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Anti-Yeast Effects of Some Commercial Essential Oils against Food-Related Yeasts

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Abstract: *In recent years, aromatic plants and their extracts have been examined to prove their effectiveness for food safety and preservation applications. In this study, aimed to evaluate the effectiveness of laurel, clove, oregano, cinnamon, marigold, pepper mint, mustard, coriander, and tea tree oil to inhibit the growth of various food-related yeasts (Candida zeyland, Candida sake, Candida keyfr, Candida lambica and Sacchoromyces cerevisiae).The essential oils were analyzed by GC-MS to determine the chemical compositions. Major compounds found in essential oils of laurel, clove, oregano, cinnamon, marigold, pepper mint, mustard, coriander, and tea tree were Eucalyptol (54.73%), Eugenol (96.16%), Carvacrol (79.8%), trans-Cinnamaldehyde (39.48%), 1,8-Cineole (24,87%), Menthol (22.45%), Allyl isothiocyanate (42.46%), Linalool (61,26%) and Terpinen-4-ol (40,75%), respectively. Anti-yeast activity was studied by using the agar disk diffusion and minimum inhibitory concentration (MIC) against tested yeast. The oil samples exhibited concentration dependent inhibition of the growth.Cinnamon, laurel, oregano, marigold and clove essential oils showed effectiveness to inhibit the growth of all studied yeast samples based on the disc diffusion and MIC values. However, the essential oils differ significantly in their activity on the tested microorganisms, cinnamon was the most effective against all of them and oregano was the second effective one according to MIC and disc diffusion. Laurel, marigold, clove had indicated similar inhibitory effect however coriander and tea tree had the least inhibition and they effected only C. zeyland and C. sake. The least effective essential oil for C. lambica, C. kefyr and S. cerevisiae was laurel (500 µg/mL). The mint oil revealed the only anti-yeast activity against C. lambica, C. zeyland and S. cerevisiae according to disk diffusion method. Mint and mustard had similar inhibition activity against S. cerevisiae but tea tree oil and coriander had no effect on it.*

Keywords:*Essential oils, MIC, disk diffusion assay, food-related yeasts, GC-MS*

I. Introduction

Food-related yeasts can grow at acidic pH values in foodstuffs with high carbohydrate contents (1) and are widely distributed in nature and cause the deterioration of a wide range of chilled and ambient stable edible substances such as wines, bakery products, jams and preserves, fruit products, vinegar, juices, beverages, salads, meat (2). They can also be found in dairy products such as yoghurt, kefir, and soft and fresh cheeses (3; 4). *Candida*, *Saccharomyces*, *Zygossacharomyces, Pichia*, *TrichosporonRhodotorula*, *Hansenula*and *Torulopsis*,are some important food spoiling yeasts (5; 6).

Contamination of foods by spoilage yeasts frequently leads to a decreased food product shelf-life due to gas production and sometimes undesirable off odors or flavors. In the recent times, food spoilage by yeasts is a prime issue in the food industries which significantly effects the cost and availability of the food (5).

Although heat treatment is one of the most effective methods for microbial control in foods, it may cause undesirable changes to sensitive food products. Therefore, products that cannot be pasteurized are usually treated with weak acid preservatives, such as sorbic or benzoic acid or their salts. However, some yeasts possess genetic or acquired resistance mechanisms to weak organic acids, including the ability to degrade them or to pump out dissociated anions (7; 8). Moreover, exposure to sublethal concentrations of organic acids may lead to subsequent resistance development (9). Therefore, new natural anti-yeast compounds are being promoted for enhancing the shelf life of foods and for avoiding infections (10).

Nowadays, there is a strong consumer demand to avoid or diminish the use of artificial preservatives.Consumers have demanded more natural foods, with low levels of chemical additives and less processed, however still possessing a long shelf-life.

In this panorama, plant-derived natural antimicrobials such as essential oils (EOs.) have emerged as effective compounds to provide microbiological safety of foods.

Essential oils (also called volatile oils) are natural volatile complex compounds that are characterized by a strong smell and are formed as secondary metabolites in edible, medicinal and herbal plants (11). Aromatic plants are rich in essential oils, which are composed of many compounds (eugenol, citral, pinene, thymol, cinnamic acid, carvacrol) characterized by a prominent antimicrobial activity (12; 13).

Plant extracts and plant essential oils (EOs), which have generally recognized as safe status (GRAS) in the world, have been repeatedly shown to exhibit antimicrobial activity against both foodborne pathogenic and

spoilage microorganisms (7; 14; 15; 11). Thus, there is potential for EOs to be used as natural antimicrobial alternatives to control spoilage yeasts in foods.

In this study, we have aimed to investigate the anti-yeast activity of 9 commercially available essential oils extracted from a wide range of plants including laurel, clove, oregano, cinnamon, marigold, mint, mustard, coriander, and tea tree oil against some yeast strains. The plants used in this study were selected on the basis of traditional and widespread culinary or domestic use.

II. Material and Methods

1.1. Essential oils

The EOs investigated in this study were from laurel (*Lauri expressum*), clove (*Eugenia caryaphyllata*), oregano (*Origanum vulgare*), cinnamon (*Cinnamomumzeylanicum*), marigold (*Calendula officinalis*), pepper mint (*Menthapiperita*), mustard (*Brassica nigra*), coriander (*Coriandrumsativum*), and tea tree (*Melaleucaalternifolia*). The essential oils were provided from SNS Gıda Kozmetik San. ve Tic. Ltd. Şti.Tekirdag, Turkey in (1/10) diluted form.stored in an air-tight sealed glass bottle at 4^{o}C till further use.

1.2. Yeast Strains

Different yeast strains (*Saccharomyces cerevisiae*, *Candida zeyland, C. sake C. kefyr*and *C. lambica*) were obtained from the strain collection of the Department of Food Engineering, Namık Kemal University, Tekirdag-Turkey and used to evaluate the effect of essential oil.

Stock cultures were maintained on Sabouraud-2% Dextrose Agar (DIFCO) slants at 4°C. Inocula were obtained from overnight cultures on Sabouraud-2% Dextrose Agar slants at 28–30°C and diluted in sterile PBS to a final concentration of 10^{6}cfu/mL (adjusted according to the turbidity of 0.5 McFarland scale tube).

1.3. GC–MS analyses of Essential oils

The essential oils of laurel, clove, oregano, cinnamon, marigold, pepper mint, mustard, coriander, and tea tree oil were analyzed with a gas chromatography–mass spectrotrometry (GC/MS). The analysis was carried out using a Shimadzu GCMS -QP2010-Ultra Model equipped with a column (5-Ms 30m 0,25mmX 0,25um) coupled to mass detector (Shimadzu, Kyoto, Japan).

Helium was used as carrier gas at a (1.0 ml/min). The column temperature was maintained at 60 °C for 5 min, then programmed to increase (4°C/min) until 260 °C and held for 10 min; and then to 320 °C (15°C/min) and held for 20 min with a 10:1 split ratio mode, and an injector temperature of 220 °C. The ionization energy was 70 eV, and the scanning mass range was 35–500 amu.

Identification of oil components was achieved based on their retention indices, and by comparison of their mass spectral fragmentation patterns with those reported on the NIST27/NIST107/NIST147/WILEY7 mass spectral library. The concentration of the identified compound was computed based on the percentage of the relative peak area (%).

1.4. Antimicrobial activity

1.4.1. Disc diffusion assay

The agar disc diffusion method was employed for the determination of antimicrobial activities of the essential oils (16). Briefly, a suspension of the tested microorganism (100 µl of 1 x 106 cfu/ml) was spread on the Sabouraud-2% Dextrose Agar media plates. Filter paper discs, 6 mm in diameter (Schleicher &Schuell, Germany) were soaked with 10 or 20 µl of the oil or dimethyl sulfoxide (DMSO; negative control) and placed on the inoculated plates and after storing at 4oC for 2 h, were incubated at 28oC for 48 h. Volume of essential oils tested was varied (10 or 20 µl) by using appropriate number of sterile discs. The diameters of the inhibition zones were measured in millimeters. All tests were performed in duplicate. The antibiotic natamycin(10 µg/disc) was used as a positive control.

1.4.2. Determination of minimum inhibitory concentration (MIC)

Yeast strains sensitive to the plant oils in disc diffusion assay were studied for their minimal inhibition concentration (MIC) values using the micro-well dilution assay method (17). MICs were defined as the lowest concentrations of the antimicrobial agents that inhibited visible growth of the microorganism.

The inoculated microbial strains were prepared from 24h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oils, dissolved in 10% DMSO, were first diluted to the highest concentration (500 µg/ml) for testing, and then serial twofold dilutions were made between the concentrations of 7.8 and 500 µg/ml in 10 ml sterile test tubes containing Sabouraud-2% Dextrose Broth.

The 96-well plates were prepared by dispensing 95 µl of the cultures medium and 5 µl of the yeast inoculum into each well. A 100 µl aliquot from the stock solutions of essential oils initially prepared at the concentration of 500 µg/ml was added into the first wells. Then, 100 µl of their serial dilutions were transferred

into seven consecutive wells. The last well, containing 195 µl Sabouraud-2% Dextrose Broth without any essential oil and 5 µl of the inoculum on watch strip, was used as the negative control. The final volume in each well was 200 µl. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated for 48 h at 30°C.

Microbial growth was determined by the presence of a white pellet in the bottom of the well and confirmed by plating 5 µl samples from clear wells Sabouraud-2% Dextrose Broth. The MIC value was defined as the lowest concentration of the essential oil required for inhibiting the growth of each microorganism. All tests were repeated two times.

III. Result and Discussion

3.1 Major Compounds of essential oils

The chemical compositions of the essential oils used in this study were analyzed by GC-MS analysis. The relative amount of components found in essential oils varied according to plant species and the particular compounds. The major compounds found in essential oils were shown in Table 1.

Table1: Chemical composition of the essential oil from laurel, clove, oregano, cinnamon, marigold, mint, mustard, coriander, tea tree analyzed by GC-MS

Essential oil	Major Compounds identified (%)*
Laurel	Eucalyptol (54.73%), α-terpineyl acetate (15.18%), Sabinene (4.86%), α-pinene (2.21%)
Clove	Eugenol (96.16%), trans (β)-caryophyllene (3.19 %),
Oregano	Carvacrol (79.8%), p-cymene (4.39%), thymol (4.29%)
Cinnamon	trans-Cinnamaldehyde (39.48%), cinnamic acid (30.99%), isobutylcinnammate (4.82%) trans-Cinnamic acid (4.28%)
Marigold	1,8-Cineole(24,87%), α- Thujene(17.45%), T-muurolol (12.57%)
Mint	Menthol (22.45%), Menthone (18.98%), 1,8-Cineole (4.06%), pulegone (2.21%)
Mustard	Allyl isothiocyanate (42.46%), Diallyltrisulfide (7.80%), 3-Butenyl isothiocyanate (4.67%)
Coriander	Linalool (61,26%), α-Pinene (7,88%) γ-Terpinene (4,21%) Camphor (4,16%)
Tea Tree	Terpinen-4-ol (40,75%), γ-Terpinene (14,88%) α-Terpinene (8,15%) p-Cymene (5.24%)

* Components showing a peak area of more than 2% relative to the total peak area on gas chromatography (GC) are listed

GC-MS analyses revealed that laurel oil was dominated by eucalyptol (54.73%) while the clove essential oil was found to be rich in eugenol (96.16%). Unlike our results, Olmedo et al. found 1,8-cineole (42.1%) as the main component of laurel (18). On the other hand, major compounds of the oregano, cinnamon, marigold, mint, mustard, coriander and tea tree were carvacrol, trans-Cinnamaldehyde, 1,8-Cineole, menthol, allyl isothiocyanate, linalool and Terpinen-4-ol, respectively.

The oil of marigold contained a high percentage of 1,8 cineole (24.87%), while mint oil had only 4.06 % of 1,8 cineole. In this study oregano had only 4.29% of thymol but 79.8% of carvacrol. Other studies have shown different percentages of these compounds in oregano EO. Kulisic et al. reported 35.0 % thymol, 32.0 % carvacrol and 10.5 % γ-terpineno(19) . Tomaino et al. found 48.9 % carvacrol, 11.7% p-cimeno and 5.0 % thymol(20). Baydar et al. found 86.9 % carvacrol, 2.9% p-cymene and 0.2% thymol(21). Mazzarino et al. also reported similar results with our findings as 68.1% carvacrol, 5.9% p-cymene and 3.7% thymol (22).

Cinnamon EO contained 39.5% trans-Cinnamaldehyde and also 30.1% of cinnamic acid. However, Tomaino et al. and Mazzarino et al. found higher percentages of main component trans-Cinnnamaldehyde than our result in Cinnamon; as 67.9% and 76.2%, respectively (20,22).

The most abundant component of the clove EO was Eugenol (96.16%). Similarly, Teixeira et al. reported 67.9% Eugenol, 10.8 % trans (β)-caryophyllene and 16.8 % Acetetgenol(23). In addition, Tomoaino et al. found 82.6% Eugenol, 7.45% trans (β)-caryophyllene and 8.03% Acetyl-eugenol (20). Unlike those studies, acetyl-eugenol was not determined as a major component of clove EO in our study. Mazzarino et al. also reported 77.8% Eugenol, 12.2% trans (β)-caryophyllene and 0.09% Caryophyllene oxide in clove EO (22).

Despite, in the previous studies on mint, limonene, isomethane, menthol, 1.8-cineole, pulegone, menthone, menthol and 1,8-cineole (eucalyptol) were similar components with varied concentrations (24,25), our study indicated that menthol (22.45%), menthone (18.98%) were the major compound in the mint EO.

Mustard essential oil, which primarily contains the active compound allyl isothiocyanate (42.46%). Allyl isothiocyanate exists in a precursor form, which by cell destruction is enzymatically hydrolysed to release the active form. Likewise, our results, Peng et al. noted that Allyl isothiocyanate is the major compound in mustard with 71.06% (26).

Another tested essential oil was coriander. Although it was found that coriander had Linalool (61,26%) in our study, it was noted that Δ3-carene was the major compound with 60.5% in coriander and the other components were γ—Terpinene (18.2%), camphor (6.5%) and α-Pinene (1.1 %) (23).

Terpinen-4-ol (40,75%) was the most abundant component in tea tree essential oil which was similar to the results of other studies. Terpinen-4-ol was found to comprise 53.7 % of the essential oil from the Brazilian

species to about 35 % in Australian, Indian and Taiwanase species. γ—Terpinene and α-Terpinene were the other major components and ranged between 14.0 -23.0% and 5.8-12.0%, respectively (27,28,29,30,22).

Sekiyama et al. compared the effect of mustard extract (90% AITC) diluted in agar with the effect obtained exposing the extract to bacteria and fungi through the vapour phase. They concluded that the AITC were most effective exposed as a vapour directly to the microorganisms (31).

3.2 Anti-yeast activity of essential oils

Anti-yeast activity of 9 different EOs against 5 food-related yeast were evaluated and their potentials were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and MIC values. The anti-yeast activities of the EOs were compared with a standard antibiotic such as natamycin, which was used as a positive control.

3.2.1 Disc diffusion assay

Antimicrobial potentials of the EOs were observed in terms of zone of inhibition generated by the diffusion of the EOs into the yeast strains inoculated agar plate. The zone of inhibition resulting from the diffusion of EOs is shown in Table 2.

Table 2. Anti-yeast activity of essential oils using disc diffusion assay (inhibition zone diameter in mm)

Essential oils	**Conc. µl/disk**	**Microorganisms**				
		C. zeyland	***C. sake***	***C. kefyr***	***C. lambica***	***S.cerevisiae***
Laurel	10	8	10	<6	8	8
	20	15	17	8	11	13
Clove	10	8	10	7	10	8
	20	14	16	16	14	12
Oregano	10	9	8	9	10	14
	20	26	14	22	20	30
Cinnamon	10	26	12	36	28	28
	20	36	36	44	38	38
Marigold	10	9	10	8	7	10
	20	14	15	12	10	15
Pepper Mint	10	8	<6	<6	9	12
	20	12	<6	<6	14	16
Mustard	10	9	<6	<6	<6	8
	20	13	<6	<6	<6	12
Coriander	10	7	<6	<6	<6	<6
	20	11	<6	<6	<6	<6
Tea Tree	10	7	<6	<6	<6	<6
	20	10	<6	<6	<6	<6
Natamycin		26	18	26	17	15

Conner and Beuchat (32) classified the inhibitory effects of essential oils according to their activity as strongly active (inhibition zone, > 12 mm), moderately active (inhibition zone, < 6 to < 12 mm), or inactive (inhibition zone, < 6 mm). In this study the zone of inhibition increased with the increasing concentrations of EOs. The most effective EO was cinnamon which, showed highest inhibition zones with a concentration of 10 µl such as *C.kefyr*(36mm) >*C. lambica*(28mm) = *S.cerevisiae*(28 mm) >*C. zeyland*(26 mm) >*C. sake*(12 mm) and of 20 µl*C.kefyr*(44mm) >*C. lamb*(38mm) = *S.cerevisiae*(38 mm) >*C. zeyland*(36 mm) = *C. sake*(36 mm) (Table 2.). However, mustard, coriander and tea tree EOs were the least effective against tested yeast strains except that *C. zeyland* that was inhibited with all three of them. Laurel, clove and marigold EOs had similar inhibition effect on tested microorganisms except that *C. keyfr* was slightly more resistant to laurel essential oil than others. Among yeast species, *S. cerevisiae*was the most sensitive microorganism against all EOs tested. In the study by Sachetti et al. (33) in which the antioxidant and antimicrobial activities of 11 EOs were evaluated, *S. pombe* and *S. cerevisiae* likewise proved to be the most sensitive strains.

When the concentration of EOs were 20µl, cinnamon and oregano were the most effective among all tested EOs. 20 µl of oregano essential oil caused significant inhibition with diameters *S.cerevisiae*(30mm) > *C. zeyland*(26 mm)>*C.kefyr*(22 mm) >*C. lambica*(20 mm) >*C. sake*(14 mm). Similarly, Souza et al. (2) also determined the effect of *Origanum vulgare L.* essential oil solution against food spoiling yeasts (*Candida albicans* ATCC 7645, *Candida krusei* ATCC 6258, *Candida tropicalis* MD 37, and *Saccharomyces cerevisiae* ATCC 2601) by solid medium diffusion and noticed the inhibition zones with increasing concentration of *O. vulgare* essential oil. Another study searching the anti-yeast activity of oregano, clove and cinnamon on *C. albicans* and *S. cerevisiae* and indicated their activity at 20% level with disk diffusion assay (34).The pepper mint oil indicated moderate activity against *C.lambica, C. zeyland* and *S. cerevisiae* and no activity against *C. sake* and *C. kefyr* based on inhibition zones. Unlike this study, Mkaddem et al. (35) has revealed that *Menthalongifolia*and*Menthaviridis* essential oil caused inhibition zone against yeast *C. albicans* (19 to 21 mm) and *S. cerevisiae* (25 to 28 mm).

Pepper mint and mustard had similar inhibition activity against *S. cerevisiae* on the other hand tea tree and coriander had no effect on it in this research. Although Hammer et al. (36) showed that tea tree oil induced inhibition of *C albicans* and *S. ceverisiae* at concentration of 0.5 and 0.25 (%v/v) respectively, and Silva et al. (37) exhibited that coriander essential oil inhibited *C. albicans* ATCC 24433 with the highest MIC (0.2%), tea tree oil and coriander did not show any activity except that *C. zeyland*(7-11 mm). Even though tea tree did not show any effect on tested yeast in this study, there is a research about tea tree oil that has antimicrobial activity against human and animal fungal pathogens (38).

3.2.2 Minimum inhibitory concentration method

The MIC values are shown in the Table 3. MIC values are parallel with the disc diffusion assay. Since pepper mint, coriander, mustard and tea tree did not indicate considerable effect with the disk diffusion assay, they were not tested in MIC method. The EOs used in this study showed strong anti-yeast activity as reflected by the low MIC obtained. The MICs determined for all the strains tested in this study oscillated between 7.8 and 500 µg/mL. Among the EOs tested, cinnamon oil was very effective in inhibiting the growth of all yeast tested, as shown by the low MIC (7.8 µl/ml) except *C. zeyland*requiring 15.6 µg/mL, which agrees with high inhibition zone in the disk diffusion assay. The antifungal properties of cinnamon oil are due to volatile components such as eugenol and cinnamaldehyde (39). These volatile phenolic compounds are able to damage the fungal cells because of their inhibition the fungal cell wall synthesizing enzymes (β-(1,3)-glucan and chitin synthases) by cinnamaldehyde (40). Also Melgarejo-Flores et al. (41) offered the cinnamon treatment to table grapes as an alternative to decrease fungal spoilage. The second effective EO against tested yeasts according to MIC results was oregano which displayed also relatively low MIC values, ranging 62.5-125 µg/mL.This results are similar with many studies evaluating antifungal effects of oregano. Khosravi et al. (42) evaluated the EO of oregano exhibited the broad spectrum of antifungal activity against *Candida* isolates with mean inhibition zone of 27.1 mm.

Table 3.MIC of essential oils for different yeast strains (µg/mL).

Essential oils	**Name of the strain**				
	C. zeyland	***C. sake***	***C. kefyr***	***C. lambica***	***S.cerevisiae***
Laurel	125	125	500	500	500
Cinnamon	15.6	7.8	7.8	7.8	7.8
Oregano	62.5	125	62.5	125	62.5
Marigold	125	125	250	250	125
Clove	250	125	125	250	125

The lowest MIC for *C. sake, C. kefyr, C. lamb, S. cerevisiae* was 7.8 µg/mL of cinnamon, whereas the least effective essential oils for *C. lambica, C. kefyr*and *S. cerevisiae*was laurel (500 µg/mL). Laurel, clove and marigold EOs showed the moderate to weak activity as their MIC values ranging between 500 and 125 µg/mL.

IV. Conclusion

According to obtained results, essential oils of cinnamon and oregano represent a good basis for the formulation of products with potential efficacy in the control of yeast. The results indicate that all tested yeast were relatively susceptible to all five oils. Additionally, using smaller amount of oils, when considering the application in large scales, could have considerable economic effects.Essential oils derived from aromatic plants are well-known in traditional medicine as antimicrobial agents and may be characterized as food preservatives because of antifungal properties based on this study.

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