International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-7, Special Issue3-April, 2016, pp200-205
http://www.bipublication.com

Review Article

A survey of the anti-microbial effect of *Mentaxpiperita* on *Saccharomyces cerevisiae* in Lemonade

Afsoon Kasraei[1], Mahnaz Hashemiravan*[2] and Peyman Rajaee[3]

[1] M.Sc. student, [3]Department of food science and Technology,
Varamin-pishva Branch, Islamic Azad University, Varamin, Iran
[2]Department of food science and Technology,
Varamin-pishva Branch, Islamic Azad University, Varamin, Iran
[3]Department of food science and Technology,
Varamin-pishva Branch, Islamic Azad University, Varamin, Iran
*Corresponding author: m _ hashemiravan @ yahoo.com

ABSTRACT

Mentapiperita of *Lamiaceae* family is full of essential oil. Some studies have shown the inhibiting activity of essential oil of *Mentapiperita* and its main compound against pathogenic microorganisms. This study investigates the anti-yeast activity of essential oil of *Mentapiperita* and growth inhibition of *Saccharomyces cerevisiae* in lemonade at temperature 4°C. Essential oil is investigated in five concentrations 2, 1.5, 1, 0.5, 0.2% and Saccharomyces yeast in 2 concentrations cfu/ml (10^3, 10^6) and 11 treatments and each of them were investigated in three replications. Brix changes, PH, acidity and sensory evaluation as aroma, flavor and total acceptance were investigated during 28 days at temperature 4°C. To investigate and compare the non-growth between control and treatments group, multiple range Duncan software was used to determine the difference between the mean of data at confidence level 5%. SAS software was used for statistical analysis. Essential oil of *Mentapiperita* in all concentrations stopped yeast growth. By increasing the essential oil concentration, this inhibition was rapid and at short-time and growth inhibition compared to control showed significant difference ($P<0.05$). The results of sensory test of lemonade with various concentrations of essential oil showed that treatment with 0.2% essential oil had highest total acceptance in terms of aroma and flavor and the treatment with concentration 0.2% of *Mentapiperita* in two concentrations of yeast cfu/ml (10^3, 10^6) was selected as the best treatment.

Keywords: Saccharomyces cerevisiae, essential oil, Pepper mint, lemon syrup

INTRODUCTION

By development and creation of new species of fungus pathogens, most of chemical controllers are weakened and due to causing cancer, severe toxicity, long decomposition, environmental pollution and adverse effects on human being, they are restricted to control pollution in food products. The increase of knowledge, importance of fungus diseases and great problems of their treatment obliged the researchers to find an alternative method to use synthetic chemical controllers (Rasooli*et al.*, 2005). One of the best alternatives of synthetic chemical controllers is using anti-fungal compounds. These compounds are not only important as preservative of food, they are important in control of human and plant diseases with fungus origin. Anti-fungal and anti-oxidative properties of oil essences and various extracts of plants have received much attention by academic researchers and food industry experts as their application as natural additives is based on increasing inclination in replacing anti-fungal and anti-oxidative factors with natural factors (Rasooli and, Owlia, 2005). Also, the physical nature of essential oils, that is, low molecular weight combined with pronounced lipophilic tendencies allow them to penetrate cell membrane more quickly than other substances (Alpsoy, 2010). Essential oils are a mixture of volatile and natural compounds

of terpenic blend. It seems that anti-fungal and anti-microbial properties of these compounds are the result of reaction of different compounds increasing each other effect (Razzaghi*et al*., 2009). Based on the adverse effects of synthetic chemical preservatives, people try to use natural preservatives derived of plant, animal and microbial sources that beside increasing durability of food, adverse effects of chemical food preservatives are avoided (Tepe*et al* ., 2005).

About 3000 types of essences are recognized until now, of which 300 types are important commercially and are used for their aroma, flavor of food. The most important medicinal herbs with essence are dedicated to mint, Coriander, chicory, pine, Cypress and other plants (Burt, 2004).

The researches show that anti-microbial effects of plant are regarding the presence of phenol compounds. It seems that anti-microbial effects of these compounds are associated to inactivity of cellular enzymes by these compounds depending upon the influence speed of these compounds on cell. Some researchers state that these compounds affect permeability of cellular membrane of microbial cells (Nazck andshahidi. 2004).

MATERIALS AND METHODS

Providing yeast

Saccharomyces cerevisiae yeast PTCC 6793 was provided as lyophilized ampoule from Medical Sciences School of ShahidBeheshti University of Tehran (Omidbeygi*et al*., 2007).

Providing *Mentapiperita* essence

Mentapiperita essence in this study was purchased from Magnolia Company with valid ID.

Providing lemonade

Lemonade was provided with concentrate formulation of lemon, sugar, water and concentrations 0.2, 0.5, 1, 1.5 and 2% essence of *Mentapiperita* in ShirinfamCompany based on health regulations and Iran standard (Binam, 2003).

Microbial test of lemonade

To determine inoculated bacterium, McFarland method is applied. To determine the number of probable bacteria, sulfuric acid 1% and barium chloride 1.175% are used. To determine the number of bacteria in each milliliter of suspension, 0.5 McFarland standards are used. One of the advantages of this method is that the number of existing cells in a liquid is counted rapidly and it is easy to do it. To determine the number of bacteria in each milliliter of suspension, 0.5 McFarland standards are used. A 0.5 McFarland standard is prepared by mixing 0.05 cc of 1.175% barium chloride with 9.95 mL of 1% sulfuric acid 1%. The turbidity is almost about 1/5 $\times 10^{8}$cfu/ml1cell of bacteria. To evaluate OD of turbidity, a visible-ultraviolet spectrophotometer, the absorbance at a wavelength of 625 nm should be 0.08 to 0.13 (Brown, 2009). Then, an amount of sterile water was poured into Falcon with sediment and was controlled by 0.5 McFarland standard. 3cc of diluted sediment of yeast were transferred to the cell of spectrophotometer and its absorbance was measured in wavelength 625nm. The bacteria absorbance should be equal to 0.5 McFarland. After pre parathion of 10^{8}cfu/ml of density were inoculated to 9mL of sterile distilled water to achieve 10^{7}cfu/ml. The diluting was continued to achieve 10^{6}, 10^{3}cfu/ml. Then, the strain was prepared for inoculation (Ashrafi, 2006). Inoculation consists of 2mL of suspension of *Saccharomyces cerevisiae* with concentrations 10^{6}, 10^{3} cfu/ml to 18ml lemonade with concentrations (2.5, 1.5, 1, 0.5, 0.2) essence of Mentapiperita under sterile conditions in Laminar Hood (Maghsudi et al., 2006).

This culture media was made on Merck commercial culture media as 43.1gr of culture media were poured into 1L of sterile distilled water and were sterilized in autoclave at temperature 120℃ for 15min. The sterilized culture media after being cooled to 60-70℃ in Hood was divided in sterile petri dishes and were dried. The prepared syrups with various concentrations of essence and dilutions of yeast strain by sampler, of each of dilutions, 100mL were taken and surface culture was performed on YGC agar. These petri dishes were incubated in incubator 1±25℃ for 3-5 days.

After a while, the number of colonies in petri dish were counted and reported (Binam, 1992). It is worth to mention that each test was performed in three replications and the mean of data was reported.

Chemical tests of study

Measurement of chemical factors was performed as Brix, PH, acidity after production, 7 hours, 7 , 14, 21, 28 days of all treatments and control samples at temperature 40°C (Binam, 2003).

Sensory test

According to 5-point Hedonic test (5=very good, 4=Good, 3=Average, 2= Bad, 1=Very bad), 10 skillful and trained analyzers were used and 2 features of aroma and flavor and finally total acceptance were evaluated (Sharifan, 2006).

Statistical analysis

For data analysis, fully randomized factorial design was applied. The concentration of lemonade in one level (20%), *Mentapiperita* essence concentration in 5 levels (0.2, 0.5, 1, 1.5, 2)% and *Saccharomyces cerevisiae* yeast concentration in two levels cfu/ml(10^6, 10^3) were used. This test had 11 treatments (10 species with 1 control sample) and all tests were performed with 3 replications. Duncan multiple range test was used to determine the difference between means at confidence interval 5%. SAS software was used for statistical analysis. All results were defined as mean of three replications± Standard deviation.

RESULTS AND DISCUSSION

The investigation of microbial test results

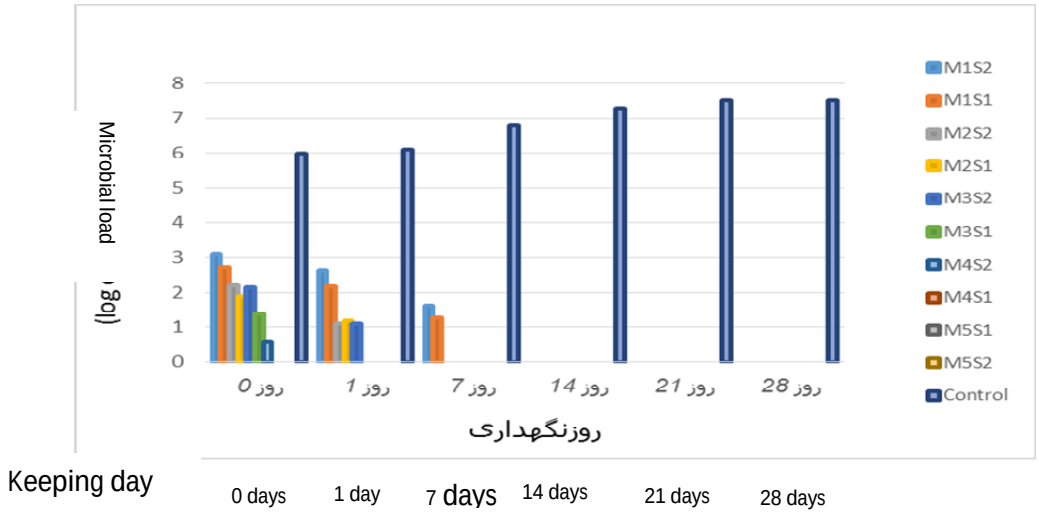

Chart 1- The effect of different concentrations of *Mentaxpiperita* essence on growth of *Saccharomyces cerevisiae* yeast in lemonade

As shown in chart 1, the investigation of the results of inhibition activity of *Mentapiperita* essence of growth of *Saccharomyces cerevisiae* yeast among treatments at zero days (after production), 7 hours after production, 7, 14, 21, 28 showed that by increase of concentration of essence, inhibition of growth of yeast is increased. Based on the results of treatment concentration with *Mentapiperita* essence and essence keeping time in lemonade, it had significant effect on *Saccharomyces cerevisiae* yeast in lemonade species ($P<0.05$). The logarithm of number of growth of these bacteria in cfu/ml is calculated. Based on the results, *Mentapiperita* essence in all concentrations had significant effect on growth of *Saccharomyces cerevisiae* yeast as after 14 days, there was no growth in any of them.

Mohammadi*et al.*, (2012) achieved some results as *Mentapiperita* essence stopped Botrytis cinerea mold at concentration 800ppm in strawberry in less than 7 days and they are consistent with the present study. Similar these results were reported in the study of Nouri*et al.*, (2009) regarding the effect of increase of essence concentration as inhibition effect of cinnamon essence and temperature of keeping on growth of Escherichia coli in hamburger was affected by different concentrations of

Cinnamon essence (0, 0.005, 0.15, 0.03)% at temperature 8, 25℃ during 21 days. The results showed that by increasing essence concentration, bacteria growth was reduced under similar temperature conditions as the highest inhibition of Escherichia coli under similar temperature conditions was dedicated to Hamburger 0.03% Cinnamon essence. Similar to this result was observed in the study of Alvandi*et al.*, (2011) as the essence in the first week of keeping stopped microorganism growth considerably. Also, the chemical composition and anti-microbial effect of *Mentapiperita* essence was evaluated and it was shown that due to the presence of menthol in essence of plant in relatively low concentrations of growth, it was effective on food decay bacteria. This essence was used in food as a natural anti-microbial compound instead of artificial preservative and it was effective. Ghalejughi*et al*., (2014) in the study of Histopathological evaluation of the effect of *Menthapiperita* essential oil on cutaneous wound healing in rats infected with *C. albicans*found that *Menthapiperita* essential oil topically, significantly reduced inflammation and migration of leukocytes, and also significantly increased in vascular regeneration, epithelialization and migration of fibroblasts, compared control group. This is consistent with the results of this study as significance of elimination of yeast compared to control group. Regarding Pharmacological investigation of methanol extract of *Menthapiperita*L. , Mathur*et al.*, 2011 showed that it had good anti-fungal activity against fungus strains of *Candida albicans*, *Saccharomyces cerevisiae* and Penicillium *notatum* and it is consistent with the results of this study regarding the effectiveness of on yeast species. Saeeidi*et al*., (2013) evaluated the comparison of the effect of three types of essential oil of *Mentapiperita*, dill and lemon on production and durability of flavored drinking water. It was shown that concentration of treatment with *Mentapiperita* and essence keeping time in drinking water had significant effect on enterococcus, E. coli in drinking water (P>0.01) and it was consistent with the results of this study. Cheraghi*et al*., (2013) evaluated the effect of inhibition of cumin essence on molding of wheatbaguette and they showed that by the increase of essence concentration, fungus was reduced and it is consistent with the following study.

- **The investigation of results of sensory test**

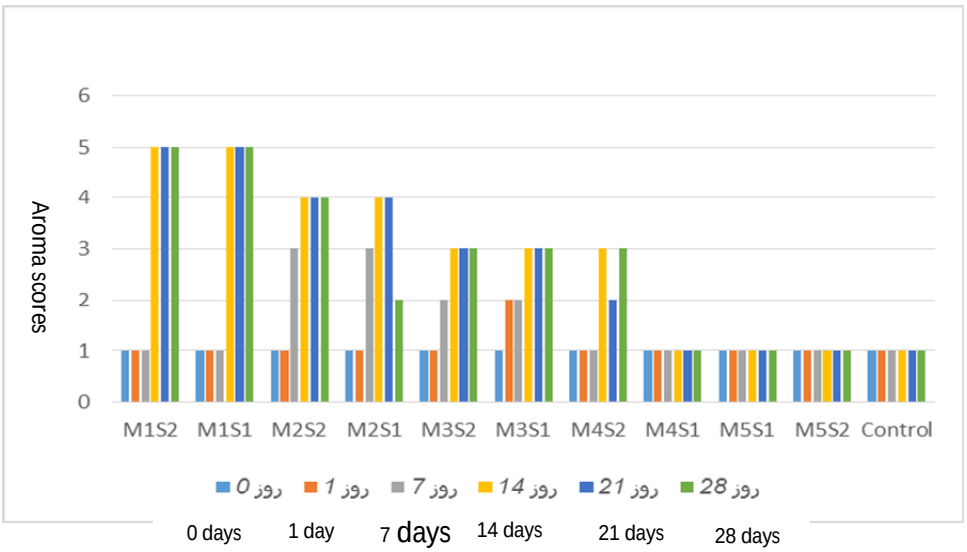

Chart 2- The aroma scores in various concentrations of essence in lemonade

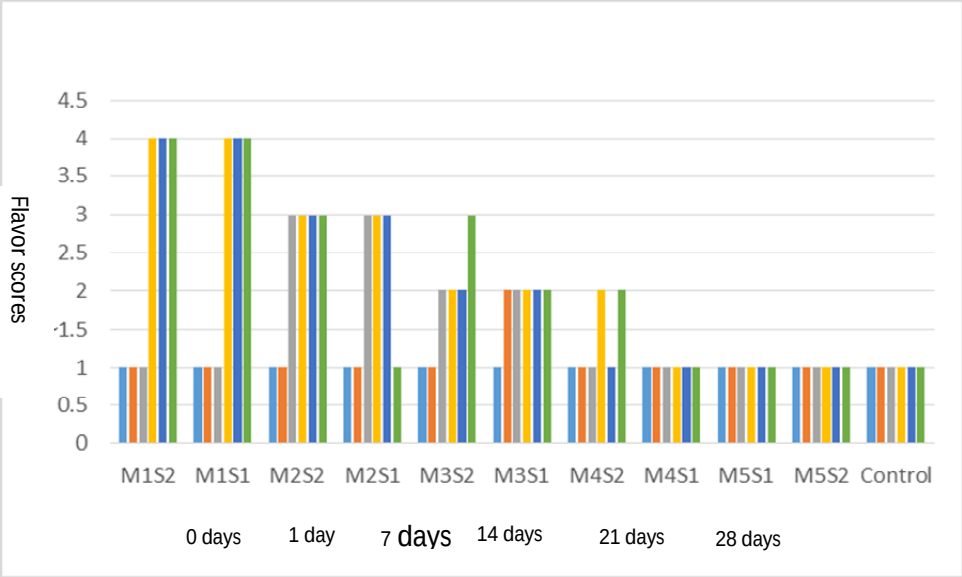

Chart 3- Flavor scores in different concentrations of essence in lemonade

Among the samples with *Mentapiperita* essence, lemonade samples with 0.2% essence were the best samples in terms of flavor, aroma and total acceptance and they had the highest mean of scores. Despite the increase of inhibition of yeast growth in treatments with concentration above 1.5, 2% of*Mentapiperita* and essence had the lowest scores and they had no good organoleptic properties.

As majority of plant essences are recognized as GRAS list, using them as food preservative has some limitations in terms of flavor and aroma (Dusan*et al.*, 2006). This is consistent with the results of present study as with the increase of inhibition of yeast growth at high concentration (1.5, 2)%, their suitability is reduced in terms of aroma and flavor and they achieve less scores compared to other treatments.

-General conclusion

The results of microbial tests in lemonade showed that by increase of essence concentration, inhibition of yeast growth was increased and the results showed thegrowth of yeast in lemonade treatments after production, 7 hours, 7, 14, 21, 28 days. The microbial test results of lemonade showed that essential oil in concentrations 0.2, 0.5, 1, 1.5, 2% could stop *Aspergillusflavus* growth and concentration 2% showed the highest inhibition as after production, no yeast growth was performed in this concentration.

According to the results of sensory test of lemonade, at high concentrations of essence, inhibition of *Saccharomyces cerevisiae* growth was increased. The aroma, flavor scores in Lemonade was reduced. The highest and lowest scores were regarding the treatment of 0.2, 1.5, 2% essence.

Based on the results of microbial test and sensory test, the treatment with 0.2% essence was selected as the best treatment. After 2 weeks, inoculated microbial population was eliminated and it was accepted from sensory aspects. According to the sensory test results of lemonade, at high concentration of essence with the increase of inhibition of Saccharomyces cerevisiae growth, aroma and flavor scores were reduced in lemonade.

REFERENCES

1- Ashrafi, F ., 2003. Practical microbiology. Ahsan publications. First edition.
2- Binam. 1993. The standard and industrial research institute of Iran. National standard of Iran. NO. 3196. Food microbiology and livestock food. Osmophilic yeasts of counting method.
3- Binam, 2003. The standard and industrial research institute of Iran, National standard of Iran, No. 2736. Juice, lemonade, features and methods of test.
4- Cheraghi, M., 2013. The comparison of the effect of three types of essential oil of with Mentaxpiperita, dill and lemon in production and durability of flavored drinking water. MA thesis of agriculture, food industry and sciences of Islamic Azad

University, VaraminPishva branch. Agriculture. 106 pages.
5- Saeedi, A., 2013. The effect of essential oil and cumin extract of Iranian plant as an anti-microbial compound in Mionese. MA thesis of agriculture. Food industry and sciences. Islamic Azad University, VaraminPishva branch. Agriculture. 167 pages.
6- Sharifan, A., 2006. The effect of essence of Thymus, thin Thymus and ShiraziThymus onStaphylococcus aureus. Phd thesis of agriculture, Food industry and sciences. Islamic Azad University, Sciences and Research branch. Agricutlure. 78 pages.
7- FarhangiGhaleJughi, N., Farahpour, M., NeyrizNaghde, M., 2015. Histopathological evaluation of the effect of Menthapiperita essential oil on cutaneous wound healing in rats infected with C. albicans. Comparative, scientific and research pathobiology. Year 11. Winter. NO. 4, 2015. 1453-1462.
8- KazemAlvandi, R., Sharifian, A., Aghazade, Meshgi, M., 2010. The investigation of the chemical compound and anti-microbial effect of Menthapiperita essence, Comparative, scientific and research pathobiology. Year 7. Winter. NO. 4. P. 355-364.
9- Maghsudi, Qobadinejad, R., Soltanzade, M., 2006. Using essential oils in keeping orange juice. 16th congress of food industry of Iran.
10- Nuri, N., F. Tuyan, N., Rokni, A., Akhundzade, A., MIsaghi, 2009. The investigation of the preservative effect of cinnamon essence and temperature of keeping on growth of E. coli O 157: H7 in hamburger by combinational technology. Journal of science and food industry. 4: 35-42.
11- Alpsoy,L.2010.Inhibitory effect of essential oil on aflatoxins. African Journal of Biotechnology9(17):2774-2481
12- Brown,A.E.(2009):Benson,s Microbiological Aplication:LaboratoryManualin general Microbiology , short Version:McGrow Hill
13- Burt, S. 2004. Essential oils their antibacterial properties and potential application in foods- areview. International Journal of Food Microbiology, 94(3): 223-253
14- Dusan, F., Marian, D. Katarine and B. Dobroslava. 2006. Essential oils _ their antimicrobial activity against Eschrichial coli and effect on intestinal cell viability. toxicology in vitro, 29: 1435_1445.
15- Mathur,A.,purohit,R.,Mathur,D.,Prasad,G., .(2011):pharmacological investigation of methanol extract of mentapiperita L. roots on the basis of antimicrobial, antioxidant and anti–inflammatory properties Der .pharmacua. sinica 2(1) : 470-477.
16- Mohammadi ,S ., Aroiee ,H. , Aminifard. M. H., Jahanbakhsh.
17- V.,(2012), In vitro and in vivo antifungal activates of the essential oils of various plants against strawberry grey mould disease agent Botrytis cinerea, Archives Of Phytopathology And Plant Protection, 45, 20, 2474–2484.
18- Naczk,M., and shahidi,F.,2004.Extraction and analysis of phenolics in food.Journal of Chromatography,1054,95-111
19- Omidbeygi, M., M. Barzegar, Z Hamidi, H. Naghdibadi.2007. Antifungal activity of thyme, summer savory, clove essential oils against Aspergillusflavus in liquid medium and tomato paste.Food Control 18:1518-1523.
20- Rasooli , I and Owlio, P.2005.Chmoprevention by thyme oils of Aspergillusparasiticus growth and aflatoxinproduction.phytochemistry 66:2851-2856
21- Razzaghi-Abyaneh, M., Shams. Jaimand, K., Alinezhad, S.,Saberi,Chemica composition and antiaflatoxigenic activity of carumcarviL.,Thymus vulgaris and citrus aurantifolia essential oils. Journal of Food control,20:1018-1024
22- Tepe, B., H. A. Akpulat. D. Daferea and M. Polissiou. 2005. J. Food Enginneering, 14: 66-447.