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Research Article

Antimicrobial effects of Rosemary essential oil with potential use in the preservation of fresh fruits and vegetables

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Different uses of *Rosmarinus officinalis* are known, and its volatile essential oil (EO) possess extensively investigated biological properties, such as antioxidant, anti-inflammatory, antiproliferative, anticancer, antiviral, antimicrobial, hepatoprotective, neuroprotective, nephroprotective, antiulcer, and many others. The aim of our study was evaluating of antimicrobial activity of *R. officinalis* essential oil in vapor phase on apples, pears, kohlrabi, and potatoes. Fruits and vegetables models were tested with Gram-positive bacteria, Gram-negative bacteria, and yeasts. Together four bacterial strains (*Salmonella enterica* subsp. *enterica*, *Yersinia enterocolitica*, *Enterococcus faecalis*, *Staphylococcus aureus* subsp. *aureus*) and four yeasts (*Candida albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis*) were tested *in situ* analyses. The most effective influence has ROEO has the most effective influence on on apples model against bacteria *Enterococcus faecalis*, and *C. glabrata*, on pears model *Salmonella enterica* and *C. glabrata*, on potatoes *Yersinia enterocolitica*, and *C. glabrata*, and on kohlrabi model *Y. enterocolitica*, and *C. albicans*. The most effective in all food models was concentration 500 μL.L^{-1}.

Keywords: bacteria, yeasts, apple, pears, kohlrabi, potatoes, *in situ* antimicrobial activity

1 Introduction

Fresh-cut fruit has become very popular, and the trend is towards fresh high-quality produce. Ready meals are also growing in popularity. The biggest obstacle to fresh-cut pears is the limited to low shelf life and susceptibility to enzymatic browning and tissue softening. Important criteria for marketability of fresh-cut products are good sensory quality as well as microbiological stability (Martín-Belloso et al., 2006).

Apples and pears are sources of various flavonoids and are attributed to have beneficial effects on human health because they contain apple procyanidin and pectin (Sanz et al., 2015; Shoji & Miura, 2014; Shtriker et al., 2018). Various studies have reported that apple and pear supplementation can cause changes in microbiota composition and metabolic activity *in vitro*. This could cause potential benefits on human health (Garcia-Mazcorro et al., 2019; Koutsos et al., 2017). Less studied is the effect of apples and pears on the microbiome. Many studies have focused extensively on plant pathogens and have mainly studied the phyllosphere (Burr et al., 1996; He et al., 2012; Liu et al., 2017; Pusey et al., 2009; Stockwell et al., 2010; Yashiro et al., 2011).

Fresh-cut vegetables can present ideal conditions for the growth of microorganisms because they are mostly low in acidity (pH between 5.8 and 6.0) (Escalona et al., 2005). The initial average of aerobic microbial, mesophilic and psychrophilic bacteria in kohlrabi slices ranged from 3.3 to 3.4 log CFU.g^{-1}. After 14 days at 5 °C in air, microbial counts increased to 4.3 to 5.6 log CFU.g^{-1} (Escalona et al., 2006). (Nguyen-the & Carlin, 1994) monitored mesophilic bacterial counts in minimally processed vegetables and counts ranged from 3 to 6 log CFU.g^{-1} and from 3 to 9 log CFU.g^{-1} after processing in cold storage.

During potato tuber growth, microbial organisms may be present on the tuber surface that have the potential to antagonize phytopathogens (Clulow et al., 1995). Previous studies with potato tuber have focused on endophytic bacteria (Sturz et al., 1999), or on bacterial and fungal pathogens of potato tuber diseases such as *Streptomyces scabies* (Loria et al., 1997), or *Dickeya* species

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(Dowley & O'Sullivan, 1991; Gudmestad et al., 2007; Pérombelon, 2002). Just (Lottmann et al., 1999) studied bacteria on the tuber surface.

Phenolic compounds present in plants and vegetables can reduce the risk of some diseases due to their antioxidant potential and inhibition of free radicals supplied by the benzene ring and hydroxyl group in their structures (Mohammed et al., 2021; Pinela et al., 2016). Rosemary (*Rosmarinus officialis* L.) oil has been used as a food seasoning in dishes (Lo Presti et al., 2005). Rosemary essential oil (ROEO) has been traditionally and extensively used as a medicinal herb with a number of properties such as anti-inflammatory, antifungal, carminative, antimicrobial, astringent, analgesic, antirheumatic, and antioxidant (Siejak et al., 2021).

Therefore, in the present research, the antimicrobial activity of rosemary (*Rosmarinus officinalis* L.) essential oil (ROEO) was investigated in the vapor phase against bacteria and yeasts on fruits and vegetables (*in situ* experiment).

2 Material and methods

2.1 Sample

The test essential oil *Rosmarinus officinalis* L. (ROEO) purchased from company Hanus s. r. o. (Nitra, Slovakia) was prepared by steam distillation of a fresh flowering plants from Tunisia. The main components of the essential oil marked by producer were 1,8-cineole 38–55%, camphor 5–15%, $\alpha + \beta$ pinene 13–23%, limonene 1–4%, borneol 1–5%. The sample was stored in the cold (4 °C) and in the dark throughout the analyses. EO was stored in a refrigerator (4 °C) protected from light, in glass vessels.

2.2 Microorganisms tested

Two gram-negative (G^-) bacteria (*Salmonella enterica* subsp. *enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671), two gram-positive (G^+) bacteria (*Enterococcus faecalis* CCM 4224, *Staphylococcus aureus* subsp. *aureus* CCM 2461) and four yeasts (*Candida albicans* CCM 8186, *C. glabrata* CCM 8270, *C. krusei* CCM 8271, *C. tropicalis* CCM 8223,) were obtained in the Czech Collection of Microorganisms (CCM; Brno, Czech Republic).

2.3 In situ antimicrobial activity

The evaluation of antimicrobial activity of ROEO on different commodities such as fruit and vegetable models (apples, pears, potatoes and kohlrabi) was carried out following the procedure reported in (Kačániová et al., 2022). This involves monitoring the antimicrobial activity against four bacteria and two yeasts. Briefly, 5 mm thick slices of fruits and vegetables were placed on solidified Mueller Hinton agar for PD (∅ = 60 mm) and microbial inoculum (0.5 McFarland) was applied. Diluted ROEO (100 µl) in ethyl acetate at 4 dilution levels (500, 250, 125, and 62.5 $\mu l.L^{-1}$) was then applied to a roll of sterile filter paper. The hermetically sealed PD was incubated for 7 days at a temperature suitable for the microorganisms to be analysed. An equivalent volume of ethyl acetate was used as a negative control. The percentage of inhibitory activity was calculated in ImageJ by the stereological method. Bulk density was calculated according to the formula:

$$Vv = P_1/p \times 100 \tag{1}$$

where: P_1 – stereological lattice of the colonies; p – the substrate

Growth inhibition was expressed as:

$$GI = \left[(C_1 - T_1)/C\right] \times 100, \tag{2}$$

where: C_1 – the growth density of control group; T_2 – the growth density in the group contained ROEO (Kačániová et al., 2022)

2. 4 Statistical analyses

Analyses and measurements were performed in triplicate. Standard deviation (SD) and mean were calculated using Microsoft Excel software. One-way analysis of variance (ANOVA) was performed using Prism 8.0.1 (GraphPad Software, San Diego, CA, USA).

3 Results and discussion

Additives used as preservatives or stabilisers, with increasing technological innovation in recent years, play an important role in food safety. However, the demand for natural food products has led to attempts to include alternatives, natural substances, rather than chemical additives in food (Hernández et al., 2016).

The Lamiaceae family is very important in terms of essential oil production with antimicrobial and antioxidant properties (Zhu, 2007). Several articles can be found in the literature regarding the antibacterial activity of rosemary essential oil against various foodborne pathogens (Ivanovic et al., 2012; Jordán et al., 2013). The antibacterial activity of the rosemary plant differs from any other due to the different chemotype composition. Therefore, it is necessary to define the chemotype (or relative abundance of its components) that works best against the most common foodborne pathogens.

This is consistent with the proposition that the antibacterial activity of an oil may be related to the chemical configuration of its constituents, the proportions in which they are present, and the interactions between them (Bajpai et al., 2012).

The results of the antibacterial activity of ROEO on apples are presented in Table 1. Intensity of the bacterial inhibition by ROEO increased with the increasing concentration of ROEO in assays across all bacteria tested. The most effective influence has ROEO on apples model against bacteria *Enterococcus faecalis* in concentration 500 µL.L^{-1} with value 80.81%.

The results of the anti-yeasts activity of ROEO on apples are summarized in Table 2. Intensity of the yest growth inhibition by ROEO increased with the increasing concentration of ROEO in assays across all yeasts tested. ROEO on apple model shows the best antimicrobial activity against yeasts with testing of *C. glabrata* in concentration 500 µL.L^{-1} with value 65.54%.

Table 3 showed antimicrobial effect of ROEO on pears. In this experiment, there was found the best antimicrobial activity against bacteria tested was found against *Salmonella enterica* in concentration 500 µL.L^{-1} with value 64.19%.

Table 4 showed results of anti-candida activity of ROEO on pears. The best anti-candida activity was found against *C. krusei* in 500 µL.L^{-1} with value 74.63%. In this experiment was found the best anti-candida effect with *C. glabrata* test in concentration 250 µL.L^{-1}.

Table 5 showed antimicrobial effect of ROEO on potatoes. The best antimicrobial activity against bacteria tested was found against *Yersinia enterocolitica* in concentration 500 µL.L^{-1} with value 67.50%.

Table 1 *In situ* analysis of the antibacterial activity of the vapor phase of ROEO in apple

Apple				
Bacterial growth inhibition (%)	**bacteria**			
Rosemary EO (µL.L^{-1})	***S. enterica***	***E. faecalis***	***Y. enterocolitica***	***S. aureus***
62.5	13.11 ±0.42^{a}	8.62 ±0.77^{a}	14.88 ±2.29^{a}	7.32 ±0.30^{a}
125	22.22 ±2.05^{b}	17.95 ±0.57^{b}	23.06 ±2.03^{b}	15.71 ±0.97^{b}
250	31.70 ±0.96^{c}	35.93 ±1.51^{c}	37.81 ±0.90^{c}	25.80 ±1.10^{c}
500	46.33 ±0.87^{d}	80.81 ±2.12^{d}	49.90 ±1.65^{d}	43.33 ±1.38^{d}

one-way ANOVA; individual letters (a–d) in upper case indicate the statistical differences between the concentrations; $p \leq 0.05$

Table 2 *In situ* analysis of the anti-yeast's activity of the vapor phase of ROEO in apple

Apple				
Mycelial growth inhibition (%)	**yeast**			
Rosemary EO (µL.L^{-1})	***C. albicans***	***C. glabrata***	***C. krusei***	***C. tropicalis***
62.5	6.77 ±1.03^{a}	12.40 ±0.45^{a}	6.92 ±1.05^{a}	13.06 ±0.71^{a}
125	13.29 ±1.22^{b}	24.77 ±1.05^{b}	13.95 ±0.61^{b}	33.10 ±1.40^{b}
250	46.22 ±2.39^{c}	44.70 ±0.96^{c}	23.33 ±1.24^{c}	45.87 ±2.11^{c}
500	55.45 ±0.69^{d}	65.54 ±0.57^{d}	42.91 ±1.00^{d}	65.29 ±1.63^{d}

one-way ANOVA; individual letters (a–d) in upper case indicate the statistical differences between the concentrations; $p \leq 0.05$

Table 3 *In situ* analysis of the antibacterial activity of the vapor phase of ROEO in pear

Pear				
Bacterial growth inhibition (%)	**bacteria**			
Rosemary EO (µL.L^{-1})	***S. enterica***	***E. faecalis***	***Y. enterocolitica***	***S. aureus***
62.5	11.51 ±0.89^{a}	8.74 ±0.79^{a}	14.50 ±0.98^{a}	8.27 ±0.55^{a}
125	23.66 ±1.06^{b}	13.61 ±1.04^{b}	24.73 ±2.10^{b}	15.71 ±1.16^{b}
250	33.70 ±0.89^{c}	24.24 ±1.57^{c}	32.69 ±1.21^{c}	33.74 ±2.00^{c}
500	64.19 ±1.37^{d}	38.87 ±0.90^{d}	43.34 ±2.18^{d}	43.66 ±1.05^{d}

one-way ANOVA; individual letters (a–d) in upper case indicate the statistical differences between the concentrations; $p \leq 0.05$

Table 6 showed results of anti-candida activity of ROEO on potatoes. The best anti-candida activity was found against *C. glabrata* in 500 μL.L^{-1} with value 87.30%.

The results of the antibacterial activity of ROEO on kohlrabi are presented in Table 7. Intensity of the bacterial inhibition by ROEO increased with the increasing concentration of ROEO in assays across all bacteria tested. The most effective influence has ROEO on kohlrabi model against bacteria *Yersinia enterocolitica* in concentration 500 μL.L^{-1} with value 73.96%.

The results of the anti-yeasts activity of ROEO on kohlrabi are summarized in Table 8. Intensity of the yest growth inhibition by ROEO increased with the increasing concentration of ROEO in assays across all yeasts tested. ROEO on kohlrabi model shows the best antimicrobial activity against yeasts with testing of *C. albicans* in concentration 500 μL.L^{-1} with value 73.80%.

The high antimicrobial activity of *R. officinalis* EO is indicated by the results obtained on previously published studies (Jordán et al., 2013; Lemos et al., 2015). Such antimicrobial activity of the tested EOs may contribute to their use in reducing alimentary pathogens and extending the shelf life of food products or as a potential natural and green substitute for synthetic antibiotics, antifungals, and preservatives in the food and cosmetic industries.

For centuries, *R. officinalis* has been used as a food preservative and flavoring agent, but only recently have the preservative mechanisms and effect been explored. Recent studies have shown very good antibacterial, antifungal and antioxidant activity of rosemary extracts

Table 4 *In situ* analysis of the anti-yeast's activity of the vapor phase of ROEO in pear

Pear				
Mycelial growth inhibition (%)	**yeast**			
Rosemary EO (μL.L^{-1})	***C. albicans***	***C. glabrata***	***C. krusei***	***C. tropicalis***
62.5	23.66 ±1.16^{a}	15.76 ±2.00^{a}	24.50 ±0.57^{a}	8.63 ±0.77^{a}
125	45.22 ±2.66^{b}	23.06 ±1.61^{b}	42.80 ±1.00^{b}	22.73 ±1.06^{b}
250	56.88 ±2.08^{c}	34.66 ±0.98^{c}	55.18 ±1.58^{c}	44.61 ±1.12^{c}
500	25.43 ±1.25^{a}	55.58 ±2.04^{d}	74.63 ±1.00^{d}	68.93 ±1.03^{d}

one-way ANOVA; individual letters (a–d) in upper case indicate the statistical differences between the concentrations; $p \leq 0.05$

Table 5 *In situ* analysis of the antibacterial activity of the vapor phase of ROEO in potato

Potato				
Bacterial growth inhibition (%)	**bacteria**			
Rosemary EO (μL.L^{-1})	***S. enterica***	***E. faecalis***	***Y. enterocolitica***	***S. aureus***
62.5	22.23 ±1.07^{a}	12.10 ±0.31^{a}	22.62 ±0.85^{a}	13.75 ±0.34^{a}
125	34.69 ±1.21^{b}	15.83 ±0.96^{b}	31.60 ±1.00^{b}	22.63 ±0.95^{b}
250	42.52 ±0.57^{c}	24.37 ±2.13^{c}	44.17 ±1.43^{c}	32.73 ±0.89^{c}
500	55.12 ±0.67^{d}	36.15 ±2.01^{d}	67.50 ±1.17^{d}	43.98 ±2.47^{d}

one-way ANOVA; individual letters (a–d) in upper case indicate the statistical differences between the concentrations; $p \leq 0.05$

Table 6 *In situ* analysis of the anti-yeast's activity of the vapor phase of ROEO in potato

Potato				
Mycelial growth inhibition (%)	**yeast**			
Rosemary EO (μL/L)	***C. albicans***	***C. glabrata***	***C. krusei***	***C. tropicalis***
62.5	6.07 ±1.56^{a}	7.75 ±1.79^{a}	6.03 ±1.35^{a}	15.94 ±2.43^{a}
125	16.22 ±2.45^{b}	21.22 ±2.61^{b}	8.02 ±2.06^{ab}	26.65 ±3.14^{b}
250	21.64 ±3.34^{b}	46.82 ±3.27^{c}	12.97 ±2.02^{b}	44.08 ±2.17^{c}
500	34.87 ±4.56^{c}	87.32 ±2.71^{d}	27.64 ±4.90^{c}	78.05 ±3.96^{d}

One-Way ANOVA, Individual letters (a-d) in upper case indicate the statistical differences between the concentrations; $p \leq 0.05$

Table 7 *In situ* analysis of the antibacterial activity of the vapor phase of ROEO in kohlrabi

Kohlrabi				
Bacterial growth inhibition (%)	**bacteria**			
Rosemary EO (µL/L)	***S. enterica***	***E. faecalis***	***Y. enterocolitica***	***S. aureus***
62.5	9.59 ±0.57^{a}	13.08 ±1.47^{a}	11.69 ±1.31^{a}	12.05 ±1.67^{a}
125	22.13 ±1.52^{b}	24.07 ±1.52^{b}	23.24 ±1.98^{b}	23.40 ±1.73^{b}
250	32.32 ±1.17^{c}	34.74 ±1.01^{c}	44.80 ±0.96^{c}	33.81 ±1.06^{c}
500	55.18 ±1.48^{d}	57.66 ±1.13^{d}	73.96 ±2.35^{d}	42.74 ±1.00^{d}

one-way ANOVA; individual letters (a–d) in upper case indicate the statistical differences between the concentrations; $p \leq 0.05$

Table 8 *In situ* analysis of the anti-yeast's activity of the vapor phase of ROEO in kohlrabi

Kohlrabi				
Mycelial growth inhibition (%)	**yeast**			
Rosemary EO (µL/L)	***C. albicans***	***C. glabrata***	***C. krusei***	***C. tropicalis***
62.5	13.62 ±0.87^{a}	11.62 ±0.92^{a}	10.82 ±0.84^{a}	12.63 ±1.12^{a}
125	24.34 ±2.82^{b}	32.73 ±1.11^{b}	24.23 ±1.06^{b}	23.74 ±0.96^{b}
250	46.00 ±2.47^{c}	45.76 ±1.13^{c}	35.40 ±3.58^{c}	34.31 ±1.88^{c}
500	73.80 ±1.05^{d}	57.61 ±0.74^{d}	55.36 ±1.46^{d}	63.36 ±1.64^{d}

one-way ANOVA; individual letters (a–d) in upper case indicate the statistical differences between the concentrations; $p \leq 0.05$

and all these combinations make the plant a very effective inhibitor of foodborne pathogens (Al-Sereiti et al., 1999; Gachkar et al., 2007; Tavassoli et al., 2011; Özcan & Chalchat, 2008). As the public becomes more sceptical of artificial food additives, the demand for safer and more natural preservatives is steadily increasing. With few side effects, rosemary has been identified as a natural preservative that could potentially replace artificial additives (Tavassoli et al., 2011).

Rosemary has shown strong antibacterial and antifungal effects in several studies. The antimicrobial and antioxidant activity of rosemary depends on the chemical composition of the essential oil which can vary greatly depending on climate, location, and time of harvest. Also, antimicrobial activity is determined not only by external factors but also by interactions between its constituents (Jordán et al., 2013).

In various studies, rosemary has been shown to inhibit the growth of bacteria such as *Escherichia coli*, *Listeria monocytogenes* and *Staphylococcus aureus* (Gachkar et al., 2007; Marinas et al., 2012; Oluwatuyi et al., 2004). However, the importance of rosemary's antibacterial effect does not end there. According to a study, rosemary also has the potential to inhibit various bacteria due to overcoming and reducing membrane impermeability (Oluwatuyi et al., 2004). This may prove to be an innovative strategy to eliminate resistant strains of bacteria. Rosemary essential oil may also increase the susceptibility of some bacteria to standard antibiotics (Marinas et al., 2012). This antibacterial activity may make *R. officinalis* a potent defense against common pathogens present in food and may represent a potential preservative that could replace artificial, chemical additives (Tavassoli et al., 2011).

R. officinalis has several different antifungal mechanisms in addition to antimicrobial and antioxidant activity. Results show that the plant essential oil can inhibit *Candida albicans* adhesion by denaturing cellular structures and thereby altering membrane permeability (Cavalcanti et al., 2011). One study suggests that rosemary may even prevent the formation of fungal biofilms. Nanoparticles of rosemary essential oil coat the structure and thus form a nanobiosystem that can significantly inhibit the adherence and thus biofilm of *Candida* fungal strains (Chifiriuc et al., 2012). New strategies are essential alternatives to traditional medicine in the treatment of drug-resistant fungi. The ability to inhibit the growth and aflatoxin production of many fungi contributes to rosemary's potential as an effective food preservative (Rasooli, 2008).

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4 Conclusions

Rosemary has been commonly used since ancient times for culinary, medicinal and ornamental purposes. The biological activities of rosemary essential oil differ from the chemotype of the tested plant. Rosemary is one of the most important natural ingredients in the food industry which is used in the form of essential oils, thanks to its antimicrobial, antioxidant, and antifungal properties. The most effective concentration in our study was 500 μL.L^{-1} and was the most effective in all food models against *Y. enterocolitica* and *C. glabrata*. The strong antibacterial activity against many foodborne pathogens of rosemary essential oil is related to the synergistic and cumulative effect of its volatile components. A successfully tested application of rosemary essential oil as a preservative was in foods with various fruits and vegetables for food packaging.

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