



Effect of Clove Oil Extract on Inhibition of various Spoilage and Pathogenic bacteria isolated from fermented pickles by Agar Well diffusion method

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Abstract

Ready-to-Eat types of food items are becoming very common now-a-days. India has become a hub for producing a variety of pickled food items. Pickling of food items allows the formation of lactic acid bacteria which are very important for the process of fermentation. Thus, physical conditions like temperature, humidity, acidity and pH should be maintained to keep the pickles safe. The variation in environmental condition leads to microbial growth. Different types of pathogenic bacteria grow at different levels of pH and acidity. In recent investigation, some of the microorganisms responsible for the spoilage of pickles were studied. The present study was undertaken to make further detailed studies on the microorganisms responsible for spoilage of pickles and their control. The samples were collected from different places and isolated various bacteria that cause spoilage on pickles and further bacterial strains identified based on morphological and physiological characters. The clove oil was collected from essential oil shops in local market and tested for inhibitory activity against the spoilage bacteria. The clove oil were also tested for inhibition on pickle bacteria by agar well diffusion method at different concentration viz., 16.00, 32.00, 64.00, 128.00, 256.00 µl. Higher concentration shows higher inhibition zone.

Keywords: Pickles, Clove oil, Bacteria, Food

INTRODUCTION

In India pickling of food items is usually done using anaerobic fermentation process. Variety of agents like acetic acid (vinegar), citric acid, brine solution (solution having high salt content) and vegetable oils are used for the process of fermentation. The application of quality and quantity of these agents may vary depending upon the item that has to be pickled. Different type of food products contains varying amount of moisture content in them. If the food items contain sufficient or high moisture content, than dry salt is added to the pickling mixture. Sometimes sugar is also added as a preservative to increase the rate of fermentation and add sweet flavor to the pickled food. *Lactobacillus bacilli* a type of Lactic Acid Bacteria (LAB) is used for the natural process of fermentation. In pickled products, acid, salt, and heating are major factors that contribute to food preservation and these factors are applied in combination. Current regulations and industrial practices regarding

acidified pickled foods are possibly out of date because of the scarcity of information on pathogen control in these foods (Lee SYe et al., 2004). Spices are used as additives to flavor, color or preserve food from ancient time in India. These are pungent or aromatic substances obtained from by dried seeds, fruits, roots, bark or leaves. Spices are the potential source of natural products and naturally derived compounds. These compounds show the antioxidant, antimicrobial properties. Ashraf AMostafa *et al.*, (2017), stated that ethanolic clove extract was potentially active against *Staphylococcus aureus*, *Vibrio parahaemolyticus* and *Pseudomonas aeruginosa* while it was inactive against *E. coli* and *Salmonella enteritidis*

MATERIALS AND METHODS

Sample collection

The three different pickle samples viz., mango pickle, lemon

pickle and cucumber pickle were collected from Ariyalur and Cuddalore District, Tamil Nadu, India. The samples were collected at different storage period based on the methods of preparation. The samples of mango and lemon pickle were taken from four to six months from the date of manufacturing and cucumber pickle sample was taken at 30-45 days old. All the samples were further stored for observation and growth of spoilage organisms. The clove oil was collected from local essential oil shops.

Isolation and Identification of spoilage bacteria from pickle sample

The samples were isolated and identified the pathogenic bacteria based on morphological and physiological characters.

Inhibitory effect of clove oil on various pickle bacterial strains

The effect of different concentration of spice oil on the growth of the bacterial was studied using clove oil. The sterilized Nutrient Agar medium was prepared and seeded with standard inoculums of the four bacterial strains viz., (PB-1), (PB-2), (PB-3), (PB-4) separately plated. Different concentrations of clove oil viz., 16, 32, 64, 128, 256, $\mu\text{l ml}^{-1}$ were prepared. On the surface of the medium wells made by using cork borer (6mm diameter) and discharged concentrations into well. The plates were incubated at temperature of 30°C and for 48 hrs. and the diameter of inhibition zone (in mm) around the well was measured. Three replications were maintained in each treatment.

RESULTS AND DISCUSSION

The characteristics of four pickle bacteria were studied by using selective medium for respective bacteria and MacConkey Agar. Based on the colony morphology, the bacteria were identified as *Bacillus spp.* (PB-1), *Clostridium spp.* (PB-2), *Staphylococcus aureus* (PB-3), and *Proteus spp.* (PB-4). Microorganisms isolated from pickles samples in this study have been earlier found in foods, environment and other places, and their pattern in similar to previous reports (Clarence et al., 2009).

Identification of the different bacterial isolates was carried out by the routine bacteriological methods i.e., By the colony morphology, Preliminary tests like Gram Staining, Capsule Staining, Endospore Staining, Motility, plating on selective media and by performing biochemical tests. Identification of purified fungal cultures were characterized by their morphology, hyphal characteristics, presence or absence of asexual spores, arrangement of conidia and reproductive structures (Beisher L, 1991) and by performing Lactophenol cotton blue mount. In addition, the necessary concentration of essential oils and their components to achieve an antimicrobial effect in foods is much higher than under in vitro conditions. The presence of salt, proteins, fat, carbohydrates, and a low aw-value cause a decrease of the

antimicrobial properties of essential oils (Klein et al., 2013).

Table 1. NZ – No Zone of Inhibition

Table 1: Effect of clove oil on inhibition of pickle bacteria and fungi by agar well diffusion method.

Clove oil conc μl	PB-1	PB-2	PB-3	PB-4	Mean
0	NZ	NZ	NZ	NZ	-
16.00	09.40	14.80	16.60	15.50	14.07
32.00	13.10	16.90	18.70	17.60	16.57
64.00	15.20	18.30	20.80	19.70	18.5
128.00	19.40	20.10	22.90	21.30	20.92
256.00	21.50	22.20	24.20	23.30	22.8
Mean	15.72	18.46	20.64	19.48	-

In the present study the growth of four pickle bacteria strains viz., *Bacillus spp* (PB-1), *Clostridium spp* (PB-2), *Staphylococcus spp* (PB-3) and *Proteus spp* (PB-4) was effectively inhibited at 256.00 μl concentration of clove oil. From the results, PB-3 shows higher inhibition zone followed PB-4, PB-2 and PB-1. The increase in concentration leads to increased zone formation.

CONCLUSION

Essential oils represent an alternative to synthetic preservatives in the food industry against spoilage of bacteria. Most investigated bacteria showed some (higher or lower) sensitivity to EOs or EO components. Therefore by using the clove oil, we can prevent the spoilage of various bacteria in fermented pickles. The fermented pickles can be packed and stored long shelf life using some of the essential oils that are not harmful to human health.

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