CIBTech Journal of Microbiology ISSN: 2319-3867 (Online)
An Online International Journal Available at http://www.cibtech.org/cjm.htm
2015 Vol. 4 (1) January-March, pp.6-12/Mishra et al.
Research Article

SYNERGISTIC ANTIMICROBIAL ACTIVITY OF ESSENTIAL OIL AND CHEMICAL FOOD PRESERVATIVES AGAINST BAKERY SPOILAGE FUNGI

***Viraj Krishna Mishra, Shivani Gupta and Ram Kumar Pundir**
Department of Biotechnology Engineering, Ambala College of Engineering and Applied Research, Ambala, Haryana, India, 133101
**Author for Correspondence*

ABSTRACT
In the present investigation two fungal strains were isolated from spoiled bread and cake samples on 2 % wheat flour agar medium. Both fungal isolates were identified on the basis of cultural and microscopic characteristics as *Penicillium oxalicum* and *Aspergillus flavus*. Effect of physical and chemical factors had been studied on growth of isolates. Essential oil of Clove (*Syzygium* sp.), Basil (*Ocimum* sp.), Neem (*Azadirachta sp.),*Ajwain also known as carom seeds (*Trachyspermum* sp.), cinnamon and chemical such as citric, benzoic, lactic and acetic acids were used to control the growth of isolates. Only clove oil showed the zone of inhibition of 45 mm against *Penicillium oxalicum* and of 15 mm against *Aspergillus flavus*. Benzoic acid, citric acid lactic acid and acetic acid showed 57.80%, 52.63%, 31.5% and 10.5% mycelial growth inhibition against *Penicillium oxalicum* respectively. Mycelial growth inhibition of acetic acid was 6.8% against *Aspergillus flavus* and no activity was observed by benzoic acid, citric acid and lactic acid. Synergistic effect of clove oil and acids had been also evaluated for control agent for growth of fungal isolates.

Keywords: *Antifungal Activity, Zone of Inhibition, MIC, Essential Oil, Synergistic Effect*

INTRODUCTION
Bakery products get deteriorated due to physical, chemical and microbiological factors. Major microbiological loss of bakery products is due to mould growth and problems that are associated with it (Saranraj and Geetha, 2012). Shelf life of bakery products is limited by growth of moulds. Blue green *Penicillium* sp. and *Aspergillus* sp. are found along with other genera of mould causing bread spoilage. Shelf life of food can be increased and spoilage can be prevented by usage of preservatives, artificial and chemical preservatives are used to inhibit the growth of food microorganisms in food. Mostly artificial preservatives are safe but some have negative and life threatening effects. For prevention of spoilage in bakery products natural preservatives are better alternative than artificial preservatives (Anand and Sati, 2013). Essential oils can be a natural alternative for chemical preservatives which is mainly extracted from plant material such as leaves, flowers, buds, twigs, bark, herbs, fruits and roots. Essential oils had showed antibacterial and antifungal activity (Burt, 2004) against many food spoiling bacteria and fungus. Essential oils were known to exhibit antifungal activity against the bread spoilage fungi too (Guynot *et al.*, 2005). The present study was conducted to evaluate the antifungal activity of essential oils and weak acid preservatives. Synergistic antifungal effect of most promising essential oil and acid food preservatives was also determined.

MATERIALS AND METHODS
Sample Collection
Fresh Bread and cake samples were procured from local shops of Ambala (Haryana, India). Essential oils were purchased from local manufacturer.
Fungal Cultivation
Spoiled Bread and cake sample were used to make serial dilution of 10^{-3}, 10^{-4}, $10^{-5}$and 10^{-6}on 2% (W/V) Wheat Flour Agar (WFA), pure cultures were transferred on 2%WFA. Culture was preserved at 4^{o}C and identification was done on the basis of cultural and morphological characterizations.

Preparation of Standard Culture

0.5 McFarland standard was used for standardization of the test microorganism. McFarland Standards was used as reference to adjust the density of microbial suspensions so that their number would be within a given range. 0.5 McFarland gives approximate cell density of 1.5 x 10^8 CFU/ml, having absorbance of 0.132 at wavelength of 600 nm. (Andrew, 2001)

Effect of Physical and Chemical Factor on Growth of Fungal Isolates

Effect of different factors on growth of isolates was studied at different pH, temperature, NaCl and water activity (a_W). pH of the medium was adjusted at 4, 4.5, 5, 5.5, 6 and 6.5. Plates were kept at different temperature i.e 25^0C, 30^0Cand 35^0C.Medium was prepared and concentration of NaCl were varied at different range 0%, 2%, 2.5%, 3%, 5%. 7% and 10%.Glycerol was added 105.8 gm, 69.1 gm and 39.1 gm in 100 ml WFA to adjust water activity to 0.80, 0.85 and 0.90 respectively.

Antifungal Activity

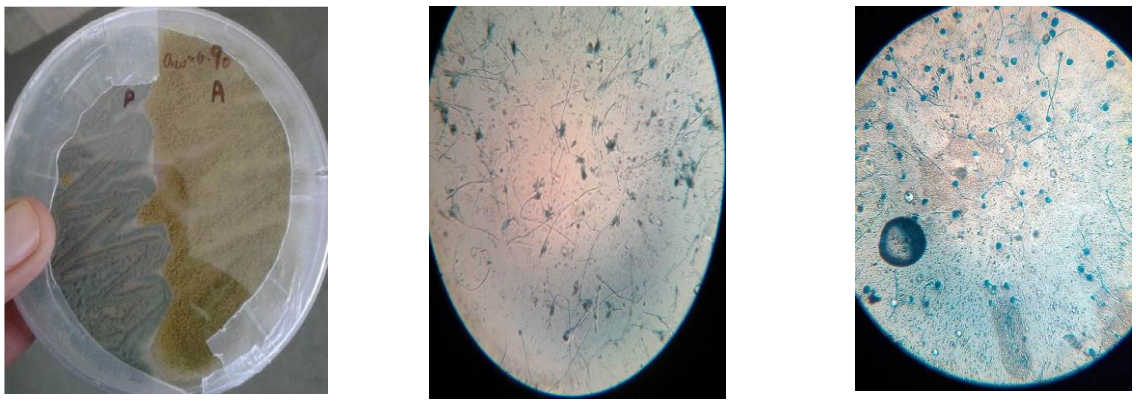
Poison food technique was used to measure the antifungal activity of chemical food preservatives and synergistic antifungal activity of acid preservative and essential oils. In poisoned food technique 90 ml WFA was prepared for each preservative and after that 10 ml of stock solution of preservatives were added to make up the volume of 100ml. For synergistic activity, weak acid preservatives and essential oil were mixed in 1:1 ratio. Plates were inoculated with fungal disc of 6 mm of diameter cut from periphery of 3 to 7 days old culture. Concentration of stock solution for citric acid and benzoic acid was 1% w/v and for acetic acid and lactic acid, it was 1% v/v. Agar well diffusion method was used to screen the essential oils which showed antifungal activity against fungal isolates. Essential oils of Clove (*Syzygium* sp.), Basil (*Ocimum* sp.), Neem (*Azadirachta sp.),*Ajwain also known as carom seeds (Trachyspermum sp.) and cinnamon were used.

Synergistic Antifungal Effects of Chemical Preservatives and Essential Oils

Effect of chemical preservative (acetic acid, benzoic acid, citric acid and lactic acid) and synergistic effect of chemical preservatives with essential oils (maximum antifungal activity shown against the tested fungus) was determined by using poison food techniques.

RESULTS AND DISCUSSION

Two most prevalent fungal isolates were obtained from bread and cake samples by using serial dilution agar method. These two fungi were identified by consulting various manuals and monographs on the basis of colony characteristics (i.e. colour, growth of colony) and sporulating structures (conidial head, types of conidiogenous cells, arrangement of conidia, sporangial head, and types of spores). From cake sample dark green coloured, powdery, compact colony identified as *Penicillium oxalicum.* From bread sample, light green coloured, cottony with white periphery colony identified as *Aspergillus flavus.* Morphological and microscopic view has been represented in Fig.1 and left sided colony was identified as *Penicillium oxalicum* and right sided colony was identified as *Aspergillus flavus.*



a) Pure culture on WFA plates b) Isolate *Penicillium oxalicum* c)Isolate *Aspergillus flavus*

Figure 1: Morphological and microscopic view of *Penicillium oxalicum* and *Aspergillus flavus*

Spoiled bread and cake products were found to contain a variety of mycoflora. *Aspergillus* sp. and *Penicillium* sp. are also responsible for causing deterioration of bakery products (Saranraj and Geetha, 2012).

Pundir and Jain (2011) isolated four fungal taxa *Aspergillus, Alternaria, Penicillium* and *Rhizopus* from fresh cakes, which had been further identified as *Penicillium oxalicum* , *Aspergillus luchuensis, Rhizopus stolonifer and Alternaria alternata*. Abellana *et al.,* (2001) had reported *Aspergillus* and *Penicillium* isolates, which spoiled sponge cake. *Aspergillusniger, A. flavus, Monilia (Neurospora) sitophila, Absidia corymbifera, Penicillium frequentans, Penicillium expansum, P. citrinum, P. stolonifer, Mucor sp., Cladosporium sp.Alternaria alternata, A. tenuissima and Scopulariopsis sp.*are the most common fungus which have been identified as bread spoilage fungi (Pundir and Jain, 2011). Similar results had also been reported by Okoko and Ogbomo (2010) and they isolated *Aspergillus niger, Aspergillus flavus* and *Rhizopus stolonifer* fungal strains from spoiled bread samples.

Essential oils are widely studied as food preservatives as alternative of chemical preservatives. Essential oil helps in cell wall degradation, weakening the membrane and increasing the membrane permeability and also react with cell membrane proteins to inhibit their function (Pundir and Jain, 2011; Judit, 2011) that leads to death of cells and control of microbial spoilage. It was found that phenolic and alcoholic essential oils modify the active sites of enzymes via hydrogen bonding to amino acid residues of active sites (Cristani, 2007; Dafera, 2000). Antifungal effect of clove, cinnamon, lemongrass cassia have been also reported against *Aspergillus* (Pundir and Jain, 2011; Judit, 2011). It is likely that essential oils may inhibit ergosterol synthesis in fungi and this leads to fungal cell death (Judit, 2011).

Effect of Physical and Chemical Factor on Growth of Isolates

Delayed growth of *Aspergillus flavus* and *Penicillium oxalicum* was observed at pH 6 and an early growth was observed at pH 4.5. At 25^0C, growth of both *Penicillium oxalicum* and *Aspergillus flavus* was observed, but at 30^0C and 35^0C only growth of *Penicillium oxalicum* was observed, *Aspergillus flavus* did not showed any growth even after fourteen days. Early growth of *Aspergillus flavus* and *Penicillium oxalicum* was observed at 3% NaCl while delayed growth was observed at 2.5% and 10% of NaCl concentration. At a_w 0.80 no growth of *Aspergillus flavus* species and *Penicillium oxalicum* were observed, at a_w 0.85 mild and delayed growth of mould was reported and at a_w 0.90 growth of both *Aspergillus flavus* and *Penicillium oxalicum* species was observed. Water activity of bread and cake is higher than other bakery products this result to early microbial spoilage of these products compared to other bakery products and allows growth of bacteria, yeast and other molds Low pH of the food materials promotes the growth of yeast and molds while at neutral or alkaline pH mostly growth of bacteria is reported.

Our results report similar observation too (Frazier, 2003; Jay 2005). The effect of NaCl and other salts like $CaCl_2$, $MgCl_2$, KCl and $MgSO_4$ was studied on *Penicillium roqueforti* and *Aspergillus niger* at 22 ^{0}C. NaCl and $MgCl_2$ showed higher inhibitory activity against these fungal strains. It was also reported that water activity also affect the inhibitory activity of these salts (Samapundo, 2010). Similar studies were also carried out by Mariona (2002-2003) to evaluate the effect of different preservation factors (environment and preservatives) on fungal growth in the presence of preservatives at different concentartion. Guynot *et al.,* (2003b) had studied the effect of pH, water activity (aw), and carbon dioxide (CO2) levels on the growth of fungus of *Eurotium sp., Aspergillus sp.,* and *Penicillium corylophilum.* Water activity at levels of 0.80 to 0.90 had a significant influence on fungal growth. As the CO_2 increases from 0% to 70%, it delayed the growth of fungus while at 100% CO_2 and at 25^0C no growth was observed till 28 days at different a_w level. The studies showed synergistic effects of modified atmosphere packaging, a_w, and pH that can be successfully used to control the growth of fungus.

Antifungal Activity of Chemical Preservatives

Out of four chemical preservatives (acetic acid, benzoic acid, citric acid and lactic acid) benzoic acid showed antifungal activity against *Penicillium oxalicum*. Mycelial growth inhibition was 57.80% (Table 1). Citric acid was found to be the second most antifungal inhibitor i.e 52.63% followed by lactic acid 31.5% and minimum mycelial growth inhibition by acetic acid i.e 10.5%. Mycelial growth inhibition of

acetic acid was 6.8% against *Aspergillus flavus* and no activity was observed by benzoic acid, citric acid and lactic acid (Table 1).

The weak organic acids, for example acetic, lactic, benzoic and sorbic acid are used as traditional food preservatives and showed inhibitory activity against bacteria and fungus. At low pH, these weak acids are permeable to the cell membrane and enter to the cell. At high pH inside the cell, acid dissociate, results in assimilation of charge within the cell (Brul, 1999; Booth and Kroll, 1989). Thus cell growth inhibition can be due to membrane disruption (Freese *et al.*, 1973; Stratford and Anslow, 1998; Bracey *et al.*, 1998) inhibition of essential metabolic reactions (Krebs *et al.*, 1983) and the accumulation of toxic anions (Eklund, 1985). Antifungal effect of acetic acid, lactic Acid, citric acid, benzoic acid and sodium acetate has been assed against bread and pickle associated fungi (*Aspergillus luchuensis, A. flavus, Rhizopus stolonifer* and *Penicillium. oxalicum*). It was reported that lactic acid, citric acid and sodium acetate also showed inhibitory activity against both *A. flavus* and *Penicillium. oxalicum* while benzoic acid did not showed any activity against *A. flavus* and *Penicillium. oxalicum* (Pundir and Jain, 2010). Our results confirmed the antifungal activity of acetic acid against *A. flavus* and *Penicillium. oxalicum.* Benzoic acid, citric acid and lactic acid only showed the inhibitory activity against *Penicillium. oxalicum* but not against *A. flavus* at used concentration, it may be due to difference in strain. The higher concentration benzoic acid, citric acid and lactic acid need to be assessed for further evaluation of inhibitory activity of this acid against isolated strain of *A. flavus.*

Table 1: Mycelial growth inhibition of bread and cake spoilage fungi by chemical preservatives

Preservative	***Penicillium oxalicum***	***Aspergillus flavus***
Acetic Acid	10.5%	6.8%
Benzoic Acid	57.80%	-
Citric Acid	52.63%	-
Lactic Acid	31.5%	-

- No growth inhibition

Suhr and Nielsen (2004) have studied the effect of weak acid preservatives at different concentration on *Penicillium roqueforti, Penicillium commune and Eurotium rubrum* they reported that calcium propionate at 0.3% (w/v), inhibited fungal growth for 2 week except when substrate pH and a_w were 4.7 and 0.97 respectively. At low a_w and pH growth was delayed till 30 days.

Synergistic Antifungal Effects of Chemical Preservatives with Essential Oil

Agar well diffusion method was used to screen the essential oils. Only clove essential oil showed antifungal activity against out of five essential oils i.e Clove (*Syzygium* sp.), Basil (*Ocimum* sp.), Neem (*Azadirachta sp.),*Ajwain also known as carom seeds (*Trachyspermum* sp.), cinnamon oil. Eugenol is the main component of clove oil which contributes to inhibitory activity. Five essential oils (cinnamon leaf, clove, bay, lemongrass and thyme essential oils) had showed antifungal activity against *Eurotium amstelodami, E. herbariorum, E. repens, E. rubrum, Aspergillus flavus, A. niger and Penicillium corylophilum* at pH 6 and at different water activity levels (a_w 80– a_w 90). It was observed that substrate also affected the inhibitory activity; these fungi were totally inhibited when wheat flour based agar medium was used but showed limited success in controlling growth of fungi when sponge cake analogues were used (Guynot, 2005). Our result did not show the broad activity of these essential oils, it may be due to strain difference or preparation method of essential oil. Higher concentrations of these essential oils need to be checked to ascertain inhibitory activity of these essential oils. Combined mixture of benzoic acid and clove oil, lactic acid and clove oil, citric acid and clove oil, acetic acid and clove oil were measured against *Penicillium oxalicum* and *Aspergillus flavus*. Percentage of mycelial growth inhibition of mixture of benzoic acid and clove oil was 73.68% followed by mixture of acetic acid and clove oil 57.8%, 10.5% by mixture of citric acid and clove oil and 5.2% by mixture of lactic acid and clove oil against *Penicillium oxalicum* (Table 2). Percentage of mycelial growth inhibition of mixture of citric acid and clove oil was 31.03% followed by 26.3% by mixture of benzoic acid and clove oil, no inhibitory

effect was shown by mixture of lactic acid and clove oil and by mixture of acetic acid and clove oil against *Aspergillus flavus* (Table 2).

Synergistic effects of essential oils of *Ocimum basilicum* L. plants (Anise, Bush, Cinnamon, Dark Opal and a commercial sample of dried basil) against yeasts and moulds were studied. Synergistic effects of low pH (pH 4·2) and salt (5% NaCl) on Anise oil was determined. As the concentration of Ansie oil was increased the growth of *Lact. Curvatus* and *S. cerevisiae* had been delayed substantially (Lachowicz, 1998).

Table 2: Synergistic antifungal activity of preservative and clove essential oil (% mycelial growth inhibition)

Preservative + Clove oil	***Penicillium oxalicum***	***Aspergillus flavus***
Acetic Acid +clove oil	57.8%	-
Benzoic Acid + clove oil	73.68%	26.3%
Citric Acid+ clove oil	10.5%	31.03%
Lactic Acid +clove oil	5.2%	-

- No Growth inhibition

Antimicrobial effects of acetic acid and propionic acids were also checked against bakery associated bacteria. Acetic acid was found to be most promising inhibitor (Rosenquist, 2002). Clove oil increases the activity of acetic acid against *Penicillium oxalicum* and it inhibited the antifungal activity of acetic acid against *Aspergillus flavus.* Benzoic acid showed highest inhibitory activity against *Penicillium oxalicum* and it also inhibited growth of *Aspergillus flavus* in presence of clove oil. Clove oil showed negative effect on antifungal effect of citric acid and lactic acid against *Penicillium oxalicum.* There was no synergistic effect of clove oil and lactic acid against *Aspergillus flavus* but clove oil had positive effect on activity of lactic acid *against Aspergillus flavus*. These synergistic and antagonistic effects are due to phenolic and alcoholic compound presence in essential oils. Synergistic effect is mainly due to similarity in phenolic compounds of essential oils (Nestor *et al.,* 2012; Bajpai, 2012; Lambert, 2001; Azeredo *et al.,* 2011) while interaction between non-oxygenated and oxygenated monoterpene hydrocarbons contribute to antagonistic effect of essential oils (Hammer *et al.,* 1999; Goñi, 2009). The most common accepted mechanism for synergistic inhibition activity are- i) sequential inhibition of a common biochemical pathway ii) inhibition of protective enzymes and iii) use of cell wall active agents to enhance the uptake of other antimicrobials (Hammer *et al.,* 1999). The factors involved in antagonistic effects are not well studied (Nestor *et al.,* 2012).

Conclusion

Essential oils are natural preservative; these oils can be successfully used to enhance the shelf life of bakery products. The wide spectrum of activity is essential for choice of EOs as preservative. Essential oils can also be helpful to improve the flavor and aroma of the bakery products along with its shelf life. Synergistic effect of weak acidic preservative can not only improve the inhibitory activity of essential oils but also the range of activity against bakery associated microorganism. More study is needed to understand the synergistic and antagonistic activity of essential oils and weak acid preservative for its commercial application.

ACKNOWLEDGEMENT

Authors are thankful to management and director of Ambala College of Engineering and Applied Research for providing necessary support and facility to complete the research work.

REFERENCES

Abellana M, Sanchis V and Ramos AJ (2001). Effect of water activity and temperature on growth of three Penicillium species and Aspergillusflavus on a sponge cake analogue. *International Journal of Food Microbiology* **71** 3151-3157.

Anand SP and Sati N (2013). Artificial Preservatives and their Harmful Effects: Looking Toward Nature for Safer Alternatives. *International Journal of Pharmaceutical Sciences and Research* **4**(7) 2496 – 2501.

Andrews JM (2001). Determination of minimum inhibitory concentration. *Journal of Antimicrobial Chemotherapy* **48** 5-16.

Arroyo M (2003). Natural Antifungal Systems for Prevention of Mould Spoilage in Bakery Products, Applied Mycology Group Institute of Bioscience and Technology, Cranfield University.

Bajpai VK, Baek KH and Baek SC (2012). Control of Salmonella in foods by using essential oils: A review. *Food Research International* **45** 722–734.

Bassolé IHN and Juliani HR (2012). Essential Oils in Combination and Their Antimicrobial Properties. *Molecules* **17** 3989-4006.

Booth IR and Kroll RG (1989). The preservation of foods by low pH, In: *Mechanisms of Action of Food, Preservation Procedures,* edited by Gould GW (Elsevier) London 119–160.

Bracey D, Holyoak CD and Coote PJ (1998). Comparison of the inhibitory effect of sorbic acid and amphotericin B on *Saccharomycescerevisiae*: is growth inhibition dependent on reduced intracellular pH? *Journal of Applied Microbiology* 85.

Brul S and Coote P (1999). Preservative agents in foods Mode of action and microbial resistance mechanisms. *International Journal of Food Microbiology* **50** 1–17.

Burt S (2004). Essential oils: their antibacterial properties and potential applications in foods-a review. *International Journal of Food Microbiology* **94**(3) 223-53.

Cristani M, D'Arrigo M, Mandalari G, Castelli F, Sarpietro MG, Micieli D, Venuti V, Bisignano G, Saija A and Trombetta D (2007). Interaction of four monoterpenes contained in essential oils with model membranes: Implications for their antimicrobial activity. *Journal of Agricultural and Food Chemistry* **55** 6300-6308.

Dafera DJ, Ziogas BN and Polissiou MG (2000). GC-MS analysis of essential oils from some Greek aromatic plants and their fungitoxicity on Penicilliumdigitatum. *Journal of Agricultural and Food Chemistry* **48** 2576-2581.

De Azeredo GA, Stamford TLM, Nunes PC, Neto NJG, De Oliveira MEG and de Souza EL (2011). Combined application of essential oils from *Origanumvulgare*L. and *Rosmarinusofficinalis* L. to inhibit bacteria and autochthonous microflora associated with minimally processed vegetables. *Food Research International* **44** 1541–1548.

Eklund T (1985). The effect of sorbic acid and esters of para-hydroxybenzoic acid on the proton motive force in *Escherichia coli* membrane vesicles. *Journal of General Microbiology* **131** 73–76.

Frazier WC and Westhoff DE (2003). *Food Microbiology,* 4th edition (Tata McGraw-Hill Publishing Company Limited) New Delhi 539.

Freese E, Sheu CW and Galliers E (1973). Function of lipophilic acids as antimicrobial food additives. *Nature* **241** 321–325.

Goñi P, López P, Sánchez C, Gómez-Lus R, Becerril R and Nerín C (2009). Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils. *Food Chemistry* **116** 982–989.

Guynot ME, Mari SN, Sanchis V and Ramos AJ (2003b). Modified Atmosphere Packaging for Prevention of Mold Spoilage of Bakery Products with Different pH and Water Activity Levels. *Journal of Food Protection* **66**(10) 1864–1872.

Guynot ME, MarÍn S, SetÚ L, Sanchis V and Ramos AJ (2005). Screening for Antifungal Activity of Some Essential Oils against Common Spoilage Fungi of Bakery Products. *Food Science and Technology International* **11**(1) 25-32.

Hammer KA, Carson CF and Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology* **86** 985–990.

Jay JM, Loessner MJ and Golden DA (2005). *Modern Food Microbiology,* 7th edition (Springer) USA 790.

JuditKrisch Rentsenkhand and Tserennadmid CsabaVágvölgyi (2011). Essential oils against yeasts and moulds causing food spoilage Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas (Ed.) 1135-1142.

Krebs HA, Wiggins D, Sole S and Bedoya F (1983). Studies on the mechanism of the antifungal action of benzoate. *Biochemical Journal* **214** 657–663.

Lachowicz KJ, Jones GP, Briggs DR, Bienvenu FE, Wan J, Wilcock A and Coventry MJ (1998). The synergistic preservative effects of the essential oils of sweet basil (*Ocimum basilicum L.*) against acid-tolerant food microflora. *Letters in Applied Microbiology* **26** 209–214.

Lambert RJW, Skandamis PN, Coote P and Nychas GJE (2001). A study of the minimum inhibitoryconcentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology* **91** 453–462.

Okoko FJ and Ogbomo O (2010). Amylolytic Properties Of Fungi Associated With Spoilage In Bread. *Continental Journal of Microbiology* **4** 1 – 7.

Pundir RK and Jain P (2010). *Screening For Antifungal Activity of Commercially Available Chemical Food Preservatives* **5**(2) 25-28.

Pundir RK and Jain P (2011). Evaluation of five chemical food preservatives for their antibacterial activity against bacterial isolates from bakery products and mango pickles. *Journal of Chemical and Pharmaceutical Research* **3**(1) 24-31.

Rosenquist H and Hansen Å (2002). The antimicrobial effect of organic acids, sour dough and nisin against *Bacillus subtilis* and *B. licheniformis* isolated from wheat bread. *Journal of Applied Microbiology* **85**(3) 621–631.

Samapundo S, Deschuyffeleer N, Van Laere D, De Leyn I and Devlieghere F (2010). Effect of NaCl reduction and replacement on the growth of fungi important to the spoilage of bread. *Food Microbiology* **27**(6) 749-56.

Saranraj P and Geetha M (2012). Microbial Spoilage of Bakery Products and Its Control by Preservatives. *International Journal of Pharmaceutical & Biological Archives* **3**(1) 38-48.

Stratford M and Anslow PA (1998). Evidence that sorbic acid does not inhibit yeast as a classic ‘‘weak acid’’ preservative. *Letters in Applied Microbiology* **27** 203–206.

Suhr KI and Nielsen PV (2004). Effect of weak acid preservatives on growth of bakery product spoilage fungi at different water activities and pH values. *International Journal of Food Microbiology* **95**(1) 67-78.