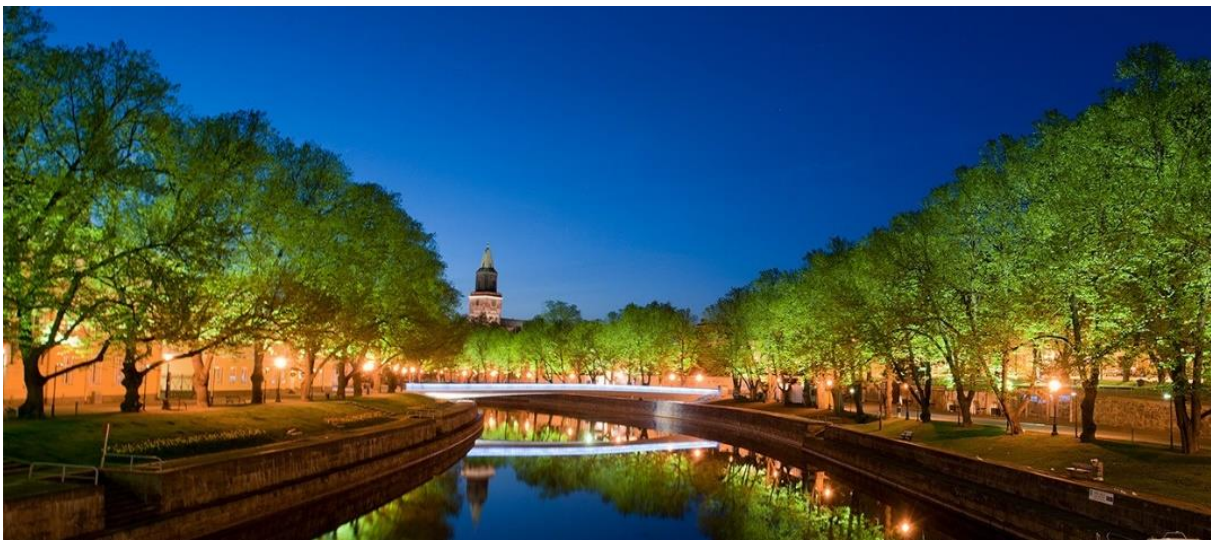

# Polyphenols Communications e-vol.1., no 1.

XXX International Conference on Polyphenols

13-15 July 2021, Turku, Finland

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*Editor:*
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*Printing:*
Painosalama Oy, Turku, Finland

ISSN 2737-064X

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O3.1

# Comparison of different extraction techniques to determine the phenolic compound concentration in olive mill waste water

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## MAIN CONCLUSION

Among the literature, the most favoured method of determining phenolic content of OMWW is through the use of ethyl acetate extraction with hexane defatting. Our study targeted 25 phenolic compounds and their isomers and it was found that with freeze drying and resuspension of the solid in methanol while shaking or ultrasonification, or acidification with ultrasonification, much higher concentrations of phenols could be detected.

## INTRODUCTION

Bio-based phenolic compounds are the subject of increasing scientific interest because of their possible beneficial effects on human health from a renewable source. One such source of bio-based phenolics is found in the olive oil production industry. During three-phase olive oil production olive oil, olive pomace, and olive mill wastewater (OMWW) are generated. Olive oil is the principal fat source of the traditional Mediterranean diet and due to its high content of polyphenols, has been associated with numerous beneficial-human health properties [1]. However, only 2% of the total phenolic content of the milled olive fruit goes to the oil phase, while most resides in the liquid OMWW (≈53%) and solid pomace (≈45%) [2]. In this way, OMWW can act as a cheap source of valuable phenolic compounds which have been investigated by several research groups using different techniques to recover and utilize them as source in natural food additives, pharmaceuticals, and cosmetics [3].

Prior to phenolic compound recovery, the quantity and identification of phenols present in OMWW must be determined. Various procedures to determine the phenol content in OMWW have been studied, but most rely on maximizing the recovery of one compound, hydroxytyrosol, and thus the complexity of the biophenols may be underrepresented. The analysis of phenols in OMWW generally follows similar methods as for phenols from other sources. However, the fact that OMWW is the result of a process that breaks cell walls and exposes the matrix to enzymes, oxygen, and mild heat (during malaxation of the paste) means that the phenol profile undergoes significant changes prior to sampling. This creates a challenge in stabilising the profile at the point of sampling to minimize further changes prior to analysis. The objective of this study was to assess various OMWW extraction methods and their effect on the identification of phenolic compounds.

## MATERIALS & METHODS

OMWW samples were collected from the Oljarna Krožera Franka Marzi olive oil mill (Srgaši, Slovenia) from a three-phase decanter centrifuge. Immediately after sampling, OMWW samples were stored in a freezer (-18 °C) prior to analysis.

Samples were treated via different methods to extract the phenolic compounds. These were characterized by high-performance liquid chromatography (HPLC) coupled to electrospray ionisation and quadrupole time-of-flight mass spectrometer (ESI-QTOF-MS). HPLC equipment incorporated a Poroshell 120 column (EC-C18; 2.7 µm; 3.0 × 150 mm). An elution gradient of 100% water/ formic acid (99.05: 0.5, v/v) (A) towards 100% acetonitrile/ methanol (50: 50, v/v) is used over a period of 20 minutes (flow rate: 0.5 mL/min; injection volume: 1 uL). Extracts were screened for phenolic

compounds previously reported for Olea europaea L. Their presence was confirmed based on accurate mass and fragmentation profiles with literature data and analytical grade standards.

## RESULTS & DISCUSSION

Phenols were extracted via different methods:

- Freeze drying of OMWW. Adding dry matter in methanol while shaking. Filter.
- Freeze drying of OMWW. Adding dry matter in methanol:water (1:1) while shaking. Filter.
- Freeze drying of OMWW. Adding dry matter in methanol. Ultrasonification. Filter
- Ethyl acetate liquid-liquid extraction of OMWW
- Filter OMWW. Get filtrate. Dissolve residue in methanol + filter.

Twenty-five phenolic compounds and their isomers were determined. It was found that the ethyl acetate extraction method, which is the most frequent method used in literature to measure phenolic compounds in OMWW detected lower amounts of phenols than other methods, even the filtration method performed better. Acidifying the OMWW didn't lead to a better performance. The highest phenol concentrations were obtained with freeze drying of OMWW and resuspension of the dry matter in methanol via shaking or ultrasonification. Freeze drying of OMWW and resuspension of the dry matter in methanol:water delivered concentrations for certain phenols similar to the filtration technique, for other compounds similar to the freeze drying with methanol resuspension technique.

Also the influence of acidification and ultrasonification was examined. Ethyl acetate liquid-liquid extraction of acidified OMWW didn't result in higher phenol detection than of normal OMWW. Also acidification of OMWW and filtration didn't increase the phenol yield. However, acidification and 5 min of ultrasonification of OMWW gave two to ten times higher phenol concentrations, depending on the phenolic compound.

Authors acknowledge the European Commission for funding InnoRenew CoE (grant agreement #739574), under the H2020 Widespread-Teaming program, and the Republic of Slovenia (investment funding from the Republic of Slovenia and the European Union's European Regional Development Fund) as well as Proenrich (Grant #792050), under Horizon 2020, Bio-based Industries PPP.

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