PRESERVATION OF POULTRY MEAT WITH THE APPLICATION OF LINALOOL

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ABSTRACT
The growth of microorganisms on the surface of solids and semi-solid food can be overcome with the application of antimicrobial agents to extend the shelf life. Linalool fall under the category of generally recognized as safe and recently trend are growing for utilization of natural antimicrobial agent to resolves issues related food spoilage. The present study was carried out to understand the effect of linalool - an essential oil - in the preservation of poultry meat. As the concentration of linalool increased from 20 to 120µl/ml shows increased zone of inhibition from 16.67 to 35mm against Escherichia coli and for Staphylococcus aureus increased from 7.6 to 45mm. The optimum concentration of linalool was 100µl/ml. The minimum inhibitory concentration of linalool was determined at pH 6. The further study was carried out to determine the effect of pH on antimicrobial property of Linalool. The maximum antimicrobial activity was observed at pH range 6-7. The total plate count of linalool treated sample increases which were comparatively low to control sample. The linalool treated chicken sample was preserved up to 4 days at 4°C.

Keywords: Essential oils, minimum inhibitory concentration (MIC), linalool, preservation, antimicrobial activity

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INTRODUCTION

An aromatic and volatile liquids extracted from plants, known as secondary metabolites, which also have defence mechanism against microbes are called essential oils (Zeković *et al.*, 2011). The seeds, stems, roots, blossoms, bark and different parts of the plant are source of essential oils. The various application of essential oil is noted as flavourings and antimicrobial.

The antimicrobial properties of fundamental oils have been depicted and, as a result of the developing interest on antimicrobials for anticipating microbial sustenance decay and bacterial contaminations. Numerous basic oils are as of now utilized as a part of the nourishment business as enhancing operators and some are known to apply antimicrobial action (Silva *et al.*,2011).

Linalool (3,7-dimethyl-1,6-octadien-3-ol) is an essential aroma concoction, much of the time utilized as a part of scented items as a result of its crisp, elegant smell. Linalool is an unsaturated hydrocarbon and is along these lines vulnerable to oxidation within the sight of air. Linalool is an optically dynamic colourless fragrant fluid, unsaturated terpene fluid liquor found in numerous essential oils.

The microbial and physical quality of poultry of meat are mostly susceptible to contamination due to sanitation during packaging and processing, slaughter process and storage system (Borche and Arinder, 2002; Selvan*et al*., 2007). Hence the present study deals to understand the effect of linalool in preservation of poultry chicken.

MATERIAL AND METHODS

Material

Nutrient broth and Agar Agar ((Hi- Media, Mumbai, Maharashtra, India), Linalool (Sigma Aldrich, Sodium chloride, NaOH and HCl (S. D. Fine - Chem Ltd., Mumbai, Maharashtra, India) were purchased. The entire chemicals were analytical grade.

Strain: *Staphylococcus aureus, Escherichia coli* were procured from NCL, Pune, Maharashtra, India. Raw chicken were procured from butcher.

Antimicrobial activity of Linalool determined by using Agar well diffusion method

The assay disc diffusion method was used to determine the antimicrobial activity of essential oils (Balouiri*et al.*, 2016; Sonawane*et al.*, 2018). The test was carried out in duplicate with triplicates on antimicrobial activity of the essential oil-Linalool.

The volume of 0.1ml (approximately 10 cells / ml) of microorganisms grown in liquid growth media at 37ºC was inoculated in Nutrient broth agar and was poured in sterile Petri plates and wells were punched with sterile cork borer on the agar and were filled with linalool, where linalool and solvent propylene glycol concentrations were made (10 to 100µl/ml). The plates were incubated at 37ºC for 48 hours. After the incubation period inhibition zones around the well were measured in millimetres. The sensitivity of the oil was determined according to the diameter of the inhibition zone. The experiment was tested for triplicates for the strains used (Babu *et al.*, 2011).

For pH range of linalool against antimicrobial activity, the pH was adjusted using a pH meter and 1N NaCl or 1N HCl as per requirement. After setting the pH, the media was autoclaved and agar well diffusion assay was performed.

Assessment of antimicrobial activity of Linalool at different pH values

For determination of antimicrobial activity at different pH values, *Staphylococcus aureus* and *Escherichia coli* were used as a test organism. 4 different pH values were maintained ranging from pH 6- pH 10. Linalool concentration of 100 µl/ml was used owing to the results obtained in MIC.

Model development for Chicken

Incorporation of linalool in marinated chicken breast

Owing to the results obtained from the pH tests, a model marinated chicken breast sample was made. The protocol followed was as below:

50 gm of chicken breast sample was weighed, 4 such samples were taken. The samples were marinated in a mixture of curd, ginger and garlic paste and finely chopped coriander. A 0th day sample from the chicken was taken for calculating its TPC and CFU. Serial dilutions of the sample were made and inoculated by spread plate technique. The samples were kept for incubation for a period of 24 hours. Four concentrations which had effective antimicrobial activity where added to the sample. For incorporation of the linalool into the chicken the amount of linalool to be added in the chicken was carefully calculated so as the resulting concentration of the linalool in the sample would be 100 µl/ml by using propylene glycol as a solvent and added in the marinating mix and then it was applied to the chicken thoroughly. The samples were then stored in a zip-lock bag and stored at 4ºC.

Assessment of total plate count of the linalool in chicken breast as a preservative

To assess the antimicrobial potential of the linalool, the samples were tested on 2^{nd}, 3^{rd}, 4^{th}, and 6^{th} day. The samples were taken out of storage, 0.5 ml from each sample was pipetted out and serial dilutions were made. The dilutions made were then plated using spread plate technique. The plates were then kept for incubation period of 24 hours. After the incubation period, the plates were observed for the number of colonies, the number of colonies was noted down and the CFU for the sample was calculated.

$$\text{CFU/ml} = \frac{\text{number of colonies} * \text{dilution factor}}{\text{Volume of culture plate}}$$

RESULTS AND DISCUSSION

Antibacterial activity of linalool: The different concentration (20 to 100 µl/ml) of linalool treated with gram-positive bacteria such as *Escherichia coli* and gram negative bacteria *Staphylococcus aureus* which shows increased in zone of inhibition as concentration increases shown in Table 1. The highest zone of inhibition observed with 100 µl/ml of linalool against *Escherichia coli* and *Staphylococcus aureus*i.e 34.33 and 44.6 respectively**.** Herman et al. (2016) proves the antimicrobial efficacy of linalool against *S. aureus, E. coli* and *C. albicans.*

Table 1. Antibacterial activity and zone of inhibition with gram-negative strain

Concentration of linalool (v/v) µl/ml	Zone of inhibition (mm) *Escherichia coli*	Zone of inhibition (mm) *Staphylococcus aureus*
20	16.67±1.53	7.6± 0.58
40	18.67±1.15	17.6± 2.31
60	26±1	26.6± 2.88
80	30±2.31	32± 1.73
100	34.33±0.57	44.6±0.58
120	35±1.15	45±1

pH range of linalool

The significant zone of inhibition was observed at pH 7 shown in Table 2. In case of *Escherichia coli*, there was no significant difference after pH 7 and in case of *Staphylococcus aureus*, zone of inhibition was decreased after pH 7. pH 4 and below were not used for testing because of improper gelling of agar below pH 5.

Table 2. Effect of pH range on zone of inhibition of bacterial strain treated with linalool

pH range	Zone of inhibition *Escherichia coli* (mm)	Zone of inhibition (mm) *Staphylococcus aureus*
6	17.33± 0.57	43.66± 0.57
7	19±1	44.66± 0.57
8	19± 2.65	16.33± 0.57
9	19± 2.65	12± 0.0
10	18± 2.35	7.6± 0.58

Effect of linalool on total plate count during storage of chicken breast

The total plate count found in control sample at 1st day was $89x10^4$ CFU/ml as compared to sample treated with linalool was $25x10^4$ CFU/ml which shows almost 71% reduction in plate count. Total plate count and colony forming unit was increased during storage day. But sample treated with 100 µl/ml of linalool concentration shows effective in reduction of plate count and colony forming unit at the 4th day.

Table 3. Effect of linalool on total plate count during storage of chicken breast

Day	Concentration of linalool (%)	Total plate count (TPC) (CFU/ml)
1st day	Control	$89x10^4$
	100 µl/ml	$25x\ 10^4$
2nd day	Control	$170x10^4$
	100 µl/ml	$63x10^4$
3rd day	Control	$352x10^4$
	100 µl/ml	$105x10^4$
4th day	Control	$480x10^4$
	100 µl/ml	$232\ x\ 10^4$
6th day	Control	$529x10^4$
	100 µl/ml	$280x10^4$

CONCLUSION

The 100 µl/ml linalool showed effective zone of inhibition against bacterial strains such as *Escherichia coli* and *Staphylococcus aureus.* The antimicrobial activity was also tested on various pH (6-10) to get a clear picture on what category of food would linalool show maximum inhibition of bacterial activity and could further result in extending the shelf life of the poultry meat. It was observed that the maximum antibacterial activity was in the range of pH 6- pH 7. During the storage up to 6^{th} day the total plate count was observed lowest as compare to control which suggest that linalool could be used as preservative up to 3 day with its optimum parameters.

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