Inhibitory Effect of Essential Oils Extracted from *citrus* Peel on Microbial Growth of Bread

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Introduction

Shelf life i.e. the period it will preserve an acceptable level of eating quality from a safety is very important for both manufacturer and the consumer. Bread occupies an important place amongst all bakery products, because the ingredients that are used and the water activity (0.96-0.98) of breads are supportive of the growth of microorganism reducing the shelf life of bread (Salim-ur-Rehman *et al.*, 2007).

To enhance the shelf life of bread, chemical antimicrobial agents have been employed but they are considered responsible for many carcinogenic and teratogenic attributes and residual toxicity. Due to these reasons, consumers tend to be doubtful of chemical additives and thus the demand for natural preservatives has been intensified (Skandamis *et al.*, 2001). The *Citrus* peel essential oil comprises one of the most versatile essential oils. It is well known that essential oils from *Citrus spp.* have pronounced antimicrobial effect against both bacteria and fungi (Sumonrat *et al.*, 2008). Also the *Citrus* peels are non edible thus discarded after extracting the juice. *Citrus spp.* can be found in most of the regions in the world (i.e. commonly found in Sri Lanka).

Methodology

Citrus peel essential oil extraction

Citrus peel EOs of lime (*Citrus aurantifolia),* sweet orange *(Citrus sinesis)* and pommelo (*Citrus maxima)* were extracted by using steam distillation method (Sumonrat *et al.*, 2008).

Investigation of antibacterial and antifungal property of essential oil:

A preliminary test was conducted to bacteria and fungi which were isolated from spoiled bread that kept at room temperature (i.e. open environment for 5-days). Pour plate method was followed for the isolation of test micro-organisms with streak plate method. The isolated and identified fungi and bacteria were grown on PDA and NA media, respectively for the treatments of three groups of EOs. Triplicates were conducted to increase the accuracy of tests.

Application of *Citrus* peel EOs on bread:

The selected high effective EO was sprayed on whole bread, sprayed on slices and sprayed on bread wrapping along with the control bread. The EOs treatments were done under 0.1% (v/v) as described by Viuda *et al.* (2007). Later, the plate count method was followed to take direct measurement of micro-organism count in each EO treated bread as well as for controls during 0 - 96 hr time period.

Sensory evaluation and data analysis:

The sensory evaluation was conducted for EOs treated breads during 0-96 hrs. The data obtained from bacterial and fungal count were statistically analyzed by ANOVA and Friedman's test, respectively.

Results and discussion

Identification of molds and bacterial morphology

On the basis of direct examination and gram staining, the arrangement and the colony morphology were taken into account and it was recorded that majority of the molds isolated during this study belonged to *Mucor spp., Aspergillus spp., Penicillum spp.*,and the bacterial *spp.* were gram-positive coccus as well as gram-positive coccus.

Effective essential oil for bread test micro-organisms

The 0.1% (v/v) *C. sinensis, C. maxima, C. aurentifolia* treated for identified bread fungi sp. such as *Mucor sp. Aspergillus sp. Penicillum sp.* and for bread bacteria *spp.* A with different volume (5 ml, 10 ml, 20 ml) and negative treatment, control treatment were showed significance difference ($p<0.05$) under the 95% confidence interval. The effective treatment for mold *sp.* and bacterial *sp.* were 10 ml and 20 mL *C. sinensis* among another treatments.

Bacterial and Fungal colony count at different storage intervals

Bacterial and fungal susceptibility to *C. sinesis* essential oil, as determined by the plate count technique, showed that treatments and storage periods had significantly ($p<0.05$) affected the bacterial count of bread. Maximum numbers of bacterial colonies were observed in bread containing no essential oil treatment (T_0). Spraying of *C. sinensis* peel essential oil on all slices (T_2) showed to be most effective treatment against bacterial spoilage than other two essential oil treatments showed in Figure 1 shown.

Sensory evaluation of bread 0 hr- 96 hr storage interval

According to the Friedman's test sensory evaluation results for each sensory attributes showed significantly different ($p<0.05$) with sum of ranks at 0 hr- 72 hr storage intervals. After the 72 hr storage interval control bread rejected due to spoilage and at 96 hr storage other treatment types (i.e T_1, T_2, T_3) taken for sensory evaluation.

As 0 hr - 96 hr storage interval's sum of ranks, 96 hr sum of rank for each sensory attributes of bread showed high values for T_2 - Spraying of *C. sinensis* essential oil on all the slices treatment type than other treatment types such as EO sprayed on whole bread and EO sprayed on wrapping of bread.

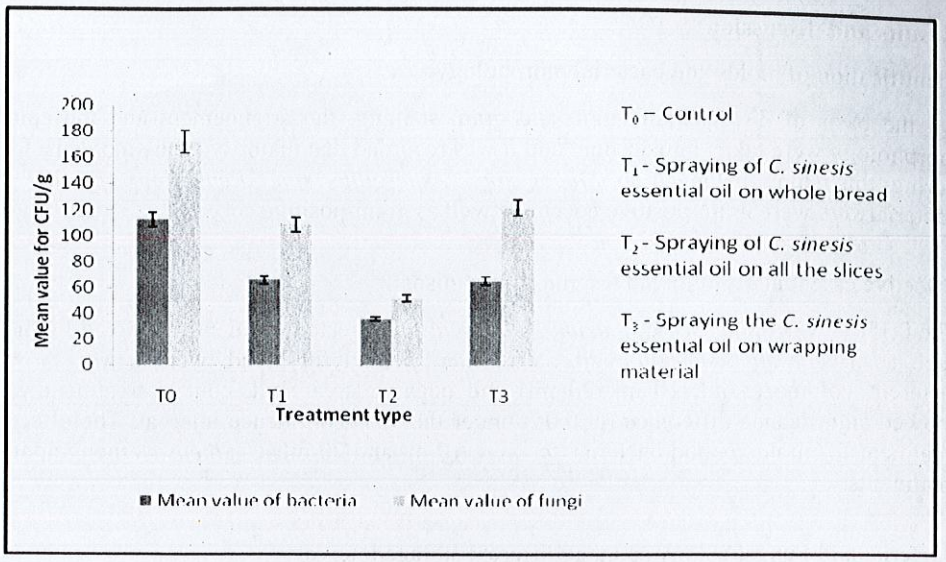

Figure 1: Mean value for colony forming unit per gram of bacteria and fungi with treatment type

Conclusions

When considering all the analyzed data and results from three type of essential oil i.e. *Citrus sinensis* (sweet orange), *Citrus maxima* (pommelo), *Citrus aurantifolia* (lime) exhibit the antimicrobial ability against bread micro-organisms while *Citrus sinensis* illustrate the high efficacy than other two types against both bacteria and molds of bread showing significant difference ($p<0.05$). The highest bacterial and mold colony count were recorded in the bread treated without the spray of *Citrus* peel essential oils. Treatment T_2 in which *C. sinensis* peel essential oil was sprayed on all slices of bread proved the most effective inhibitory treatment against bacterial and fungal spoilage of bread significantly ($p<0.05$).

References

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