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## **Effect of cyclodextrins on the physical properties and stability of crude olive pomace extracts**

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### **Abstract**

Poor technological properties of raw olive pomace extracts (OPE) significantly limits its utilization as nutraceutical and functional food ingredient. Increasing the share of the main olive pomace biophenols (OPB) and improving physical properties of crude OPE are prerequisites for obtaining a usable extract. To achieve that, we examined encapsulation of OPE with different types and amounts of cyclodextrins (CDs).

To determine physical parameters of freeze-dried OPE, CIE LAB color and particle size distribution were analyzed. Stability of crude OPE was examined under accelerated degradation conditions (60 °C, 75% humidity, 45 days). Stability parameters were: 1) total phenolic content; 2) antiradical potential; 3) the amount of OPB determined with HPLC-FLD. In comparison to the native sample (no CD), all the samples containing CDs were significantly lighter, redder, and yellower. It was impossible to carry out particle size analysis for the native sample due to the loss of its powder properties soon after the exposure to the air (the sample absorbed moisture and turned into gummy consistency). The studied stability parameters revealed that randomly methylated  $\beta$ -CD (RAMEB) and hydroxypropyl  $\beta$ -CD (HPB) provided the greatest protection from extracts' disintegration. The examined CDs significantly improved the technological properties of OPE. RAMEB and HPB could be considered as the most appropriate encapsulating agents.

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### **Introduction**

Olive pomace (OP) is a solid residue deriving from the olive oil production. OP cannot be disposed in the environment without prior treatment because of its

high content of polyphenols (PP). The main PP in OP (tyrosol (TS), hydroxytyrosol (HTS), and oleuropein (OLE)) have many positive pharmacological effects but the poor technological properties of raw OPE

significantly limits its utilization as nutraceutical and functional food ingredient.

The addition of encapsulation agents to extraction solvents (such as CDs) represents novel, unconventional approach that enables obtaining extracts with improved technological properties [Ho et al., 2017].

The aim of this study was to investigate both the PP yield and physical properties of crude OPE after encapsulation with different types and amounts of CDs.

## Materials and Methods

The initial raw sample was frozen OP obtained from several two-phase olive mills in Croatia. PP from dried, defatted, and sifted OP were extracted by a previously optimized ultrasound assisted extraction procedure [Vitali Čepo et al., 2016]. Different types and amounts of the CDs were investigated:  $\beta$ -CD (B), randomly methylated  $\beta$ -CD (RAMEB), hydroxypropyl  $\beta$ -CD (HPB), and  $\gamma$ -CD (G), in two concentration levels (8 mg/ml and 16 mg/ml\*).

To determine physical parameters of freeze-dried extracts, CIE LAB color and particle size distribution were analyzed.

Stability of the extracts was examined under accelerated degradation conditions (60 °C, 75% humidity, 45 days) [Gomez-Plaza et al., 2006] [Durante et al., 2016]. Stability parameters were: 1) percentage of total phenolic content (TPC) examined with Folin-Ciocalteu method [Singleton and Rossi, 1965]; 2) antiradical potential examined with TEAC method [Re et al., 1999]; 3) the content of TS, HTS, and OLE determined by using high performance liquid chromatography with fluorescent detector (HPLC-FLD) [Vitali Čepo et al., 2017].

## Results and Discussion

In comparison to the native sample (no CD), all the samples containing CDs were significantly brighter, redder, and yellower (Figure 1). Investigating OPE as an antioxidative functional food ingredient, the brightness of the sample might be considered as a preferred characteristic since it diminishes the possibility of affecting original food color. Perceived difference in color ( $\Delta E$ ) was higher than 12 in all the

encapsulated samples indicating very big difference comparing to the native sample.

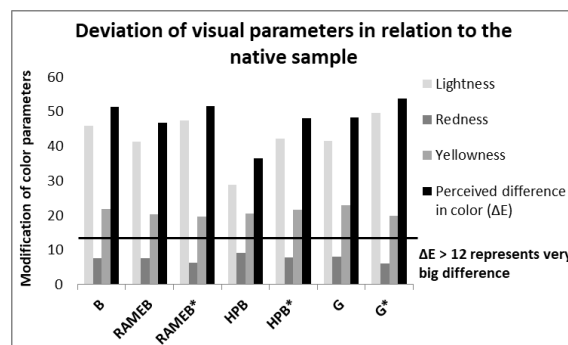


Figure 1 CIE-LAB analysis of crude olive pomace extracts.

It was impossible to carry out particle size analysis for the native sample due to the loss of its powder properties soon after exposure to the air (the sample absorbed moisture and turned into gummy consistency). Addition of CDs significantly changed the appearance of OPE (extracts appear as fine powders after freeze drying). Analysis of extracts containing CDs (Figure 2) showed that the one extracted with G (at the concentration level of 8 mg/mL) had the largest diameter (44.31  $\mu\text{m} \pm 5.18 \mu\text{m}$ ), while the samples extracted with RAMEB\* (at the concentration level of 16 mg/mL) had the smallest diameter (13,35  $\mu\text{m} \pm 4,98 \mu\text{m}$ ).

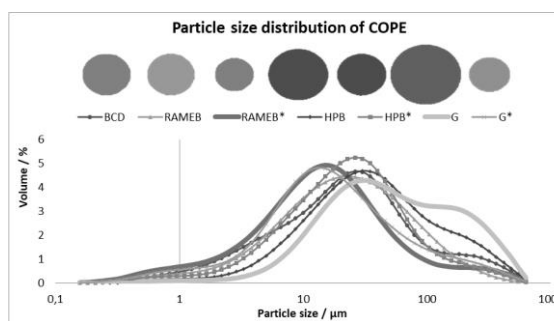
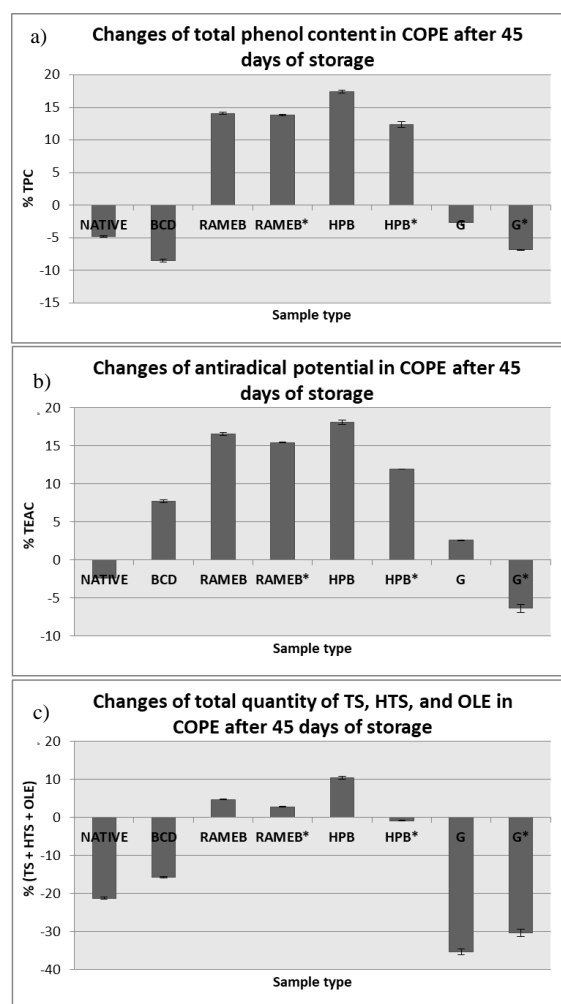


Figure 2 Particle size distribution analysis of crude olive pomace extracts that contains different types and amounts of CDs. Circles indicate the graphical representation of their particle size distribution mutual ratio.

With the reference to TPC, Figure 3a reveals that RAMEB and HPB increased the final proportion of phenols on average by 19 % (in comparison to the native sample during storage), while B and G did not improve extracts' stability. The same trend was observed studying antiradical potential with TEAC analysis (Figure 3b) and determining TS, HTS, and OLE share with HPLC-FLD (Figure 3c).

B and G did not produce any protective effect during storage. The results can be explained with the fact that the protective effect is mainly depended on the structure of the formed complexes between excipient and the target compound. If the reactive moiety of the molecule of interest is located inside of the cyclodextrin cavity, its degradation is reduced. Since the cavity of G is wider than the cavity of B and its derivatives (HPB and RAMEB), our assumption is that CDs with smaller cavity provide better protection of OPB. Furthermore, reactive groups on the cyclodextrin core also interact with molecule of interest and could catalyze its degradation.



**Figure 3** Changes of a) TPC; b) antiradical potential; c) total quantity of TS, HTS, and OLE in COPE after 45 days of storage.

## Conclusion

The possibility of utilizing CDs for the improvement of technological properties and the stability of crude OPE has been successfully applied for the first time. Our work showed that CDs significantly improve the

technological properties of crude OPE. Among them RAMEB and HPB could be considered as the most appropriate encapsulating agents since they show the greatest protective effect regarding stability.

## Acknowledgements

This work has been fully supported by Croatian science foundation (HRZZ) under the project UIP-2014-09-9143 (Valorization of olive waste in sustainable food innovation (NutriOliWa)).

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