Anti-yeast activity of mentha oil and vapours through in vitro and in vivo (real fruit juices) assays

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ABSTRACT

The anti-yeast activity of mentha oil and vapours was evaluated against 8 food spoiling yeasts through disc diffusion, disc volatilisation and micro broth dilution method. The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) varied from 0.28 to 2.25 and 1.13 to 4.5 mg/ml, respectively. Furthermore, the anti-yeast efficacy of mentha oil alone and in combination with thermal treatment was evaluated in a real food system i.e. mixed fruit juices. The samples treated with a combination of mentha oil at the MIC, ½ MIC and ¼ MIC levels and thermal treatment enhanced the reduction viability. Chemical characterisation of mentha oil by gas chromatography-mass spectrometry (GC–MS) revealed that the dominant compounds were cis-menthone (27.43%), menthol (24.3%), trans-menthone (9.23%), limonene (3.84%), menthofuran (4.44%) and isomenthol (3.21%). Present results established the superior performance of integrated treatment over individual exposure for fruit juice preservation.

1. Introduction

Food spoilage by the yeast is substantially responsible for microbiological spoilage of a wide range of chilled and ambient stable edible substances such as wines, milk, cheese, bakery goods, jams and preserves, fruit products, vinegar, beverages, juices, salads and meat (Souza, Stamford, Lima, & Trajano, 2007). Nowadays, food spoilage by yeasts is a prime issue in the food industries, which significantly influences the cost and availability of the food (Belletti et al., 2007).

The use of synthetic antimicrobial agents and chemical food preservatives is one of the oldest techniques for controlling food spoilage. However, due to growing evidence about the harmful effects of chemicals, there is a continuous pressure to reduce the amount of synthetic preservatives (Burt, 2004) and provide minimally processed food products, without compromising food safety. Therefore, alternative sources of safe, effective and acceptable natural preservatives need to be explored, such as essential oils.

Mentha piperita (family Lamiaceae) is well known for its medicinal and aromatic value. Mentha spp. from different parts of the world have been previously investigated for their essential oil compositions and biochemical activities (Iscan, Kirimer, Kurkcuoglu, Baser, & Demirci, 2002; Mahboubi & Haghetti, 2008). The antimicrobial efficacy of mentha oils has been found to vary from moderate to significant, and often correlates with the composition of the oil (Iscan et al., 2002; Mahboubi & Haghetti, 2008; Mimica-Dukic, Bozin, Sokovic, Mihajlovic, & Matavulj, 2003; Schelz, Molnar, & Hohmann, 2006; Vadeagarinia et al., 2006). Among twelve essential oils, the in vitro anti-yeast activity of mentha oil was found to be strongest (Abdel-Mallek, Bagy, & Hasan, 1994) against five pathogenic yeasts strains (Candida albicans, Candida stellatoidea, Candida tropicalis, Torulopsis candida and Torulopsis versatilis).

However, due to the relatively low antimicrobial activity of essential oils, the recent reports have followed a more rational approach of integrating the essential oils/plant extracts with other treatments (Van Vuuren, Suliman, & Viljoen, 2009). The combination of a mild thermal treatment with the presence of natural compounds can be an important strategy to inhibit or delay microbial growth in many food products, avoiding the problems of organoleptic impact on food/juices. Temperatures above 55 °C increase the vapour pressure of the volatile compounds, enhancing their ability to solubilise in the plasma membrane of yeasts and, in turn, enhancing their bioactivity (Lanciotti et al., 2004). Therefore, combination of essential oils with heat treatment can be explored for developing more effective food preservation techniques.

In the present study, the effect of mentha oil against different food spoiling yeast species was studied through in vitro (disc diffusion method, disc volatilisation method, MIC/MFC) as well in vivo
(preservation of mixed fruit juices) anti-yeast assays. Furthermore, for reducing the quantity of essential oil in the real food system, combined effect of the mentha oil with thermal treatment was also evaluated. The chemical composition of mentha oil has been analysed by GC–MS.

2. Materials and methods

2.1. Chemicals and strains

The essential oil was procured from Erbamea, Italy and stored in an air-tight sealed glass bottle at 4 °C until further use. Growth media and Tween 80 were purchased from Oxoid Ltd. Basingstoke, Hampshire, England and Merck, Schuchardt, Germany, respectively.

Different yeast strains (Saccharomyces cerevisiae SPA, Zygosaccharomyces bailii 45, Aureobasidium pullulans L6F, Candida diversa TSD, Pichia fermentans T2A1, Pichia kluyveri TjA, Pichia anamala, Hansenula polymorpha CBS 4732) were obtained from the strain collection of the Dipartimento di Scienze degli Alimenti of Bologna University, Italy and used to evaluate the effect of essential oil. The yeast strains were grown in Yeast Peptone Dextrose (YPD) medium at 28 °C for 24 h in an orbital shaking incubator (Universal table shaker 709, Milan, Italy) at 120 rpm.

2.2. Gas chromatographic mass spectrometry (GC–MS) analysis of mentha oil

Gas chromatography-mass spectrometry (GC–MS) analyses were carried out on an Agilent 7890 gas chromatograph (Agilent Technologies, Palo Alto, CA) coupled to an Agilent 5975 mass selective detector operating in electron impact mode (ionisation voltage, 70 eV). A CP-Wax 52 CB capillary column (50 m length, 0.32 mm inner dia, 1.2 μm film diameter) was used. The temperature program started from 50 °C, then programmed at 3 °C/min to 240 °C, which was maintained for 1 min. Injector, interface, and ion source temperatures were 250, 250, and 230 °C, respectively. Injections were performed in split mode and helium (1 ml/min) was used as the carrier gas. The mass selective detector was operated in the scan mode between 20 and 400 m/z. Data acquisition started 4 min after injection.

Five millilitres of 100 ppm solution of the mentha oil was placed in 10 ml vials and the vials were sealed by PTFE/silicone septa. One microlitre of the samples were injected directly into the column with a split ratio of 1:100. Component separation was achieved following the method described above. The identification of the molecules was based on comparison of mass spectra of compounds both with those contained in available databases (NIST version 2005) and with those of pure standards (Sigma–Aldrich, Milan, Italy) analysed under the same conditions.

2.3. Anti-yeast activity of mentha oil by liquid and vapour phase

2.3.1. Disc diffusion method

The agar disc diffusion method was employed for the determination of antimicrobial activities of the essential oils (National Committee for Clinical Laboratory Standards (NCCLS), 1997). Briefly, a suspension of the tested microorganism (100 μl of 1 × 10⁶ cfu/ml) was spread on the YPD agar media plates. Filter paper discs, 6 mm in diameter (Schleicher & Schuell, Germany), were soaked with 10 μl of the oil and placed on the inoculated plates. After storing at 4 °C for 2 h, the discs were incubated at 28 °C for 48 h. The volume of essential oils tested was varied (10, 20 or 30 μl) by using appropriate number of sterile discs. The diameters of the inhibition zones were measured in millimetres.

2.3.2. Disc volatilisation method

A standard experimental set-up as described by Lopez, Sanchez, Battle, and Nerian (2005) was used. Briefly, a 100 μl portion of each suspension containing approximately 10⁶ cfu/ml was spread over the surface of YPD agar plate and allowed to dry. A paper disc (diameter 6 mm, Schleicher & Schuell, Germany) was laid on the inside surface of the upper lid and 10 μl mentha oil was placed on each disc. The plate inoculated with microorganisms were immediately inverted on top of the lid and sealed with parafilm to prevent leakage of mentha oil vapour. Plates were incubated at 28 °C for 48 h and the diameter of the resulting inhibition zone in the yeast lawn was measured. The volume of mentha oil tested was varied (10, 20 or 30 μl) by using appropriate number of sterile discs.

2.3.3. Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

Broth microdilution assays were performed as recommended by NCCLS, 1997. All tests were performed in YPD agar supplemented with Tween 80 (final concentration of 0.5% v/v). Yeast strains were cultured overnight at 28 °C in YPD broth. Test strains were suspended in YPD to give a final density of 1 × 10⁶ cfu/ml and these were confirmed by viable counts. Geometric dilutions ranging from 0.036 mg/ml to 72.00 mg/ml of the mentha oil were prepared in a 96-well microtiter plate, including one growth control (YPD broth + Tween 80) and one sterility control (YPD broth + Tween 80 + test oil). Plates were incubated at 30 °C for 48 h. The yeast cell growth was indicated by the presence of a white pellet on the well bottom. The MIC values were determined as the lowest concentration of oil preventing visible growth of microorganisms. MFC was defined as the lowest concentration at which no growth was observed after sub-culturing into fresh media.

2.4. Mixed fruit juice preservation by mentha oil and thermal treatment

2.4.1. Preparation of fruit juice mixture inoculated with S. cerevisiae

Apples (Golden delicious) and oranges at commercial maturity were purchased from a local market (Ipercoop, Cesena). After being washed, apples were cut into about 35 mm slices, then 5 mm slices, then 2.5 mm slices, then 1 mm slices, then 0.5 mm slices, then 0.25 mm slices, then 0.125 mm slices. The suspension of the yeast strain (S. cerevisiae) was mixed with fruit juice mixture to result in final concentration of 10⁵ cfu/ml and the inoculated juice mixtures were transferred in 10 ml sterilised glass vials.

2.4.2. Effect of thermal treatment

The effect of thermal treatment was studied by exposing the mixed juice samples at 80 °C for 30, 60 and 90 s. Subsequently, the treated vials were stored at room temperature up to 8 d and samples were drawn on 0, 2nd, 4th and 8th d.

2.4.3. Effect of mentha oil

One percent ethyl alcohol solution of mentha oil was mixed in 10 ml inoculated fruit juice mixture at MIC level (1.13 mg/ml), ½ MIC level (0.57 mg/ml) and ¼ MIC level (0.30 mg/ml). Fruit juice sample inoculated with S. cerevisiae alone were considered as positive control. Subsequently, the treated vials were stored at room temperature up to 8 d and samples were drawn on 0, 2nd, 4th and 8th d.
2.4.4. Effect of mentha oil and thermal treatment

A set of inoculated fruit juice mixtures vials added with three different concentrations of mentha oil was exposed to thermal treatment (80°C) for 30, 60 and 90 s. Each sample was treated in triplicate. Subsequently, the treated vials were stored at room temperature up to 8 d and samples were drawn on 0, 2nd, 4th and 8th d. All treated samples were serially diluted and plated on PDA. The plates were incubated for 72 h at 80°C and samples were made. The efficacy of the thermal treatment alone and combination with different doses of mentha oil were quantified by the variation in log cfu of the inoculated yeast strains with time.

2.4.5. Sensory analysis

Anticipating the organo-leptic impact of mentha oil, sensory analysis was conducted for selected samples during storage. Non-inoculated mixed fruit juice samples treated with different concentrations of mentha oil, as well as thermal treatment, were subjected to sensory evaluation by a four member sensory panel composed of staff from the laboratory. The same trained persons were used in each evaluation, and all were blinded to which sample was being tested. The sensory evaluation was carried out in artificial light and the temperature of samples approximated the ambient temperature. Special attention was given to the colour and the presence of exudates in the glass vials prior to opening and the assessment of abnormal odours during the opening of the sample. Assessment was designed to identify the impact of aroma due to mentha oil as well as spoilage conditions. Odours typical of mixed fruit juice, as exemplified by special samples that were retaining colour prior to each sensory evaluation, were regarded as acceptable. Distinct putrid, sweet, sour or cheesy odours were regarded as indicative of spoilage and therefore unacceptable. Bright colours typical of fresh oxygenated juices were considered acceptable. A persistent dull appearance, unusual colour or appearances were considered unacceptable.

2.5. Statistical analyses

All the experiments were done in triplicate and repeatability was established. Significance of differences among treatments (P < 0.05) was analysed using one way ANOVA (SPSS, 10.0 version). For all experiments, three replicates were used and the data presented here represents the mean of these replicates with standard error or deviation.

3. Results and discussion

3.1. Chemical composition of mentha oil

Qualitative and quantitative analysis of the mentha oil is listed in Table 1. A total of 20 components were identified, which represented 15.8% monoterpenic hydrocarbons and 80.9% oxygenated monoterpenes, and about 96.7% of the total detected constituents. A portion (3.3%) of the total composition was not identified. The major constituents of the oil were cis-menthone (27.43%), menthol (24.3%), trans-menthone (9.23%), limonene (5.84%), 1,8-cineole (5.63%), methyl cyclopentane (4.88%), menthofuran (4.44%) and isomenthol (3.21%). The presence of all these compounds in the Mentha sp. was reported by Rohloff (1999); Yadegarinia et al. (2006).

The presence of significant amount of oxygenated monoterpenes (80.9%), represented by menthol and menthone compounds, indicates high antimicrobial potential of mentha oil (Iscan et al., 2002). Therefore, the anti-yeast activity was evaluated against the food spoiling yeasts.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical components</th>
<th>RT (min)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Methyl cyclopentane</td>
<td>2.23</td>
<td>4.88</td>
</tr>
<tr>
<td>2</td>
<td>α-Pinenene</td>
<td>7.08</td>
<td>1.37</td>
</tr>
<tr>
<td>3</td>
<td>β-Pinenene</td>
<td>9.91</td>
<td>1.93</td>
</tr>
<tr>
<td>4</td>
<td>β-Phellandrene</td>
<td>10.35</td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td>Limonene</td>
<td>13.43</td>
<td>5.84</td>
</tr>
<tr>
<td>6</td>
<td>1,8-Cineole</td>
<td>13.85</td>
<td>5.63</td>
</tr>
<tr>
<td>7</td>
<td>3-Carene</td>
<td>15.36</td>
<td>0.40</td>
</tr>
<tr>
<td>8</td>
<td>m-Cymene</td>
<td>16.47</td>
<td>0.63</td>
</tr>
<tr>
<td>9</td>
<td>3 Octanol</td>
<td>21.41</td>
<td>0.78</td>
</tr>
<tr>
<td>10</td>
<td>Terpineol</td>
<td>24.05</td>
<td>0.66</td>
</tr>
<tr>
<td>11</td>
<td>p-Menthone (cis)</td>
<td>25.01</td>
<td>27.43</td>
</tr>
<tr>
<td>12</td>
<td>Menthofuran</td>
<td>25.74</td>
<td>4.44</td>
</tr>
<tr>
<td>13</td>
<td>p-Menthone (trans)</td>
<td>26.18</td>
<td>9.23</td>
</tr>
<tr>
<td>14</td>
<td>Methyl acetate</td>
<td>28.84</td>
<td>1.31</td>
</tr>
<tr>
<td>15</td>
<td>Isopulegol</td>
<td>29.29</td>
<td>0.22</td>
</tr>
<tr>
<td>16</td>
<td>Trans-Isopulegole</td>
<td>29.58</td>
<td>0.22</td>
</tr>
<tr>
<td>17</td>
<td>Isomenthol</td>
<td>30.14</td>
<td>3.21</td>
</tr>
<tr>
<td>18</td>
<td>Isopulegole</td>
<td>30.80</td>
<td>0.51</td>
</tr>
<tr>
<td>19</td>
<td>Menthol</td>
<td>31.89</td>
<td>24.3</td>
</tr>
<tr>
<td>20</td>
<td>Pulegone</td>
<td>32.53</td>
<td>1.32</td>
</tr>
</tbody>
</table>

Monoterpane hydrocarbons 15.8%
Oxygenated monoterpane 80.91%
Total % of identified compounds 96.71%

RT, retention time (min), relative area percentage without using the FID response correction factor, (results are based on GC-MS; MS acquisition started after 4 min).

3.2. Anti-yeast activity of mentha oil

3.2.1. Disc diffusion method

Antimicrobial potential of the mentha oil was observed in terms of zone of inhibition generated by the diffusion of the essential oil components into the yeast strains inoculated agar plate. The zone of inhibition increased with the increasing concentrations (i.e., 10, 20, and 30 µl) of mentha oil on sterile discs (Fig. 1). The inhibition zone due to 10 µl mentha oil was A. Pullulans (40 mm) > P. kluyveri (40 mm) > C. diversa (18 mm) > P. anomala (18 mm) > Z. bailii (16 mm) > H. polymorpha (14 mm) > S. cerevisiae (12 mm) > P. fermentans (9 mm). However, complete growth inhibition of any yeast strain was not found at the maximum concentration (30 µl) of mentha oil tested. Earlier, Origanum vulgare essential oil showed 16 mm zone of inhibition against several food spoiling yeasts at 10 µl concentration (Souza et al., 2007). These zones were substantially smaller than those observed here for mentha oil at the same concentration.

The hydroalcoholic extract and essential oil of M. piperita were recently evaluated for anti-yeast activity against 50 strains of C. albicans, 10 strains of C. glabrata, 10 strains of C. tropica1s, 8 strains of Candida parapsilosis and 2 strains of Candida krusei (Carretto, Aleida, Furlan, Jorge, & Junqueira, 2010), where mentha oil showed the strongest inhibitory activity against the strains C. albicans, C. tropicalis and C. parapsilosis. The above discussion points out variability in the response of yeasts depending upon the strains tested as well as the composition of essential oil used.

3.2.2. Disc volatilisation method

The zone of inhibition resulting from the exposure to mentha oil vapours is shown in Fig. 2. As observed in earlier assays using mentha oil in liquid phase, the zone of inhibition due to mentha oil vapours also increased with increasing concentration of oil and followed the same trend with respect to the different yeast strains. However, as compared to the liquid phase, mentha oil vapours resulted in a significantly larger zone of inhibition (P < 0.05) for all the yeast strains tested. Remarkable resistance against mentha oil vapours was seen in case of P. fermentans (10 mm), < H.
polymorpha (28 mm), < P. anomala (30 mm) yeast strains and complete growth inhibition was not observed, even at 30 µl mentha oil vapours. However, P. kluyveri and A. pullulans were completely inhibited in presence of the vapours generated by 20 µl mentha oil (Fig. 2).

It can be inferred that the anti-yeast activity of the essential oil vapours can be achieved to a lesser degree than essential oil in the liquid phase. Hence, essential oil vapours have higher application potential than the corresponding oil. Integration of eugenol, thymol, or menthol has been attempted with MAP for preservation of table grapes (Valverde et al., 2005). The addition of volatile compounds inside the packages was effective in reducing the microorganism proliferation in table grape, the effect being higher for yeast and molds than for mesophilic aerobes.

3.2.3. MIC and MFC of mentha oil

The MIC of mentha oil was determined against different food spoiling yeast strains (S. cerevisiae, Z. bailii, A. pullulans, C. diversa, P. fermentans, P. kluyveri, P. anomala, H. polymorpha). The MIC and MFC values are shown in Table 2. The oil exhibited concentration dependent inhibition of the growth and the MIC of mentha oil varied from 0.28 to 2.25 mg/ml (Table 2). The lowest MIC (0.28 mg/ml) was shown by P. kluyveri and highest MIC (2.25 mg/ml) was shown by H. polymorpha and P. fermentans. The MFC of the yeast

<table>
<thead>
<tr>
<th>Yeast Strains</th>
<th>10 µl</th>
<th>20 µl</th>
<th>30 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cerevisiae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z. bailii</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. pullulans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. diversa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. fermentans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. kluyveri</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. anomala</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. polymorpha</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Anti-yeast potential of mentha oil; disc diffusion method, zone of inhibition due to the different concentration (10, 20, and 30 µl) of mentha oil against S. cerevisiae, Z. bailii, A. Pullulans, C. diversa, P. fermentans, P. kluyveri, P. anomala, H. polymorpha. (column height represents the mean of triplicate results and error bar represents the standard error).

Fig. 2. Anti-yeast potential of mentha oil vapour; disc volatilisation method, zone of inhibition due to the different concentration (10, 20 and 30 µl) of mentha oil vapour against S. cerevisiae, Z. bailii, A. Pullulans, C. diversa, P. fermentans, P. kluyveri, P. anomala, H. polymorpha. (column height represents the mean of triplicate results and error bar represents the standard error).

Table 2

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Name of the strain</th>
<th>MIC (mg/ml)</th>
<th>MFC (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saccharomyces cerevisiae SPA</td>
<td>1.13</td>
<td>2.25</td>
</tr>
<tr>
<td>2</td>
<td>Zygosaccharomyces bailii 45</td>
<td>1.13</td>
<td>2.25</td>
</tr>
<tr>
<td>3</td>
<td>Aureobasidium pullulans 16F</td>
<td>1.13</td>
<td>2.25</td>
</tr>
<tr>
<td>4</td>
<td>Candida diversa TSD</td>
<td>0.56</td>
<td>1.13</td>
</tr>
<tr>
<td>5</td>
<td>Pichia fermentans T2A1</td>
<td>2.25</td>
<td>4.5</td>
</tr>
<tr>
<td>6</td>
<td>Pichia kluyveri T1A</td>
<td>0.28</td>
<td>1.13</td>
</tr>
<tr>
<td>7</td>
<td>Pichia anomala</td>
<td>0.56</td>
<td>1.13</td>
</tr>
<tr>
<td>8</td>
<td>Hansenula polymorpha CBS 4732</td>
<td>2.25</td>
<td>4.5</td>
</tr>
</tbody>
</table>
strains also varied from 0.56 to 4.5 mg/ml and showed a similar pattern i.e. H. polymorpha, P. fermentans, (4.45 mg/ml) > S. cerevisiae, Z. bailii, A. pullulans (2.25 mg/ml) > P. kluveri, C. diversa, P. anomala (1.13 mg/ml). In the present study, the highest MIC/MFC was shown by H. polymorpha and P. fermentans.

The MIC of Brazilian M. Piperita essential oil against Candida spp. was 1.1 mg/ml (Duarte, Figueira, Sartoratto, Rehder, & Delarmelina, 2005), which was very similar to that evaluated in present study. Attempts were made to compare the present MIC/MFC values with those for M. piperita essential oil of Indian origin, used in our previous study (Tyagi & Malik, 2011). The MIC of M. piperita oil (1.13 mg/ml) against S. cerevisiae was similar to the MIC of mentha oil against S. cerevisiae, Z. bailii and A. pullulans observed in the present study. However, the MIC of M. piperita against C. albicans (1.13 mg/ml) was substantially higher than MIC of mentha oil against C. diversa (0.56 mg/ml) observed in the present study. In agreement with this, the zone of inhibition resulting from the exposure to M. piperita oil (20 μl) vapours against C. albicans through the disc volatilisation method was significantly smaller (58 mm) than that due to the same concentration of mentha oil against C. diversa measured (71 mm) in the present study. This difference in activity could be attributed to different yeast species and composition of the oils used. The quantity of major antimicrobial compounds (oxygenated monoterpenes in mentha oil used is higher (80.9%) than that in M. piperita oil (63.3%) used in the previous study.

The oxygenated monoterpenes can increase the permeability of the membrane, leading to leakage of the cell contents (Bennis, Chami, Chami, Bouchikhi, & Rennal, 2004; Burt, 2004; Cristani et al., 2007). Cell membrane degradation, loss of cytoplasmic components and inhibition of respiratory activity due to certain terpenes (α-terpinene and limonene) have been reported in C. tropicalis (Adegoke, Iwahashi, Komatsu, Obuchi, & Iwahashi, 2000; Uribe, Ramirez, & Pena, 1985). The presences of monoterpenes as major compounds could be a reason for higher anti-yeast activity of mentha oil. The different concentrations at which mentha oil exerted significant anti-yeast effect indicate that there may be possibilities for the use of mentha oil as an additive to foodstuffs, where a reduction in food spoilage yeast is required. Hence, further experiments were conducted to validate the efficacy of mentha oil in conjunction with other hurdle technologies in a real food system.

3.3. Mixed fruit juice preservation by mentha oil and thermal treatment

The process of juice pasteurisation can be accomplished by different time–temperature combination treatment. In an earlier study, apple juice from eight different varieties of apples was heated at high-temperature (60–90 °C) and short-time (20–100 s) combinations (HTST). The best stability of cloud and colour in relation to heat impact was achieved by HTST treatment between 70 °C/100 s and 80 °C/20 s (Krapfenbauer, Kinner, Gossinger, Schonlechner, & Berghofer, 2006). Therefore, in the present study, exposure to higher temperature (80 °C) for 30, 60 and 90 s has been selected for thermal pasteurisation of mixed fruit juices.

3.3.1. Effect of thermal treatment

As shown in Fig. 3a, thermal treatment for 30 and 60 s at 80 °C was not effective for S. cerevisiae load reduction of the mixed fruit juice samples. Only 0.49 log cfu reduction was observed in 90 s thermal treated samples after eight days. Hence, the temperature–time combination used for thermal treatment was almost ineffective for preserving juice spoilage by S. cerevisiae.

3.3.2. Effect of varying concentration of mentha oil

As shown in Fig. 3b, complete growth inhibition of S. cerevisiae was observed in mixed fruit juice mixture at MIC level. The viable count of yeast cells in mixed juice increased with decreasing concentration of mentha oil. In untreated samples, the enhancement of yeast load (4.59 log cfu) was significantly higher than that in ½ MIC (0.60 log cfu) and ¼ MIC (3.63 log cfu) treated samples.

Tserennadmid et al., (2011) studied the anti-yeast activity of several essential oils such as clary sage, juniper, lemon and marjoram. The MIC of essential oils in juices was significantly higher than in vitro MIC values. Since high concentrations of different essential oils are required to achieve useful antimicrobial activity, unacceptable levels of inappropriate flavours and odours may be present (Gutierrez, Barry-Ryan, & Bourke, 2009). Interestingly, in the present study, MIC of mentha oil against S. cerevisiae was the same for both in vitro and in vivo (real fruit juice) assessments. This is in contrast to the above report (Gutierrez et al., 2009), where the MIC in juices was higher than the MIC in growth media. It may be due to lower pH (3.2) of fruit juices. To further reduce the required mentha oil concentration for controlling the yeast load in mixed juices, the interaction between essential oil and thermal treatment was also studied.

3.3.3. Combined effect of mentha oil and thermal treatment

The log reduction in cfu count of the S. cerevisiae due to the combined effect of mentha oil at MIC level, ½ MIC level and ¼ MIC level along with thermal treatment at 80 °C for 30, 60 and 90 s of mixed juices was recorded for a particular time interval (i.e., after 2, 4 and 8 d). In samples exposed to a combination of thermal treatment and MIC level/½ MIC level mentha oil, complete growth inhibition of S. cerevisiae was observed on first sampling after two days (Fig. 4). Furthermore, growth reoccurrence was found on ½ MIC level treated samples however no growth was
observed in MIC level treated samples up to eight days of storage. Hence, the combination of thermal treatment with essential oil reduced the oil dose requirement significantly. Even in the samples treated with ¼ MIC level of mentha oil, the combination of thermal treatment for 90 s enhanced the log reduction by 1.03log cfu as compared to only mentha treated samples. Hence, the combination of thermal treatment with mentha oil can provide better juice preservative with minimal impact on the organoleptic properties of juices.

Belletti, Kamdem, Tabanelli, Lanciotti, and Gardini, (2010) observed that neither the thermal treatment alone, nor the presence of the terpenes at their maximum concentrations in the absence of the thermal treatment, was able to guarantee the microbial stability of the beverages against S. cerevisiae. However, when used in combination, mild thermal treatment (55 °C, 15 min) enhanced the bioactivity of terpenes (citral, linalool and β-pinene) caused an increasing of their vapour pressure, which in turn increased their possibility to solubilise in the yeast cell membrane. The present study also recorded the enhancement in anti-yeast activity of mentha oil up on combination with thermal treatment. By exposure to higher temperature (80 °C), the vapour pressure of the phenolic compounds (major components of mentha oil; menthol and menthone) would have increased, thereby causing higher damages to the cell membrane of tested strains. Our previous study employing SEM, TEM and AFM showed that the height of the C. albicans cells was drastically reduced in vapour treated cells as compared to the oil treated ones (Tyagi & Malik, 2010). Hence lemon grass oil vapours were more potent than the lemon grass oil for causing irreparable damage to Candida cells. These results are in agreement with notably higher antiyeast activity of mentha oil vapours (Section 3.2.2) over mentha oil observed in the present study.

The combination of thermal treatment with essential oils offers a very useful synergy, whereby increase in temperatures during storage could enhance the vapour phase concentration of volatiles, thereby enhancing the antimicrobial activity for better food preservation. The combination of thermal treatment with mentha oil treatment has not been previously evaluated for preventing fruit juice spoilage. Our results established that mentha oil can be very well used with thermal treatment for the food preservations.

3.3.4. Sensory analysis

The colour of the MIC level treated samples remained acceptable for a longer period than ½ MIC and ¼ MIC level treated samples. The addition of mentha oil did not undesirably alter the odour and colour of the mixed juice. In particular, the juicy odour of mixed juice supplemented with mentha oil persisted eight days longer in comparison to untreated samples. Furthermore, colour remained stable in samples treated with MIC level mentha oil. In ½ MIC level treated samples, juice odour and colour persisted up to 4 days, while in ¼ MIC treated samples juice odour and colour persisted up to two days only.

4. Conclusion

In the present study, the significant anti-yeast potential of the mentha oil against the food spoiling yeasts has been established using in vitro and in vivo tests. Furthermore, higher efficacy of mentha oil vapours over mentha oil was also demonstrated. Interestingly, the MIC of mentha oil against S. cerevisiae was the same for in vitro and in vivo (real fruit juice) assessments. The anti-yeast activity of mentha oil in mixed juice mixtures reinforces its applicability in food preservation, since this essential oil is considered toxicologically safe.

The combination of mentha oil and mild thermal treatment for mixed juice preservation could have additional advantages such as efficacy without alteration in organoleptic properties of the edible material/food. Further utilisation of mentha oil in different food systems is required to confirm the antimicrobial activity of mentha oil for preservation and/or extension of shelf life of raw and processed food.

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