

Inhibitory Effect of Citrus Peel Essential Oils on the Microbial Growth of Bread

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Abstract: A study was conducted to determine the effect of citrus peel essential oils on the microbial growth and sensory characteristics of bread. Citrus peel essential oils extracted by cold expression from malta (*Citrus sinensis*) and mossumbi (*Citrus sinensis*) were applied in different forms (treatments) separately. The essential oils significantly affected sensory characteristics such as symmetry of form, character of crust, colour of crumb, colour of crust, taste, texture, aroma and grain of bread. They also inhibited and delayed the microbial growth in the bread. Maximum inhibitory effect was achieved against molds and bacteria by spraying the malta peel essential oil on bread.

Key words: Bacteria, mossumbi, malta, bread, mold

Introduction

In baking industry, bread occupies a unique position both in production and utilization as compared to other bakery products. The ingredients of bread are supportive to growth of microorganisms and multiplication at different stages of bread production, slicing and wrapping. The main types of microbial spoilage of bread are moldiness and ropiness which are troublesome for bakers. Mold growth often begins within a loaf of sliced bread, where more moisture is available than at the surface, especially in the crease (Liaqat, 1988).

Since, bread is an important part of our daily diet; therefore, ways and means should be explored to increase its shelf life. The shelf life of bread can be increased by improving the hygienic conditions of mixing and baking premises and ensuring sterilized environment. To enhance the shelf life of bread, several 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 doubtful of chemical additives and thus the demand for natural preservatives has been intensified (Skandamis *et al.*, 2001).

The citrus peel essential oils are the most versatile essential oils. These oils are mainly used for flavouring fruit beverages, confectioneries, soft drinks, perfuming eau de cologne, soaps, cosmetics and household products. They are also used in medical treatments as immune stimulating as well as being anti-inflammatory agents. The citrus peel essential oils are known to exhibit antimicrobial properties such as antifungal, antibacterial, antiviral and antiparasite (Duccio *et al.*, 1998; Burt and Reinders, 2003; Moreira *et al.*, 2005). The investigation of naturally occurring antimicrobials for

food preservation receives greater attention due to consumer awareness of natural food products and growing concern of microbial resistance towards conventional preservatives (Schuenzel and Harrison, 2000). The present study was undertaken to observe the effect of citrus peel essential oils extracted from malta (*Citrus sinensis*) and mossumbi (*Citrus sinensis*) peels on microbial growth and sensory characteristics of bread.

Materials and Methods

Wheat flour was purchased from local market of Faisalabad.

Extraction of citrus peel essential oils: Citrus peel essential oils were extracted from the peels of malta (*Citrus sinensis*) and mossumbi (*Citrus sinensis*) by using cold expression method as described by Braddock (1999) with some modifications (Ahmad *et al.*, 2006; Rehman *et al.*, 2007). Fresh malta and mossumbi fruits were purchased from local market. Peels were removed with a sharp knife and pressed with a hydraulic press (Carver Laboratory Press, Model: C) at 10,000 psi. Emulsion extracted was centrifuged at 10,000 rpm. Essential oil was separated from sludge with the help of micropipette. It was packed in amber colour bottles and stored in a refrigerator.

Preparation of bread: The breads were prepared according to straight dough method as described in AACC (2000). Flour (100g), salt (1g), sugar (3g), yeast (1g) and shortening (5g) were mixed together with Hobart mixer. Dry ingredients were thoroughly blended at low speed for 2 minutes before the addition of water

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Table 1: Citrus peel essential oil treatments

Treatments	
T ₀	Control
T ₁	Addition of malta peels essential oil in the dough.
T ₂	Spraying of malta peels essential oil on the whole loaf.
T ₃	Spraying of malta peels essential oil on all the slices.
T ₄	Spraying of malta peels essential oil on wrapping material
T ₅	Addition of mossumbi peels essential oil in the dough.
T ₆	Spraying of mossumbi peels essential oil on the whole loaf
T ₇	Spraying of mossumbi peels essential oil on all the slices
T ₈	Spraying of mossumbi peels essential oil on wrapping material

Table 2: Colony forming units of bacteria/g of bread at different storage intervals

Treatments	0 h	24 h	48 h	72 h	96 h	Means
T ₀	6×10 ¹	9×10 ¹	1.6×10 ²	2.1×10 ²	2.7×10 ²	15.8 ^a
T ₁	5×10 ¹	8×10 ¹	1.5×10 ²	2.0×10 ²	2.5×10 ²	14.6 ^{ab}
T ₂	-	8×10 ¹	1.4×10 ²	1.9×10 ²	2.2×10 ²	12.6 ^c
T ₃	-	-	-	5×10 ¹	8×10 ¹	2.6 ^f
T ₄	-	-	6×10 ¹	9×10 ¹	1.2×10 ²	5.4 ^e
T ₅	6×10 ¹	9×10 ¹	1.6×10 ²	2.1×10 ²	2.6×10 ²	15.6 ^a
T ₆	-	8×10 ¹	1.5×10 ²	2.0×10 ²	2.4×10 ²	13.4 ^{bc}
T ₇	-	-	5×10 ¹	8×10 ¹	1.5×10 ²	5.6 ^e
T ₈	-	5×10 ¹	8×10 ¹	1×10 ²	2.0×10 ²	8.6 ^d
Means	1.89 ^e	5.22 ^d	10.56 ^c	14.78 ^b	19.89 ^a	

Table 3: Colony forming units of mold/g of bread at different storage intervals

Treatment	0 h	24 h	48 h	72 h	96 h	Mean
T ₀	-	2.1×10 ²	2.4×10 ²	2.7×10 ²	2.9×10 ²	20.2 ^a
T ₁	-	1.2×10 ²	1.6×10 ²	2.3×10 ²	2.6×10 ²	15.4 ^{bc}
T ₂	-	8×10 ¹	1.4×10 ²	1.8×10 ²	2.4×10 ²	12.8 ^d
T ₃	-	-	-	1×10 ²	1.5×10 ²	5.0 ^f
T ₄	-	-	9×10 ¹	1.6×10 ²	2.0×10 ²	9.0 ^e
T ₅	-	1.4×10 ²	1.7×10 ²	2.5×10 ²	2.7×10 ²	16.6 ^b
T ₆	-	8×10 ¹	1.5×10 ²	2.0×10 ²	2.5×10 ²	13.6 ^{cd}
T ₇	-	-	1×10 ²	1.5×10 ²	2.1×10 ²	9.2 ^e
T ₈	-	5×10 ¹	1.4×10 ²	1.7×10 ²	2.2×10 ²	11.6 ^d
Mean	0.0 ^e	7.5 ^d	13.2 ^c	19.0 ^b	23.2 ^a	

and then mixed at fast speed for 5 minutes. After mixing, the dough was allowed to rest for 2 h at 29°C. The dough was divided into pieces of equal weight, moulded into loaves by hand and placed into pre-greased pans. The dough pieces were kept in a proofing chamber for 50 min at 30°C and 70% humidity. The bread loaves were baked in a gas oven at 255°C for 20 min and were allowed to rest for a while to cool down before depanning. These were cooled, sliced and packed in polyethylene bags.

Application of citrus peel essential oils on bread: The malta (*Citrus sinensis*) and mossumbi (*Citrus sinensis*) peel essential oils were applied in the dough and breads (Table 1). The dose of essential oil at the rate of 0.1% was applied in each case as described by Kita *et al.* (2003).

Total bacterial count: Bacterial count in bread was determined at 0, 24, 48, 72 and 96 h intervals of storage on nutrient agar medium by plate count method (Harrigan and McCance, 1976).

Mold counting: Molds count in bread was determined at 0, 24, 48, 72 and 96 h intervals of storage on sabouraud agar medium by plate count technique (Beneke, 1962).

Identification of Molds: Slide culture technique was adopted to identify the molds that appeared on the surface of the plate (Awan and Rehman, 2000).

Sensory evaluation of bread: Sensory evaluations of bread were carried out by a panel of 6 trained judges for external characteristics i.e. volume, colour of crust, symmetry of form, evenness of bake, characters of crust and internal characteristics i.e., grain, colour of crumb, aroma, taste, texture at 0, 24, 48, 72 and 96 h storage intervals (Land and Shepherd, 1988).

Statistical analysis: The data were statistically analysed using ANOVA. A least significant difference (LSD_{0.05}) was used to test the effects of treatments when the F-test was statistically significant at p<0.05 (Steel *et al.*, 1997).

Results and Discussion

Bacterial colony count at different storage intervals:

The results on the colony count of bacteria in bread at different storage intervals are shown in Table 2. Bacterial susceptibility to two essential oils, as determined by the agar plate 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 (T₀). There were 6×10¹ colony forming units/g of bread at 0 h which increased to 2.7×10² colony forming units/g of bread at 96 h storage. Spraying of malta peel essential oil on all slices (T₃) proved to be most effective treatment against bacterial spoilage. First colony appeared after 72 h of storage. In case of spraying of malta peel essential oil on wrapping materials (T₄), colonies were observed at 48 h of storage which increased to 1.2×10² colony forming units/g at 96 h of storage. It indicated that spraying of malta peel essential oil on slices and on wrapping materials gave better control against bacterial inhibition. The reportable observations were obtained from spraying of mossumbi peel essential oil on slices of bread which showed low susceptibility (T₇). First colony appeared at 48 h storage and increased to 1.5×10² colony formed units/g of bread at 96 h of storage. Duncan's Multiple Range test helped to rank the values of different treatments in ascending order. T₀ (Control) was placed at the top with mean scores of 15.80 followed by T₅ (application of mossumbi oil in the dough) with 15.60 scores. The minimum score (2.60) was assigned to T₃ (spray of malta peel essential oil on all slices), so it proved to be most effective treatment against bacterial spoilage followed by T₄ (spray of malta

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Table 4: Effects of citrus peel essential oils on sensory characteristics of bread

Parameter	T ₀	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈
Volume	6.7 ^{abc}	6.78 ^{ab}	6.70 ^{bc}	6.84 ^a	6.74 ^{ab}	6.64 ^{bc}	6.68 ^{bc}	6.68 ^{bc}	6.54 ^c
Symmetry of form	3.1 ^e	3.18 ^d	3.18 ^d	3.4 ^a	3.34 ^{ab}	3.18 ^d	3.24 ^{bc}	3.30 ^{bc}	3.28 ^{bc}
Character of crust	2.9 ^e	2.96 ^{bc}	2.80 ^d	3.14 ^a	3.08 ^a	2.90 ^c	2.70 ^d	3.00 ^b	2.96 ^{bc}
Colour of bread crumb	6.6 ^c	6.72 ^{bc}	6.80 ^{bc}	7.04 ^a	6.90 ^{ab}	6.62 ^c	6.72 ^{bc}	6.92 ^{ab}	6.78 ^{bc}
Colour of crust	6.6 ^c	6.70 ^{bc}	6.84 ^{ab}	6.96 ^a	6.78 ^{abc}	6.70 ^{bc}	6.78 ^{abc}	6.84 ^{ab}	6.68 ^{bc}
Evenness of bake	2.24	2.14	2.24	2.24	2.16	2.12	2.18	2.18	2.16
Taste	139 ^d	14.12 ^{bcd}	14.32 ^{abc}	14.56 ^a	14.40 ^{ab}	13.98 ^{cd}	14.12 ^{cd}	14.34 ^{abc}	14.20 ^{abc}
Texture	11.5 ^c	11.68 ^{bc}	11.92 ^{ab}	12.14 ^a	12.02 ^a	11.56 ^c	11.82 ^{abc}	11.98 ^{ab}	11.82 ^{abc}
Aroma	6.0 ^{cd}	6.04 ^{bcd}	6.04 ^{bcd}	6.32 ^a	6.18 ^{ab}	5.92 ^d	5.92 ^d	6.14 ^{bc}	6.06 ^{bcd}
Grain	10.4 ^{bc}	10.68 ^{ab}	10.36 ^{bc}	10.88 ^a	10.58 ^{abc}	10.42	10.28 ^c	10.60 ^{abc}	10.46 ^{bc}

peel essential oil on wrapping materials) with mean scores of 5.40. The maximum score of bacterial count for storage interval was 19.89 at 96 h and minimum 1.89 at 0 h.

The results are supported by the findings of Al-Mohizea *et al.* (1987). The initial microbial loads, immediately after baking were found to be low primarily due to higher oven temperature and thin layer of dough. The microbial load of air and relative humidity inside the packing played a major role in bread spoilage. Earlier research in the essential oils field has incredibly increased chiefly with respect to the antimicrobials used to control food pathogens and food native microflora (Sabulal *et al.*, 2006; Sharma and Tripathi, 2006) and the knowledge of possible mechanism of action of these oils (Singh *et al.*, 2002). These results revealed the potential of essential oils as natural preservatives in food products.

Viable mold colony count at different storage intervals:

The molds are the microbes widely spread in nature. They may be found growing on foods such as fruits, cakes, preserves and bread and responsible to cause spoilage in such foods (Jay, 1990). The data on mold colony count are presented in Table 3. The results were highly significant for treatments, storage intervals and their interactions with each other. Maximum number of mold colonies was observed in T₀ (Control). There were 2.1×10² colonies/g of bread at 24 h and increased to 2.9×10² colony forming units/g of bread at 96 h of storage. Treatment T₃ indicated that spraying of malta peel essential oil on slices of bread has highest inhibitory effects on mold growth. First colony appeared after 72 h of storage of bread and load was 1.5×10² colony forming units/g of bread at 96 h of storage. In case of T₄ (spray of malta peel essential oil on packing material), the colonies appeared after 48 h and final count was 2.0×10² colony forming units/g of bread at 96 h of storage. On the other hand, spraying of mossumbi peel essential oil on all slices of bread showed low susceptibility against mold (T₇). First colony appeared at 48 h storage and increased to 2.1×10² colony forming units/g of bread at 96 h of storage.

Duncan's Multiple Range test was applied which helped in rank the mean values of different treatments in ascending order. T₀ (Control) was placed at the top with

the highest scores (20.20) followed by T₅ (application of mossumbi oil in the dough) with 16.60 scores. The minimum score (5.0) was assigned to T₃ (spray of malta peel essential oil on all slices). So, it proved to be most effective treatment against mold spoilage followed by T₄ (spray of malta peel essential oil on packing material) with mean scores of 9.0. The maximum score of mold count for storage interval was 23.22 at 96 h and minimum at 0 h. The results are in close concurrence with the findings of Agarwal *et al.* (1979) who reported that essential oil from the seeds of *Nigella sativa* was active against *Aspergillus flavus* and *A. niger*.

Identification of molds and colonial morphology: The isolated molds were classified through the prescribed slide culture method as described by Awan and Rehman (2000). On the basis of direct examination and staining through lactophenol blue method, the arrangement and the colour of the spore along with the type of hyphae were taken into account and it was recorded that majority of the molds isolated during this study belonged to *Aspergillus flavus* followed by *A. fumigatus*, *Penicillium spp.*, *Rhizopus* and *Mucor* (Sharma and Tripathi, 2006).

Aspergillus spp. showed white colonies which became greenish blue and black or brown as culture matured. *Penicillium spp.*, on maturity showed greenish or blue green colonies. *Rhizopus spp.* exhibited rapidly growing white coloured mold swarms over entire plate. Aerial mycelium, cottony and fuzzy with black sporangium were depicted. Roots like hyphae (rhizoid penetrating the medium) were found when examined under microscope. *Mucor* colonies were resembled with the colonies of *Rhizopus* but Rhizoids were absent (Jay, 1990).

The same as well as *Aspergillus niger*, *Neurospora sitophila* and green molds from a loaf of commercial bread were isolated by Sharma and Tripathi, 2006. Al-Mohizea *et al.* (1987) also presented the similar results that at 22-24°C, *Penicillium* and *Aspergillus spp.* predominated, followed by *Rhizopus* and *Neurospora spp.*

Sensory evaluation: Sensory evaluation of bread was carried out by a panel of 6 judges for external and

internal characteristics at 0, 24, 48, 72 and 96 h storage intervals (Table 4). The essential oils affected sensory characteristics i.e., symmetry of form, character of crust, colour of crumb, colour of crust, taste, texture, aroma and grain of bread significantly but no effect of oil was observed on evenness of bake of bread. The bread slices sprayed with malta peel essential oil got the highest over all scores which were not significantly different with the scores of bread having malta peel essential oil treated wrapper. The aroma of bread having mossumbi peel essential oil application was affected significantly.

Conclusion: The highest bacterial and mold colony counts were recorded in the breads treated without the spray of citrus peel essential oils. Treatment T₃ in which malta peel essential oil was sprayed on all slices of bread, proved to be most effective inhibitory treatment against the bacterial and fungal spoilage of bread.

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