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Research Article

Effect of Extracts from *Lentinula edodes* and *Pleurotus sajor caju* Mushrooms and *Anas platyrhyncha* Egg Shell Membrane on Preservation of Fresh Meat

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Abstract

Keeping food longer and safe in its natural state for human consumption is important to avoid disease, waste and scarcity. The preservation potentials of methanolic extracts of the mushrooms Shiitake (*Lentinula edodes*), *Pleurotus sajor-caju* and aqueous extract of duck (*Anas platyrhyncha*) egg shell membranes was examined for meat inoculated with three species of bacteria; *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 20971), *and Salmonella enterica* (ATCC 14028). Buffered saline wash from the meat inoculated with the bacteria species and treated with the extracts were evaluated for microbial load (Log) reduction. All three extracts significantly ($p < 0.05$) reduced the counts of the three-bacteria species used to inoculate the meat. When compared with the mean log count (5.6) obtained for the control, a mean log reduction difference of 2.6, 3.2 and 3.3 for the bacteria *Salmonella typhi*, was recorded from the wash of all three extracts; *Anas platyrhyncha* egg shell membrane, *Pleurotus sajor-caju* and *Lentinula edodes* (Shiitake) respectively after 24 hours of treatment. A similar trend followed in the results from the meats inoculated with *Salmonella enterica* and *Escherichia coli* and treated with the three extracts. Our findings show that these organic products have promising potentials for preserving meat from bacterial contamination.

INTRODUCTION

The need to have safe food devoid of pathogenic microorganisms and their attendant toxins and other spoilage microorganisms has become a huge concern in food security globally. Foods when fermented keep longer; a well-preserved food should last for a considerable length of time, up to two to three weeks. Unfortunately, not all foods can be preserved easily through fermentation.

Foods including meat are susceptible to changes which may result from oxidation or direct microbial activities including their multiplication in such foods which can cause undesirable physical and sensory changes thereby affecting the flavor and aroma, texture and functionality (proteins), and loss of relevant nutrients as vitamins [1] leading to food spoilage.

More importantly, food-borne infections have been a key issue in food maintenance and safety. Fundamentally, food is preserved to maintain the appearance, inherent natural characteristics and extend shelf life. To do this, producers of food have introduced and used different chemical agents including organic acids. Use of less or preferably, non-toxic chemical preservatives are particularly important in food as consumers are becoming more and more aware of the consequences of chemical preservatives in food. Synthetic preservatives have received negative publicity and people now prefer organic foods and those tagged "preservative free" to cut down their intake of presumed chemicals [2]. The use of natural organic by-products in food preservation has re-emerged with a shift from use of chemicals and other synthetic products [3]. This shift in trend has been attributed largely to the minimal or no toxic side effects associated with these nature-based or ethno-medicinal products; as is now practiced in many developing countries where dearth in current trends of standard and good food preservation methods still persist.

Fermented foods are assumed preserved and so can keep much longer than those not fermented. These are however, not without their negative physical outcomes on the food texture and acceptability.

To deal with issues arising from fermentation and chemical preservation, natural products such as lactic acid from plants have been used in food preservation [4]. Biopreservation and use of natural antimicrobials as well as high hydrostatic pressure (HHP) have also been adopted to extend shelf life of foods [5].

In meat preservation, a major challenge is how to maintain

the flavor and the often-pinkish color expected from cured meats. Researchers are studying different natural preparations that can be safely used to prevent bacterial pathogens from surviving in meat without altering the natural colour and flavor.

According to Day [6], some tests for new products have proved successful in this regard as observed in the use of cherry powder combined with celery powder for meat preservation.

Carbon dioxide and ozone have been used to discourage the growth of surface micro-organisms on beef carcasses during prolonged storage at chilling temperatures. Although ozone leaves no toxic residues in meat, its use in a production environment can be dangerous for personnel; moreover, it accelerates the oxidation of fat and is more effective against air-borne micro-organisms than against those on meat [7].

Other organic acids apart from lactic acid, which is a frequently used inhibitory agent that is effective in fresh meat preservation, have been found to be responsible for discolouration and production of pungent odours in meat [8]. Sodium salts have been used in the meat industry because of their ability to increase flavour, prolong shelf life, and improve the microbiological safety of products [9].

Degradation of meat whether by microorganisms or by other oxidative substances results in color change which in turn affects visual appeal. This makes it imperative to find effective solutions to these rather cosmetic problems associated with meat preservation and of course the main issue of spoilage and foodborne infections. Before now, organic materials have been assessed for their ability to preserve meat [6]. In addition, extracts from some of these organic products are reported to possess antimicrobial properties [10].

This study will therefore, seek to determine the inhibitory properties of extracts from duck egg shell membranes and from two species of edible mushrooms against *Escherichia coli*, *Salmonella typhi*, *and Salmonella enterica* used to inoculate fresh meat. This will be carried out by determining the degree to which they can reduce microbial loads of these potential bacterial pathogens in artificially infected meat.

MATERIALS AND METHODS

Meat

Fresh lean meat procured from the local abattoir in Omu-Aran town of Kwara State, Nigeria, was placed in sterile food pack and transported to the laboratory. The meat was washed with sterile distilled water using a spray bottle and then processed aseptically to avoid and minimize surface contamination with bacteria. The meat was cut in 10 g amounts and placed in sterile glass bowls.

Preparation of extracts

Methanolic extracts of edible mushrooms: Shiitake (*Lentinula edodes*) and *Pleurotus sajor-caju* and aqueous extract of freshly laid duck (*Anas platyrhyncha*) egg shell membrane were prepared for use as follows:

Mushrooms extract

Fifty grams (50 g) of powered mushroom was weighed and transferred into a clean and sterile muslin bag. Using the ratio 1:4 (w/v), 200ml of methanol was measured into the solvent flask of the Soxhlet apparatus; the water bath of the Soxhlet apparatus was set to heat at 60°C for 8 hours. The extract obtained was thereafter, concentrated using a rotary evaporator (MRC: ROVA-100) and freeze dried using a lyophillizer (LYOTRAP -Sterling. LTE Scientific Limited, Greenfield, Oldham OL3 7EN).

Duck shell membrane extract

Freshly laid *Anas platyrhyncha* eggs were collected and washed thoroughly in clean water to remove external debris. The washed eggs were cracked open and the contents emptied into a clean receptacle. The shells were then carefully washed three times in clean water to remove albumin residue. The membrane attached to the shells was carefully stripped off manually from the shells. The eggshell membranes were air dried in clean trays for 7 days and ground into powder in a Warren blender at high speed.

The finely ground duck egg shell membrane powder (50g), was weighed into conical flask and dissolved in 500ml of distilled water: ratio of 1:10 (w/v) and mixed properly in an orbital shaker (Stuart Orbital shaker SSL1) for 12 hours. The bulk extract was then filtered and the filtrate concentrated in the rotary evaporator. The concentrated extract was freeze dried using a lyophillizer (LYOTRAP -Sterling. LTE Scientific Limited, Greenfield, Oldham OL3 7EN).

Bacteria species used

Three bacteria species; *Escherichia coli* (ATCC 25922), *Salmonella typhi* (ATCC 20971), *and Salmonella enterica* (ATCC 14028) were cultured in brain heart infusion broth (LAB-M, LAB049) and used at 10^3CFU/ml.

Treatment of meat with bacteria and extracts

Four groups of meat in 10g amounts with each group having a set of three for each extract and bacterial treatments were adopted in the study with three replicates per treatment. Group A, methanolic extract of *Lentinula edodes* (Shiitake); Group B, methanolic extract of *Pleurotus sajor-caju*; Group C, aqueous extract of duck (*Anas platyrhyncha*) egg shell membrane and group D, controls with no extract treatment. Each group (set of three and their replicates) was inoculated with 10ml of 10^3CFU/ml of each of the bacteria pathogens respectively in PBS and incubated at 37°C for 8 hours. Controls were treated with PBS before incubation. After 8 hours, excess inoculum was drained off the meats in all the samples A, B, C and D. Bacteria inoculated meat samples in groups A, B and C were then sprayed with 10ml (50 mg/ml) reconstituted freeze-dried extracts of *Lentinula edodes*, *Pleurotus sajor-caju* mushrooms and duck *Anas platyrhyncha* egg shell membrane respectively in sterile PBS. The control group was sprayed with sterile buffered physiological saline (PBS) of pH 7.4. The meats infected with the three bacteria species and now treated with corresponding extract solution and the controls were again incubated for 24 hours at 37°C.

Determination of bacterial load reduction

After incubation, the meats (tests and controls) were removed and washed by teasing in sterile PBS (pH 7.4). The wash

was made up to 10ml using BPS solution. From the wash, Log dilutions of test and control in saline; 10^{-1} to 10^{-7}, was carried out on nutrient agar plates. The mean values of the viable bacterial counts were recorded after incubation.

Meat colour change

Mark of microbial effect leading to spoilage in the meat was undertaken by observing for colour change in the meat appearance adopting the $L^*a^*b^*$ colour space system; a procedure of the International Illumination Commission [11].

Statistical analysis

Results for the bacterial load reduction were analysed statistically using the Paired samples t-Test at 0.05 level of significance.

RESULTS AND DISCUSSION

There were no discernible colour changes in the meats at the expiration of the experiment in the groups inoculated with bacteria and treated with the extracts. This apparent lack of effect on the colour of meat treated with the extracts gives credence to their use in meat preservation. However, we are unable to conclude that this effect can be sustained if the period of treatment is further extended. The meat in the control group appeared darkened with an offensive odour; an evidence of sustained microbial activity.

The two extracts from *Lentinula edodes* (Shiitake) and *Pleurotus sajor-caju* mushrooms and aqueous extract of *Anas platyrhyncha* egg shell membrane, appreciably reduced the viable bacterial loads of all three-bacteria species used to inoculate the meat.

When compared to the control; meats which were inoculated with the three bacteria organisms but left untreated with the extracts, those inoculated with *Salmonella typhi* and treated with duck egg shell membrane aqueous extract showed a 2.6 Log reduction in the bacteria load. Similarly, a 3.2 and 3.3 Log reductions of same bacteria; *Salmonella typhi,* were recorded from the wash of *Pleurotus sajor-caju* and *Lentinula edodes* (Shiitake) methanolic extract treated meats respectively. The differences (Figure 1A) shows the mean values of the total viable counts from both control and test while (Figure 1B) shows the Log differences between control and tests.

Similar to the result obtained for extract effect on *Salmonella typhi*, all three extracts from *Anas platyrhyncha* egg shell membrane, *Pleurotus sajor-caju* and *Lentinula edodes* (Shiitake), reduced the load of *Salmonella enterica* by 2.4, 3.5, 2.8 Logs respectively. The load of *Escherichia coli* on the meat treated with extracts from *Anas platyrhyncha* egg shell membrane, *Pleurotus sajor-caju* and *Lentinula edodes* (Shiitake) was reduced by 3.0 Log, 5.4 log and 4.0 Log respectively (Figures 2A, 2B, 3A, 3B). For all the treatments, the differences in the bacterial Log reduction compared to the control, were statistically significant ($p<0.05$).

Many non-edible mushrooms exist, but the two studied mushrooms are edible and are also considered as medicinal mushrooms in some forms of traditional medicine.

Previous studies on the *in-vitro* and *in-vivo* antimicrobial

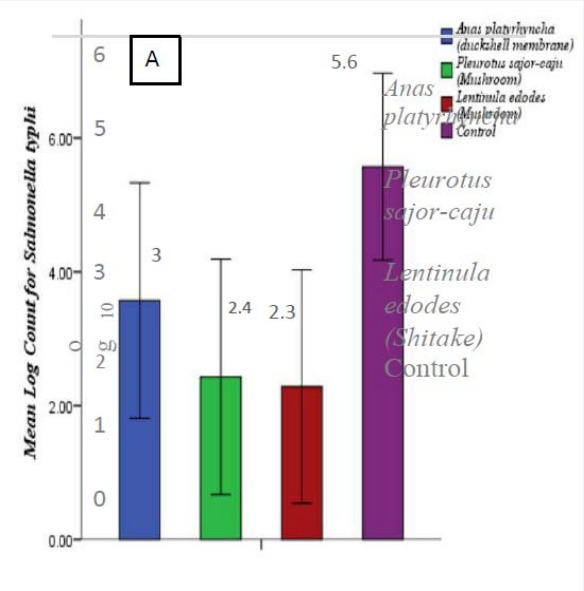

Figure 1a Average (Log10) count for *Salmonella typhi* treated with and Control extracts.

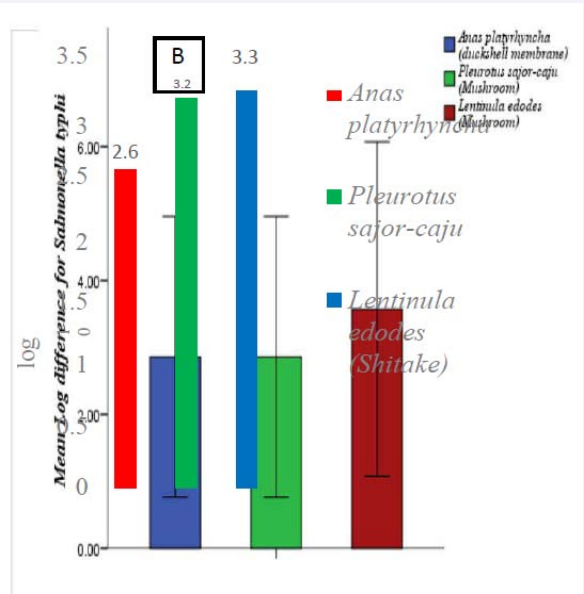

Figure 1b Bacterial Log10 Reduction Rate (Average count for Control – Average count of Tests) for *Salmonella typhi.*

activities of mushrooms extracts on bacteria have been made [10] though Lima and others [12] have suggested the need for further investigations to confirm claims of the antimicrobial activity of the extract of one such mushroom; *Agaricus blazei* against Gram negative and Gram positive bacteria. Overall, the methanolic extracts of the edible mushrooms *Lentinula edodes* (Shiitake) and *Pleurotus sajor-caju,* investigated in this study were more potent in inhibiting the growth and survival of all three-bacteria species tested, particularly against *E. coli.*

Similarly, egg shells are reported to be used to treat food poisoning; a practice by the Indians who administer the ashes

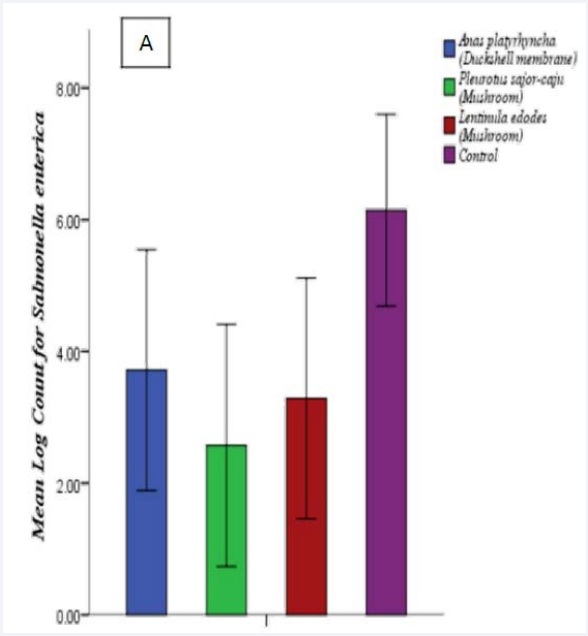

Figure 2a Average (Log10) count for *Salmonella enterica* treated with extracts and control.

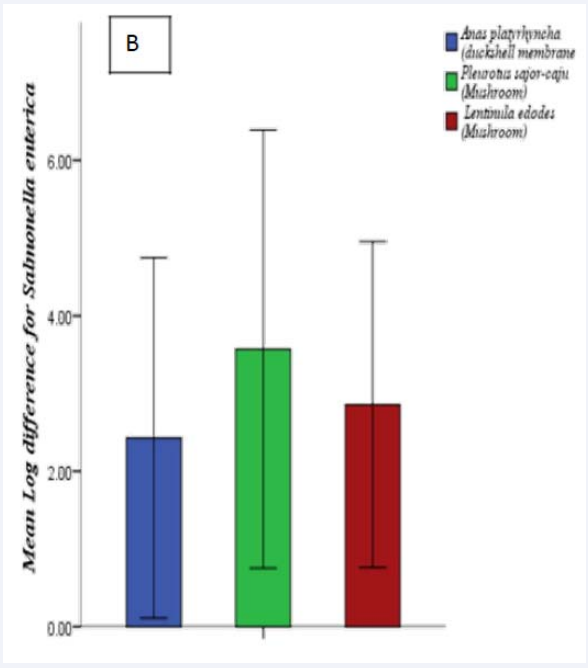

Figure 2b Bacterial Log10 Reduction Rate (Average count for Control – Average count of Tests) *Salmonella enterica.*

from chicken eggshells which are left after hatching [13]. Eggshell membrane contains antimicrobials which include the matrix proteins and polysaccharides; about 70% of the matrix is protein and 11% is polysaccharide that contains dermatan sulphate, keratin sulphate, chondroitin sulphate A and B, uronic and hyaluronic acids [14,15]. Some of these matrix proteins are able to prevent the passage and growth of Gram negative spoilage bacteria within the egg [16]. Ovotransferrin and lysozyme are antimicrobial active components in the eggshell membrane; both proteins are present in the shell matrix [14,15]. We suspect that these proteins and polysaccharides in the shell matrix may be responsible for the antimicrobial activity of the eggshell membrane on the three bacteria tested. Lysozyme has found use in food and pharmaceutical industries; used in the preparation of aerosols for the treatment of bronchopulmonary diseases and as prophylactics in conditions relating to dental caries. It has inhibitory properties against *Clostridium tyrobutyricum.* Other workers [17] have reported the use of a combination of lysozyme from egg and EDTA against *Salmonella typhimurium* in broiler eggs. These reports support our findings on the activity of the

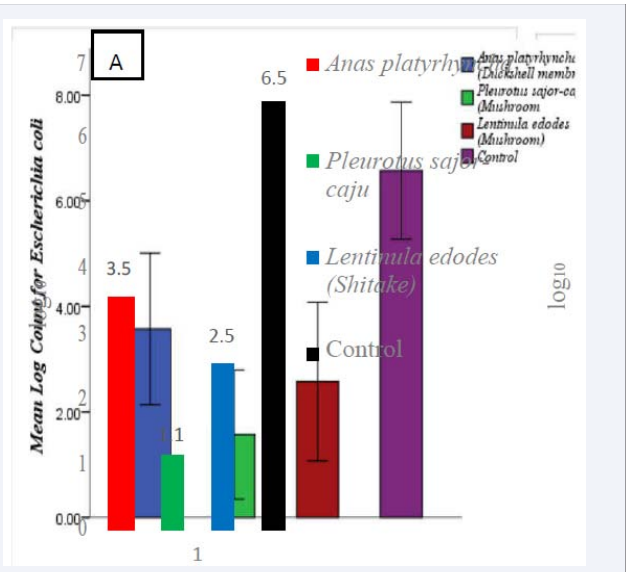

Figure 3a Average (Log10) count for *Escherichia coli* treated with extracts and control.

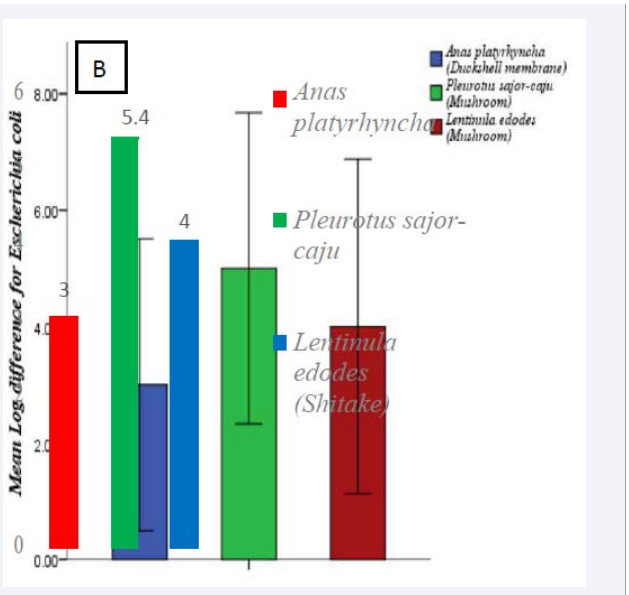

Figure 3b Bacterial Log10 Reduction Rate (Average count for Control – Average count of Tests) for *Escherichia coli.*

extract from the duck egg shell membrane on the three bacteria used to inoculate the meat.

Scientific evidence is available that support the production of antibiotic and antiviral substances from the *Pleurotus* mushrooms. Extracts of mushrooms are now prepared as supplements, tinctures, hot water preparations, fruit body mycelium and many more, and used against a broad range of ailments. They contain various medicinal compounds such as triterpenoids, glycoprotein, natural antibiotics, and enzyme inhibitors that can be used to maintain good health [18] with evidence of vitamin production [19]. Ergosterol, known to play important role in the fluidity of cell membrane and in aerobic growth of fungi**,** is a major product of sterol biosynthesis present in fungi.

Several efforts have been made to preserve meat and meat products using different preservation methods in the past including chilling. Unfortunately, though, some of these techniques have not produced favourable results. Reasons for this are often tied to nature of products used. Generally, chemical products used in food preservation are the main culprits as some of them have been incriminated in cancer. Among such are butylated hydroxytoluene, butylated hydroxyanisole, and *tert*-butylhydroquinone [20].

From our study, the two mushrooms; *Pleurotus sajor-caju* and *Lentinula edodes* (Shiitake) showed evidence to protect meat from contamination by pathogenic bacteria through the appreciable reduction in the bacterial load recorded of the three organisms earlier inoculated on the meat.

Since the mushrooms and duck eggshell membranes are edible, the ability of their extracts to inhibit the growth and multiplication of potential bacterial pathogens makes them good candidates for use in extending shelf life of meat and consequently, its preservation.

CONCLUSION

Our findings therefore, on use of *Pleurotus sajor-caju, Lentinula edodes* (Shiitake) and *Anas platyrhyncha* (duck) egg shell membrane, for meat preservation presents another very important way these products can be utilised in the food industry. The mushrooms are easily cultured and the duck bird is also easily reared and the egg shells can be harvested and used in food preservation rather than allowed to waste and litter the environment. It is hoped that success recorded from this study will help to overcome the very harmful effects of some chemical agents such as benzoates, butylates, butylated hydroxytoluene (BHA) and caramel used as food preservatives. A spray application of extracts from these products on meat which showed no visible colour impairment makes the products acceptable and friendly to use for meat preservation.

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