ANTIOXIDATIVE AND ANTIMICROBIAL POTENTIAL OF BITTER ORANGE *Citrus aurantium* L.

Hrvoje Pavlović[1], Iva Mračić Raič[2], Leona Golubov[1], Nela Nedić Tiban[1*]

[1]*Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 18, 31000 Osijek, Croatia*

[2]*University of Dubrovnik, Department for Mediterranean Plants, Marka Marojice 4, 20000 Dubrovnik, Croatia*

[*] *nela.nedic@ptfos.hr*

Introduction

Despite advancements in food production and hygiene, food safety remains a critical public health concern since food-borne illnesses affect up to 30% of individuals in industrialized nations annually. New approaches for decreasing or eliminating food-borne infections are needed, possibly in conjunction with existing methods Essential oils (EOs) are fragrant, oily liquids that can be extracted, fermented, or expressed from plant material such as flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots (Burt, 2004). In addition to their aromatic qualities, essential oils exhibit several medicinal actions, including antioxidant and antibacterial capabilities, along with numerous health advantages, and they have multiple applications in the food, pharmaceutical, perfumery, and cosmetic sectors (Yousefi et al., 2019). The citrus species, a member of the *Rutaceae* family, is an annual plant that abundantly grows in warm areas around the world but is primarily found in the Mediterranean countries of the Middle East and Southern Europe. The majority of citrus species are tiny trees or huge evergreen shrubs, and their fruits rank among the world's most significant tree fruit crops. Due to their widely recognized sensory qualities and numerous health benefits, both oranges could be used as functional ingredients in human diets since they are a source of several major bioactive compounds, such as alkaloids, polyphenolics, carotenoids, and limonoids, with numerous applications in the food, agricultural, and nutraceutical industries. *Citrus aurantium* L. (Figure 1.) - sour orange or bitter orange, is also a source of many active components such as limonene, coumarins, synephrine and flavonoids, including neohesperidin, naringin and tangaretin (Farag et al., 2020).



Figure 1. Bitter orange

The essential oil, extracted from leaves, ripe and unripe peels through different extraction methods (Figure 2.) contains bioactive compounds predominantly composed of linalool and limonene, along with oxygenated and hydrocarbon monoterpenes.

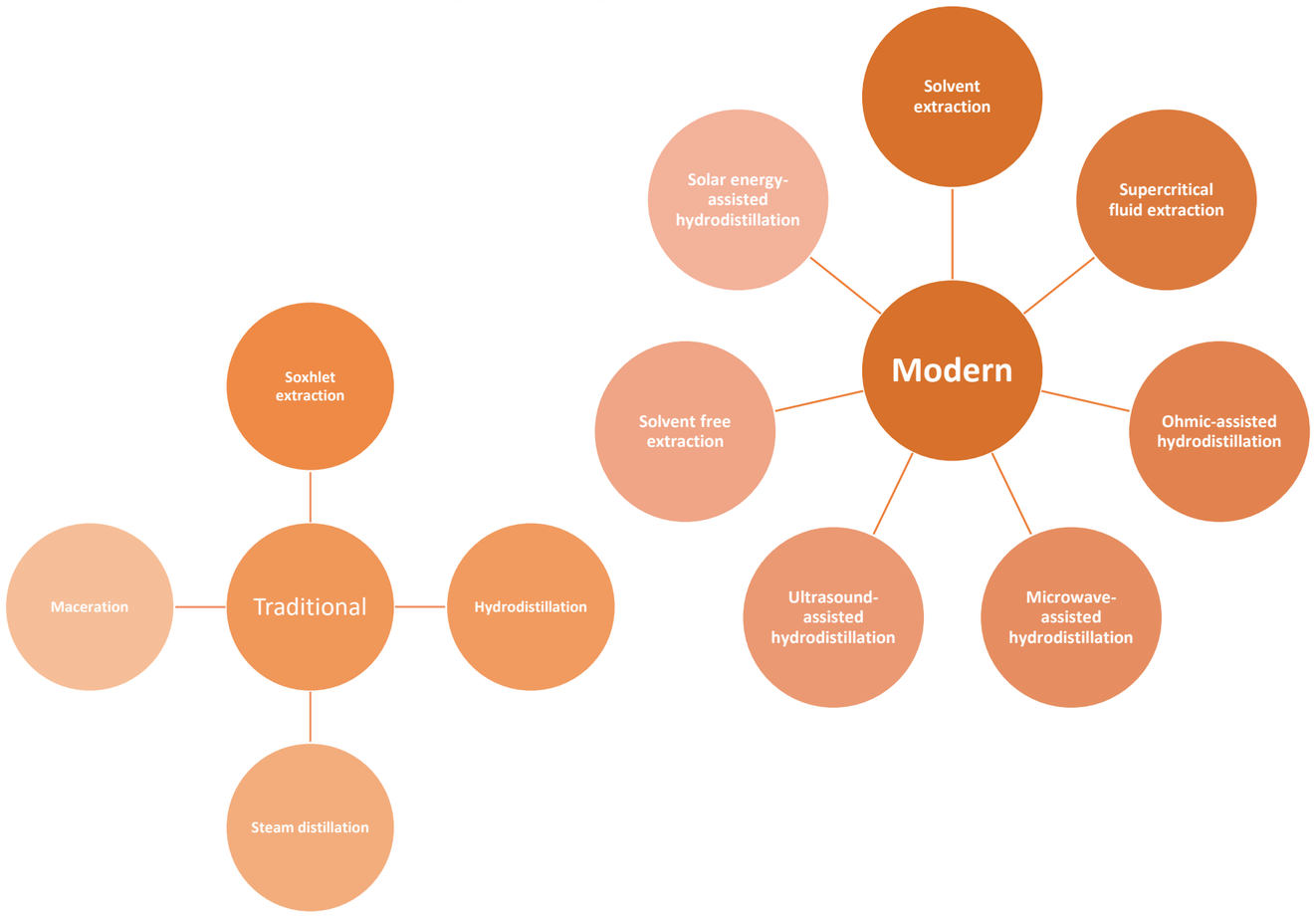

Figure 2. Different bioctive compounds extraction methods (Katekar et al., 2023)

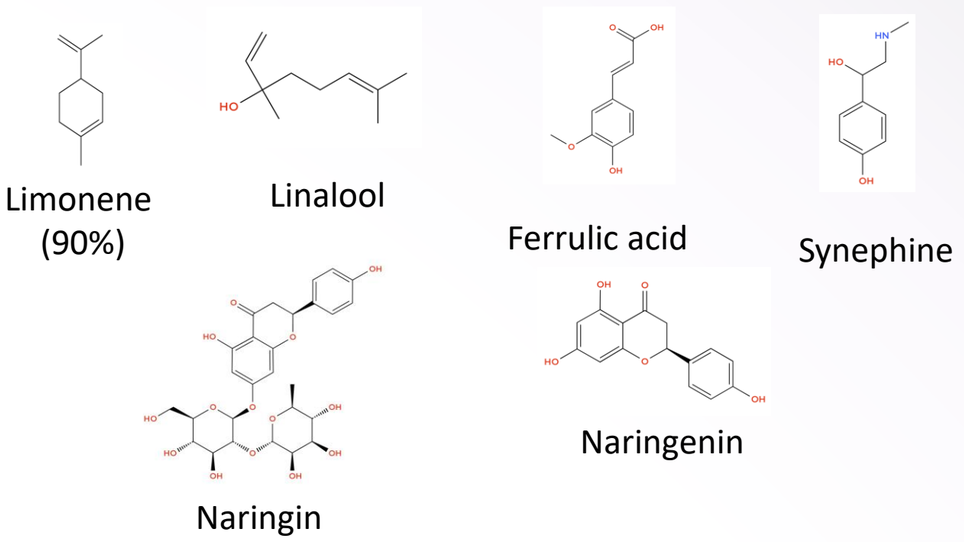

Figure 3. Bioctive compounds in *Citrus aurantium* L.

Antioxidative and antimicrobial activity

The antioxidant capacity of bitter orange essential oils (BOEOs) varies according to the part of the plant and the method of distillation; the strongest activity is noticed in oils extracted from the leaves by steam distillation. Oils from the flowers and bark are rich in limonene and other terpenes that may reduce oxidative stress and inflammatory indicators like TNF-α, even if some leaf extracts may have a reduced phenol concentration. By inhibiting the enzyme acetylcholinesterase, the oils also stand out as possible neuroprotectants. Antioxidant activity frequently correlates with the total concentration of phenolic substances (Elhawary et al. 2024; Hsouna Oulebsir et al. 2022).

BOEOs have broad-spectrum antibacterial action that works against both Gram-positive and Gram-negative bacteria, including *Salmonella enterica* and *Escherichia coli*, as well as *Bacillus cereus* and *Listeria monocytogenes*. Oils from the leaves frequently exhibit more bactericidal activity than those from the bark, with minimum inhibitory concentrations (MICs) ranging from 1.64 to 7.80 mg/mL. It has been demonstrated that the oils prolong the shelf life of foods, including raw chicken, by preventing bacterial growth during storage. The mechanism of action involves disruption to the bacterial cell membrane. They are highly effective against forms of bacteria that are resistant to antibiotics, which makes them intriguing natural preservatives and medicinal agents (Al-Aamri et al. 2018; Ellouze et al., 2024; Hsouna et al., 2013).

The antifungal effect was observed on molds of the genus *Penicillium*, with the species *P. crustosum* being the most sensitive, and the species *P. expansum* and *P. citrinum* being more resistant to the effects of the oil (Kačániová et al., 2020). Strong antifungal inhibition zones of bitter orange essential oil was observed on *Asperillus flavus* and *Fusarium graminearum* (22 mm) while *A. nidulans* and *Alternaria alternata* were more resistant (15 mm) to oil activity (Hsouna et al., 2013).

Conclusions

- ✓ The main bioactive ingredients of BOEOs are limonene, linalool, ferulic acid, synephine, naringin and naringenin.
- ✓ BOEOs can be used to improve antioxidant, antimicrobial and nutritional properties of food.
- ✓ The use of bitter orange by-products in packaging systems is important in promoting sustainability and circular economy.

References

1. Burt S. Essential oils: their antibacterial properties and potential applications in foods-a review. International Journal of Food Microbiology. 2004 Aug;94(3):223–53.
2. Yousefi M, Rahimi-Nasrabadi M, Pourmortazavi SM, Wysokowski M, Jesionowski T, Ehrlich H, et al. Supercritical fluid extraction of essential oils. TrAC Trends in Analytical Chemistry. 2019 Sep;118:182–93.
3. Farag MA, Abib B, Ayad L, Khattab AR. Sweet and bitter oranges: An updated comparative review of their bioactives, nutrition, food quality, therapeutic merits and biowaste valorization practices. Food Chemistry. 2020 Nov;331:127306.
4. Elhawary EA, Nilofar N, Zengin G, Eldahshan OA. Variation of the essential oil components of *Citrus aurantium* leaves upon using different distillation techniques and evaluation of their antioxidant, antidiabetic, and neuroprotective effect against Alzheimer's disease. BMC Complement Med Ther. 2024 Feb 2;24(1):73.
5. Hsouna AB, Hamdi N, Halima NB, Abdelkafi S. Characterization of Essential Oil from *Citrus aurantium* L. Flowers: Antimicrobial and Antioxidant Activities. J Oleo Sci. 2013;62(10):763–72. doi:10.5650/jos.62.763
6. Kačániová M, Terentjeva M, Galovičová L, Ivanišová E, Štefániková J, Valková V, et al. Biological Activity and Antibiofilm Molecular Profile of *Citrus aurantium* Essential Oil and Its Application in a Food Model. Molecules. 2020 Aug 30;25(17):3956.
7. Oulebsir C, Mefti-Korteby H, Djazouli ZE, Zebib B, Merah O. Essential Oil of *Citrus aurantium* L. Leaves: Composition, Antioxidant Activity, Elastase and Collagenase Inhibition. Agronomy. 2022 Jun 18;12(6):1466.
8. Al-Aamri MS, Al-Abousi NM, Al-Jabri SS, Alam T, Khan SA. Chemical composition and in-vitro antioxidant and antimicrobial activity of the essential oil of *Citrus aurantifolia* L. leaves grown in Eastern Oman. Journal of Taibah University Medical Sciences. 2018 Apr;13(2):108–12.
9. Ellouze I, Ben Akacha B, Mekinić IG, Ben Saad R, Kačániová M, Kluz MI, et al. Enhancing Antibacterial Efficacy: Synergistic Effects of *Citrus aurantium* Essential Oil Mixtures against *Escherichia coli* for Food Preservation. Foods. 2024 Sep 27;13(19):3093.
10. Katekar VP, Rao AB, Sardeshpande VR. A cutting-edge assessment of recent advancements in essential oils extraction technologies for energy and ecological sustainability: A comprehensive review. J Food Process Engineering. 2023 Sep;46(9):e14414.

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