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# A performance comparison of olive oil mill wastewater enzymatic treatments

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ABSTRACT

*Aspergillus niger* enzyme preparation, locally produced, and six commercial fungal enzyme preparations were assessed for olive oil mill wastewater (OOMW) hydrolytic treatment. The processes performances were evaluated in terms of hydroxytyrosol (HT) and reducing carbohydrates (RC) release yields, and enzyme productivities. Enzymatic hydrolysis of OOMW of the tested enzyme preparations had shown an increase of HT and RC release yields. These ranged between 0.42 and 0.87% (g HT produced/100 g dry matter), and between 3.06 and 9.7% (g RC/100 g dry matter), respectively. The kinetic parameters (maximal release rate and half-saturation constant) determined by an alternative approach to the classical Michaelis-Menten model has shown that produced enzyme preparation had an initial release rate of 0.02 g HT/L min, which is comparable to rates obtained in the presence of Viscozyme L, Novarom Blanc and Peclyve Lif Plus. The antioxidant activity of the ethyl acetate extract of OOMW, measured by the DPPH method, was drastically enhanced after the different enzymatic treatments. This study demonstrates that enzymatic treatment is a potential method for the valorization of OOMW in order to produce valuable compounds with promising applications in the food industry.



## 1. Introduction

Olive oil mill wastewater (OOMW) is the liquid effluent produced during the extraction process of olive oil. The annual production of OOMW worldwide was estimated to be over 20 million $m^3$, and its disposal represents a huge environmental problem. The typical phytotoxicity and low biodegradability of OOMW are mainly due to the high organic matter contents as well as the presence of relevant amounts of polyphenols (up to 10 g/L) (Dammak et al., 2015a).

A number of reports have appeared in the literature attempting the valorization of OOMW to recover valuable compounds from OOMW (Dammak et al., 2016; Takaç and Karakaya, 2009) and to find a solution for the management of this liquid residue (Justino et al., 2010; Khoufi et al., 2009).

Enzymatic treatment is an interesting method for the valorization of OOMW due to several reasons; firstly, because of the release of monomeric polyphenols from glycosides, several phenolic compounds are contained in glycoside's chemical structure through a phenolic group bounded to the carbohydrate group via a glycosidic bound; and, secondly, the saccharification of carbohydrates found in OOMW (around 20 g/L) to fermentable carbohydrates stream rich in glucose that will be useful for further anaerobic digestion (Hamdi, 1993). The enzymatic treatment is advantageous compared with the chemical treatment, due to its mild operating conditions regarding pH and temperature and the absence of toxic organic solvents.

Among olive polyphenols, hydroxytyrosol is the major monomeric polyphenol in OOMW known by its high

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**Nomenclature**

*Abbreviations*

| | |
|---|---|
| BHT | butylated hydroxytoluene |
| CMC | carboxymethyl cellulose |
| DM | dry matter |
| DPPH | 1,1-diphenyl-2-picrylhydrazyl |
| EP | enzyme protein |
| HT | hydroxytyrosol |
| OOMW | olive oil mill wastewater |
| RC | reducing carbohydrates |

*Variables*

| | |
|---|---|
| $[E_0]$ | initial enzyme concentration (mg EP/g DM) |
| $C_{HT\ final}$ | final concentration of hydroxytyrosol after 6 h of treatment (g/L) |
| $C_{HT\ initial}$ | initial concentration of hydroxytyrosol (g/L) |
| $DM_{initial}$ | initial dry matter content in OOMW sample (g/L) |
| $IC_{50}$ | half maximal inhibitory concentration (μg/mL) |
| $K_e$ | half-saturation constant (mg EP/g DM) |
| $M_{enz}$ | mass of enzyme protein added in the experiment (mg) |
| $P_{enz}$ | enzyme productivity (g HT/mg EP) |
| $V_{final}$ | final volume of OOMW sample (L) |
| $Y_{HT}$ | hydroxytyrosol release yield (g HT/g DM) |

*Subscripts*

| | |
|---|---|
| enz | enzyme |
| HT | hydroxytyrosol |
| RC | reducing carbohydrates |

*Greek symbols*

| | |
|---|---|
| $\nu_{max}$ | maximal release rate (g/L min) |
| $\nu_0$ | initial release rate (g/L min) |

antioxidant activity compared with the other olive polyphenols (Obied et al., 2005a). Oleuropein, which is an ester of hydroxytyrosol and elenolic acid glycoside, is a typical glycoside contained in OOMW in high amounts (60–90 mg/g dry matter) (Dammak et al., 2014).

Oleuropein and other olive polyphenols (e.g. verbascoside and hydroxytyrosol glucoside) contained hydroxytyrosol moieties in their chemical structure, which could be released by the action of β-glucosidase and esterase (Fig. 1) (Dammak et al., 2015b; Hamza et al., 2012; Khoufi et al., 2011; Boskou et al., 2006).

β-glucosidases (EC 3.2.1.21) catalyze the hydrolysis of carbohydrates and break the bonds present in glycosides, such as verbascoside and hydroxytyrosol glucoside, releasing hydroxytyrosol or glucose (Bhatia et al., 2002). β-glucosidases are enzymes produced by various filamentous fungi, mainly *Aspergillus* and *Penicillium* in the presence of cellulose. The major commercial applications of β-glucosidases are flavor enhancement of fruit juices and wine through the release of aroma compounds from glycoside precursors (Daenen et al., 2008). β-glucosidases' application in OOMW might contribute to the release of hydroxytyrosol by the action of β-glucosidases on oleuropein, which would be beneficial, since this compound is supposed to have significant antioxidant power (Visioli et al., 2002; Hamza et al., 2011).

**Table 1 – Physical parameters and composition of olive oil mill wastewater (OOMW) used in this study. (Values are means ± standard deviation of three determinations).**

| Parameter | Value |
|---|---|
| pH (–) | 4.59 ± 0.02 |
| Viscosity at 25 °C (mPa s) | 5.30 ± 0.06 |
| Conductivity at 25 °C (mS/cm) | 11.1 ± 0.10 |
| Fat (kg/m$^3$) | 4.57 ± 0.21 |
| Reducing carbohydrates (kg/m$^3$) | 32.77 ± 1.26 |
| Dry matter (kg/m$^3$) | 178.5 ± 2.84 |
| Density at 25 °C (kg/m$^3$) | 1040 ± 0.04 |

While different studies have shown that fungal enzyme preparation is technically possible to apply for the increase of hydroxytyrosol amount in OOMW, no work has yet studied the process performance and reaction kinetics for eventual process optimization. Little consideration has been given to the process performance parameters and how various enzyme preparations could be applied to show its different impact on hydroxytyrosol of major products and the reducing carbohydrates. Most enzyme hydrolysis studies have attempted to show the effect of enzyme preparation for the enhancement of hydroxytyrosol quantity, irrespective of process performance, with the assumption that β-glucosidase is the principal enzyme responsible for the release of hydroxytyrosol. Optimization of OOMW hydrolysis by enzymes requires a good knowledge of the kinetic reaction. The complexity of the enzymic hydrolysis of OOMW was observed from the fact that they are heterogeneous insoluble substrates, and, thus, their enzymic hydrolysis is always limited.

In this study, enzymatic hydrolysis of OOMW was investigated by using different fungal enzyme preparations. The hydrolysis efficiency of OOMW was evaluated in terms of hydroxytyrosol and the reducing carbohydrate release yields and enzyme productivities. Kinetic parameters of hydroxytyrosol and the reducing carbohydrate releases were also determined using an alternative approach to the Michaelis-Menten model. Antioxidant activities of ethyl acetate extract from hydrolyzed OOMW were analyzed and compared to untreated sample.

## 2. Materials and methods

### 2.1. Materials

OOMW sample was collected from an olive oil producing plant located in Sfax (southern Tunisia) during the olive oil crop season (November, 2014). A three-phase cycle modular machine (Gruppo Pieralisi, Jesi, Italy) was used for extracting the olive oil. The composition of OOMW sample is shown in Table 1. Collected OOMW sample was defatted using hexane at room temperature to remove the remaining olive oil. Then the defatted OOMW sample was kept frozen at −20 °C before enzymatic hydrolysis to avoid any chemical changes (Dammak et al., 2016; Obied et al., 2005b).

A local enzyme preparation rich in β-glucosidase was used for OOMW hydrolysis. This preparation was obtained through the solid state fermentation of wheat bran by *Aspergillus niger* ATCC 16404. Wheat bran was used as sole carbon source. After 7 days of static culture in wheat bran, a sample of 100 g of fermented medium was supplemented with 1 L of acetate buffer $50 \times 10^{-3}$ mol/L (pH 4.8). The mixture was stirred for 3 h at 160 rpm at room temperature. The suspension was filtered and