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ARTICLE

Activity of essential oils in vapor phase against bread spoilage fungi

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ABSTRACT The antifungal activity of marjoram and clary sage essential oils (EOs) against bread spoilage molds *Aspergillus niger, Penicillium chrysogenum* and *Rhizopus spp*. was investigated by the reversed Petri dish method in malt extract medium and on wheat, wheat-rye mixed, and rye bread slices modeling an active packaging. Changes in colony size and colony growth rate were evaluated as markers for growth inhibition effect of the EOs. In vitro the most insensitive mold was *Rhizopus spp*. showing no significant changes after EO treatment compared to the untreated control. However, growth of all of the investigated molds was significantly reduced on EO vapor treated bread slices. To overcome problems rising from the strong aroma of EOs further experiments are needed to find the best EOs with acceptable aroma and high effectiveness to be used in combined technologies

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KEY WORDS

sliced bread,
essential oil,
vapor phase,
reversed Petri dish

In bakery products, especially in sliced bread, spoilage by filamentous fungi is a frequent problem. Insufficient hygiene in the factory is the main cause of fungal spoilage. Fungal spores are inoculated mostly by the slicing machine and, after 6-8 days, fungal colonies will appear on the crust of the bread. To overcome this problem preservatives, such as sorbic acid or propionic acid and their salts are added to the dough. However, consumers do not like artificial preservatives in their food, especially not in their everyday bread. The use of modified atmosphere packaging (MAP) or active packaging (AP) represents a good alternative solution to extend bread shelf life and to avoid the use of chemical preservatives. The recommended gas mixture for MAP of bakery products is 60 % CO_2 and 40 % N_2. Without oxygen absorber, however, the residual O_2 trapped in the spongy structure of bread is 3-5% which can be sufficient for spore germination (Piergiovannia and Fava 1997; Haasum and Nielsen 1998). In active packaging, essential oils in vapor phase can be used as natural preservatives without direct contact to the foodstuff (Nielsen and Rio 2000; Rodriguez et al. 2008). Suhr and Nielsen (2003; 2005) found that lemongrass and mustard oil were, in their volatile form, the best growth inhibitors against rye bread spoilage fungi.

In our study the antifungal activity of marjoram and clary sage oil against *Aspergillus niger, Penicillium chrysogenum* and *Rhizopus spp*. isolated from spoiled white bread was investigated by the reversed Petri dish method using malt extract medium. To prove the antifungal activity of the essential oil vapors, the spoilage fungi were inoculated also on bread slices - wheat, wheat-rye mixed, and rye bread - in a closed Petri dish system modeling an active packaging.

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Materials and Methods

Essential oils

The essential oils (EOs) investigated in this study were clary sage and marjoram. Their main components are linalool (27%) and linalyl-acetate (51%) for clary sage and terpinen-4 -ol (30%), γ-terpinene (16%) and cis-sabinene-hydrate (19%) for marjoram (Tserenadmid et al. 2011). The EOs were provided from Aromax Natural Products (Budapest, Hungary).

Sliced breads

Wheat bread (100% wheat flour), wheat-rye mixed bread (85% wheat and 15% light rye flour) and rye bread (80% dark rye and 20 % wheat flour) were purchased from a local market in Szeged. Sliced breads contained no preservatives and were the product of CERES Kft., Győr, Hungary.

Moulds and culture conditions

Aspergillus niger, *Penicillium chrysogenum* and *Rhizopus spp.* were from the culture collection of the Institute of Food Engineering and were grown on malt extract medium (ME; 0.4% malt extract, 1% glucose, 0.1% yeast extract) and incubated at 30°C for 3 days. The strains were maintained on ME agar slants at 4°C.

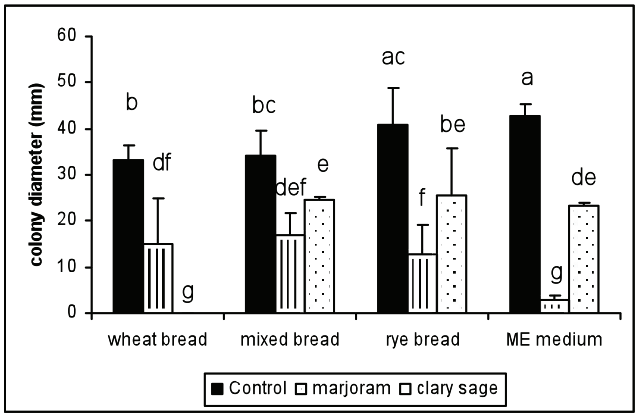

Figure 1. Colony diameter of *Aspergillus niger* growing on malt extract medium or bread slices in the presence and absence of marjoram and clary sage essential oils. Different letters on the top of columns represent significant deference ($p<0.05$).

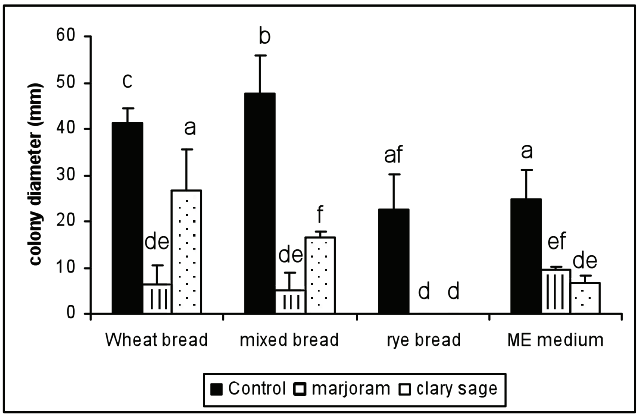

Figure 2. Colony diameter of *Penicillium chrysogenum* growing on malt extract medium or bread slices in the presence and absence of marjoram and clary sage essential oils. Different letters on the top of columns represent significant deference ($p<0.05$).

Antifungal activity tests by reversed Petri dish method

Petri dishes containing ME medium were point inoculated with the investigated fungi. In the lid of the dish a sterile paper disc (10 mm diameter) was placed and 30 µl pure EO was added to the disc to give the final concentration of 940 µl/l air. Controls were prepared by adding 30 µl distilled water to the paper disc. Petri dishes were sealed with parafilm and incubated in reversed position for 4 days at 30°C. Each day colony diameters were measured and colony growth rates were calculated. Experiments were repeated three times.

Effect of essential oil vapors on the shelf life of bread slices

Bread slices were aseptically punched out with a circular mould of 90 mm diameter and were placed in sterile Petri dishes. Bread slices were inoculated with 30 µl fungal spore suspensions (10^4spore/ml). Height of the slices was 9-10 mm, so the free headspace in a dish was 31.8 cm^3. In the lid of the dish a sterile paper disc (10 mm diameter) was placed and 30 µl pure clary sage or marjoram EO was added to the disc. Petri dishes were sealed with parafilm and incubated for 14 days at 20°C (room temperatures). Control dishes contained a paper disc with 30 µl sterile water. Bread slices were observed for colony formation and every second day colony diameters were measured. Experiments were repeated three times

Table 1. Number of days to visible growth of the fungus.

	wheat bread		wheat-rye mixed bread		rye bread	
	Mar-joram	Clary sage	Mar-joram	Clary sage	Mar-joram	Clary sage
A. niger	9	ng	10	10	9	6
P. chrys.	9	4	10	4	ng	ng
Rhizopus spp.	8	10	8	8	ng	ng

ng. No growth

Sensory evaluation

On the sixth day of storage non-inoculated bread slices were evaluated for taste and odor by an untrained panelist team of five persons.

Statistical analysis

Statistical analysis (one-way ANOVA followed by LSD testing) was performed to determine significant differences ($p<0.05$) by using SPSS 9.0 software.

Results

Antifungal activity in ME medium

The effective volume / paper disk (30 µl) of essential oils in a closed Petri dish was established in previous experiments (data not shown). In this study the growth of *A. niger* and *P. chrysogenum* was inhibited by both of the EOs; colony diameters and growth rates of treated molds decreased significantly compared to the untreated controls (Fig 1, 2 and 5, 6). On the other hand the investigated parameters (colony size and growth rate) of *Rhizopu*s spp. were not affected by either of the EOs (Fig 3, 4). Marjoram EO delayed colony formation for two days but after this period the growth of the fungus followed the same pattern as the control. Guynot et al (2003) used 50 µl essential oils / paper disk in reversed Petri dish system against bread spoilage *Eurotium*, *Aspergillus* and *Penicillium* species and have found that cinnamon leaf, clove, lemongrass, bay and thyme EOs totally inhibited the growth of the investigated molds. Other EOs (lemon, mandarin, ani-

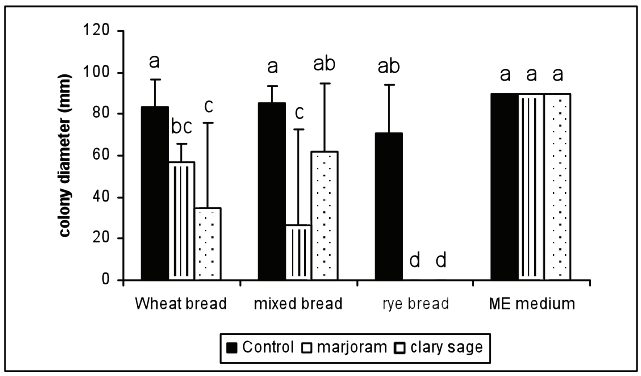

Figure 3. Colony diameter of *Rhizopus spp.* growing on malt extract medium or bread slices in the presence and absence of marjoram and clary sage essential oils. Different letters on the top of columns represent significant deference ($p<0.05$).

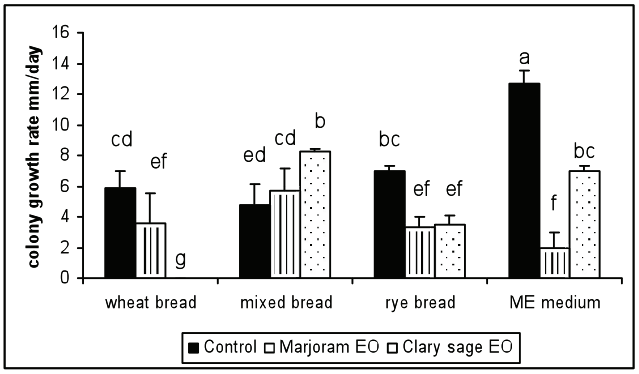

Figure 4. Colony growth rate of *Aspergillus niger* growing on malt extract medium or bread slices in the presence and absence of marjoram and clary sage essential oils. Different letters on the top of columns represent significant deference ($p<0.05$).

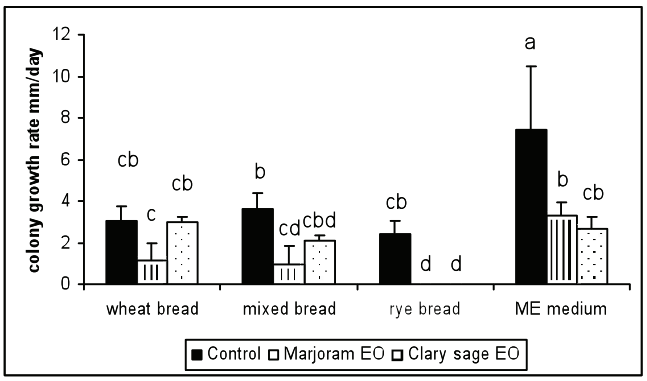

Figure 5. Colony growth rate of *Penicillium chrysogenum* growing on malt extract medium or bread slices in the presence and absence of marjoram and clary sage essential oils. Different letters on the top of columns represent significant deference ($p<0.05$).

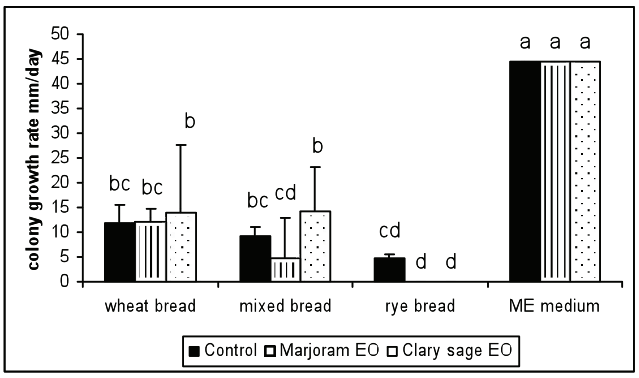

Figure 6. Colony growth rate of *Rhizopus spp.* growing on malt extract medium or bread slices in the presence and absence of marjoram and clary sage essential oils. Different letters on the top of columns represent significant deference ($p<0.05$).

seed, rosemary, basil, sage) had no or limited effect on the growth of the molds. In our study marjoram and clary sage EO had only growth reducing effect without total inhibition.

Effect of essential oil vapors on the shelf life of bread slices

The investigated molds grew well on non-treated bread slices but marjoram and clary sage EO inhibited their growth. On Table 1 the number of days to visible growth of the fungi is shown. It can be seen that EO vapors delayed colony formation of all of the molds and no growth of *Penicillium chrysogenum* and *Rhizopus spp.* was observed on treated rye bread during the 14 days of investigation. Colony size of EO vapor treated *A. niger* and *P. chrysogenum* decreased significantly similar to the results obtained in ME medium. In all cases treatment with marjoram EO vapor resulted in the smallest colonies (Fig. 1, 2, 3). Except for *A. niger* colony growth rates of treated or untreated molds growing on bread slices showed no significant differences but were in all cases significantly lower than that of the control (non-treated) in ME medium (Figs 5 and 6).

Sensory evaluation

The odor of bread slices after removing from the closed system was very strong and had almost the same intensity after staying one hour at room temperature on a plate. All panelists had the opinion that taste and odor of EO vapor treated breads was strange and unacceptable.

Discussion

Marjoram and clary sage EO vapor treatment in a closed system modeling active packaging showed good antifungal effect against bread spoilage molds *A. niger, P. chrysogenum*

and *Rhizopus spp*. Shelf life of the sliced breads declared by the producer is 8 days and in most cases during this time no mold growth occurred on the infected and EO vapor treated slices. On the other hand sensory analysis showed that the effective concentration of the EOs is too high resulting in unacceptable changes in taste and odor. Nielsen and Rios (2000) and Suhr and Nielsen (2005) used mustard essential oil in 1, 2 or 3 µl / bread slices concentration for the shelf life elongation of wheat and rye bread. Only bread treated with l µl mustard oil /slice was accepted by the panelists. Volatile compounds of the EOs are absorbed by the bread as observed for cinnamaldehyde by Rodriguez et al (2008). There was a strong correlation between cinnamaldehyde concentration in the bread and growth inhibition of molds thus the absorbed compounds are responsible for the antifungal activity but also for the sensory changes. Solution can be the use of combined treatments, for example essential oils combined with other preservatives or with modified atmosphere packaging. Further investigations are needed to find the best EOs with acceptable aroma and high effectiveness to be used in hurdle (combined treatments) technologies to prevent mold growth on sliced bread.

Acknowledgement

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