, but the addition of green tea extract or ginkgo nut extract to the film further reduced the number of bacterial colonies formed, and the green

tea extract incorporated into the gelatin film and wrapped around meat could reduce the number of bacterial colonies in the meat more than that of ginkgo nut extract incorporated into the gelatin film. The Kruskal-Wallis test was conducted, and the p-value was 0.001, and this being less than 0.05, shows a significant difference in the number of colony forming units isolated from the meat due to the type of food wrapper used.

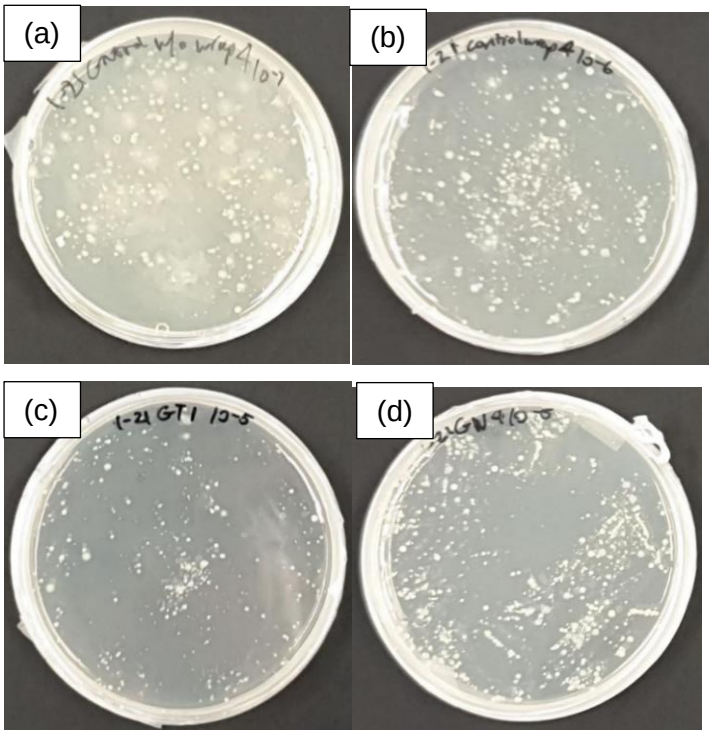

Fig. 4.2: Plates showing number of bacterial colonies isolated from the meat from the (a) unwrapped control setup, (b) plain gelatin wrap setup, (c) green tea extract gelatin wrap and (d) ginkgo nut extract gelatin wrap. Dilution factors for setups (a), (b), (c) and (d) were 10^{-7}, 10^{-6}, 10^{-5} and 10^{-5} respectively.

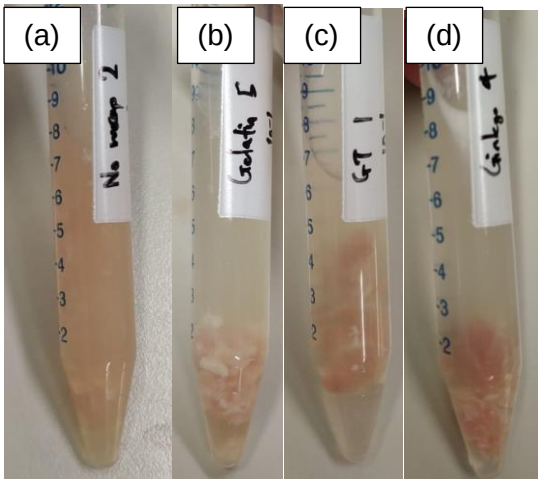

Fig. 4.3: Tubes showing first dilution of meat from the (a) unwrapped control setup, (b) plain gelatin wrap setup, (c) green tea extract gelatin wrap and (d) ginkgo nut extract gelatin wrap.

It was observed that the meat in (a) turned to a reddish hue from the original grayish-pink color of the minced pork, and was slimy and sticky, indicating rancidity, and the first dilution showed a red and cloudy suspension, with high turbidity. The other tubes in (b), (c) and (d) showed meat with a more grayish-pink color, but still slightly slimy and sticky indicating that the meat was more well-preserved, and the meat had a lesser extent of rancidity. The turbidity of the suspension in tubes (b), (c) and (d) for the first dilution is observed to be lower, with a lower

turbidity than in (a), and a white suspension instead of red, showing the film was effective at reducing bacterial growth in the meat, and preserving its freshness.

Discussion

The data shows that cinnamon extract is only effective against the Gram-positive bacteria, *B. cereus* and *S. epidermidis*, contradicting research conducted by Nabavi et al. (2015), which actually showed that cinnamon extract was also effective against *E. coli*, which it did not show antibacterial activities against, with no zone of inhibition shown during the well diffusion test. It did however, exhibit good radical scavenging activity of 93.5% as shown in Table 1, and as shown in the DPPH test, supporting the findings of Rao and Gan (2014).

However, the antibacterial and antioxidant activity of the green tea extract is shown to be better, being effective against the same bacteria, *B. cereus* and *S. epidermidis*, both of which are Gram-positive, as cinnamon extract was in the well diffusion test, with significantly greater zone of inhibition for the *S. epidermidis* setup than the cinnamon extract, and cinnamon extract only had a slightly greater zone of inhibition against *B. cereus* compared to the green tea extract, and even had a slightly greater radical scavenging activity of 93.6% as shown in Table 1, so green tea extract is shown to have better antibacterial and antioxidant activities, supporting data by Chan, Soh, Tie and Law (2011) that it had good antibacterial and antioxidant properties, even reducing the number of colony forming units in meat wrapped in the gelatin film incorporated with green tea extract the most compared to the ginkgo nut film and plain gelatin film, so it is the best extract to be incorporated into gelatin film.

Ginkgo nut extract was able to inhibit the growth of the more common food pathogens *B. cereus* and *E. coli*, as shown from the well diffusion test, supporting data from Chassagne, Huang, Lyles and Quave (2019), but it had a low radical scavenging activity of 1.91% as shown from Table 1, contradicting data from Goh and Barlow (2002). It could also significantly reduce the number of colony forming units in the meat, but not more than the green tea film. Thus, although it showed good antibacterial activity against the more common food pathogens, the poor antioxidant activity and ability to reduce the number of colonies in meat made the green tea extract a more viable option as the extract to be incorporated into the gelatin film, as it would potentially be able to reduce oxidative degradation of lipids in the meat and prolong its shelf-life more effectively.

Conclusion

Based on the data shown above, it can be shown that the green tea extract is the most ideal to be placed in the antimicrobial food wrapper as it has displayed good antibacterial activity

against the Gram-positive bacteria *B. cereus* and *S. epidermidis*, and has good antioxidant activity, so it could potentially also slow the peroxidation of lipids in the meat, and it has also displayed the most significant reduction in the number of bacterial colony forming units isolated from the meat, so despite its ineffectiveness at inhibiting the growth of Gram-negative bacteria, it is better than ginkgo nut extract due to its antioxidant activity and higher percentage reduction in the number of bacterial colonies in meat although ginkgo nut was effective in inhibiting the more common food pathogens *B. cereus* and *E. coli*, which are Gram-positive and Gram-negative respectively. Cinnamon extract had similar properties to green tea extract, except that green tea extract was comparatively slightly better than cinnamon extract in terms of both antibacterial and antioxidant abilities.

The limitations of this study were that the solvent used for the preparation of extracts was water, a polar solvent, which would not extract the non-polar compounds from the plant material, which may have been the main contributor to the antioxidant abilities of ginkgo nut and the antibacterial abilities of cinnamon against Gram-negative bacteria, which are shown to be absent based on the data, contradicting past research. However, the use of non-polar organic solvents may not be viable as most of them are toxic, with acetone and hexane being known non-polar organic solvents with high toxicity to cells and humans. Also, the use of different batches of meat may result in different initial number of bacterial colonies, so the number of bacterial colonies formed may not be consistent. In addition, the use of different batches of plant material may result in inconsistent results as they may have different chemical structures and quality.

An application of this project is the antimicrobial food wrapper. It can be used to wrap meat for storage in the freezer, and when thawing, or as candy wrappers and other types of food packaging to prolong the shelf-life of the product and keep it fresh by reducing the chances of bacterial contamination and slowing down the oxidative degradation of compounds in the food.

As an extension of this study, the lipid peroxidation test can be conducted, to determine the extent of oxidative degradation of the meat with the antimicrobial food wrapping, and determine its effectiveness at prolonging the shelf life of the food and keeping it fresh, and quantify its effectiveness at doing so. More investigation of the properties of the antimicrobial food wrapping can also be done, such as the determination of the tensile strength of the food wrapper by the mechanical strength test, and also determining the extent of biodegradability of the wrapper.

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