Consideration of Onion Solid By-Products Homogenate in Phenolics Decontamination of Olive Mill Waste Management.

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Abstract

The aim of this thesis was to treat olive oil mill waste with onion peroxidase with regard to the removal of polyphenols and decolonization. Determination of the optimum conditions for the treatment of olive mill wastewater was another objective of this study. Results showed a pH optimum of around 2.65 and a concentration of H_2O_2 of around 2.58 mM. Moreover o-diphenols could be removed in a short period of time in comparison to the total phenols. The results showed that 3 hours were sufficient for 75% removal of o-diphenols. Dilution 1 over 20 of the olive mill wastewater and the addition of some additives (NaCl and PEG) in the treatment of Olive Mill Waste (OMW) could enhance the efficiency of total phenol removal as well as, a decrease in the color intensity of the olive oil wastewater. In addition, HPLC analyses were carried out on samples representing OMW before and after the treatment. Results showed that the degradation of some phenols was carried out with the use of onion peroxidase, as well as with the use of the optimal conditions regarding the pH and the concentration of hydrogen peroxide. It was concluded that onion peroxidase could catalyze the degradation of total phenols and o-diphenols and that such degradation could also influence the change in color and the antioxidant activity of the olive oil mill wastewater**.**

Keywords: Homogenate**,** Olive Mill Waste, Management, Onion By-Product, Phenolics, Wastewater.

1. Introduction

Current methods for removing phenols from industrial waste waters include solvent extraction, microbial degradation, adsorption on activated carbon, chemical oxidation, and so on. Although these methods are effective, they suffer from such shortcomings as high cost, incompleteness of purification, formation of hazardous byproducts, and applicability to only a limited concentration range (Tong et al., 1997, Aparicio and Harwood, 2013).

Wastewater sludge can be viewed as a two-phase system-a solid network of hydrophilic polymeric materials enclosing a liquid (water) within. It is possible to enzymatically attack this complex bioreactor in order to recover valuable resources, remove toxic materials and recover the water (Whiteley and Lee, 2006).

The idea of using peroxidase, laccase and/or tyrosinase enzymes for the removal of phenols and aromatic amines from wastewater emerged in the 1980(s), and research in this field is still going on (Kauffmann et al., 1999). The majority of studies have focused on using horseradish peroxidase (HRP), which has a proven ability to remove a variety of phenolic contaminants. However, one of the major challenges associated with peroxidaseis the prohibitive cost of the enzyme (Duran and Esposito, 2000).

This problem is then circumvented by using a less expensive source of enzyme such as soybean peroxidase (SBP) since the seed coat of the soybean was identified as a rich source of peroxidase (Wright and Nicell, 1999). Also, as wide varieties of aromatic compounds were oxidized by other kind of peroxidases such as the *Coprinus cinereus* (CiP), *Coprinus macrorhizus* (CMP), and *Arthromyces ramosus* (ARP), (Villalobos and Buchanan, 2002).

A major obstacle in the commercial application of peroxidase for environmental purposes is its limited stability and reusability, which means that a continuous supply of a large amount of fresh enzyme is required. Enzyme immobilization is one of the strategies known to increase enzyme stability and reusability (Garcia-Orenes et al., 2017). For example, the results showed that after the use of immobilized bitter ground peroxidase (BGP), a maximum removal of phenols with the presence of hydrogen peroxide was observed. This observation was in agreement with those earlier reported by using HRP, SBP, and Turnip peroxidase (TP), (Akhtar and Husain, 2006).

Olive Oil Wastewaters (OOMW) contains large amounts of organic and mineral matter that are hard to degrade (Ayed et al., 2005). The use of peroxidase enzymes and hydrogen peroxide for the treatment of wastewaters has been the focus of extensive research since 1980 (Wright and Nicell, 1999, Elhag et al., 2017).

In the presence of hydrogen peroxide (H_2O_2), which acts as an electron acceptor, peroxidase enzymes (PE) catalyze the oxidative polymerization of phenols, anilines and other aromatics to insoluble oligomers. These insoluble oligomers can then be removed through a simple sedimentation or filtration system (Kennedy et al., 2002). Oxidative enzymes such as peroxidase play an important role in the decontamination of effluents. Although very little research on the use of plant materials (roots, tissues, etc.) as an enzyme source has been carried out, these are still good alternatives due to their potentially lower cost (Duran and Esposito, 2000).

Onion (*Allium cepa* L.) has played an important role in culinary, as well as in dietary, medicinal and religious roles for centuries. It may be one of the first cultivated crops because of its growing versatility and portability, and can be dried and preserved for long periods. However, during storage, onion bulbs are exposed to environmental and atmospheric conditions which can affect

their physiology and biochemistry. During this period, high catabolism is considered to be the main cause of changes in qualities such as sprouting and rotting (Benkeblia and Shiomi, 2004).

For many years, it has been believed that onion waste has no value, but recently a window of opportunity for onion waste was opened allowing it to access a whole range of market applications including the extraction and valorization of many photochemicals from this waste (Garrote et al., 2004).

The general aim of this work was to evaluate the effect of using peroxidase extracted from onion and to test its capacity in reducing the organic and phenolic contents of olive mill wastewater. The major task is to use of additives such as sodium chloride. In this way, instead of using distilled water for the dilution of the OOMW, sea water could be used; and the use of polyethylene glycone could help in the removal of phenolic compounds.

2. Materials and Methods

2.1. Collection of OOMW and Onions

The OOMW was collected during the 2014-2015 harvest season in the Chania region, Crete, from mills using two-phase extraction. OOMW was filtered using a filter paper and kept at 4°C immediately after receipt. For all determinations, OOMW was diluted 1:100 with water, except for the color determination, where the sample was measured without dilution. For all treatments OOMW was dilute 1/20. Meanwhile, Onions were obtained from a local catering facility, and stored at room temperature in the dark.

2.2. Determination of total Phenols

The content of peroxidase is higher in the apical trimmings and lowest in the outer dry layers in onions (Liao and Ku, 2012). An aliquot of 0.1ml of quercetin (0.5 mM) was mixed with 0.1 ml of the cell free extract and then 0.8 ml of H_2O_2 (3 mM in the buffer solution) was added and the absorbance was obtained at 370 nm for quercetin each 5 sec up to 1 min. One enzyme unit was defined as AA370 per sec and the specific activity was defined as U per mg of protein.

2.2.1. Preparation of the standard solution

100 mg of Caffeic acid was dissolved in 20 ml of DMF, with initial concentration of 5000 mg/1, and then dilution of the solution was carried out to obtain the following concentrations: 12.5, 25, 50, 100 and 200 mg/1.

2.2.2. Sample preparation

Olive oil wastewater was diluted into 1/1000 for the analysis. Subsequently 0.3 ml of water was added, 0.5 ml of the diluted OOMW, 0.1 ml of 4-AAP, and 0.1 ml of PF. The analysis was done in triplicate.

2.2.3. Measurement of Total Phenols

A colorimetric method was developed to measure phenol concentration using two reagents:

• 4-aminoantipyrine solution (20 mM in 0.25M NaHCOa),

• Potassium ferricyanide solution (85 mM in 0.25M NaHCOs),

The assay used 100 µl of PF, 100 µl of 4AAP, 500 µl of the sample and 300 µl of water.

The absorbance was obtained at 510 nm.

2.3. Determination of 0-Diphenols

An initial solution of chlorogenic acid, of 12 mg/1 was prepared and further dilutions were done to obtain concentrations 0.75, 1.5, 3, 6 and 12 mg/1. OOMW was diluted into 1/100 for the analysis. The analysis was done in triplicate.

2.3.1. Measure of the o-diphenol

An aliquot of 0.1ml sample was mixed with 0.2 ml HC1 (0.5M), 0.2 ml sodium nitrite (10%w/v), 0.2 ml sodium molybdate (10% w/v), 0.2 ml NaOH (2M) and 0.1 ml distilled water. The mixture was incubated at room temperature for 10 mn, and the absorbance was obtained at 525 nm.

2.4. Color Determination

For the color determination, the sample was measured directly without any preparation and the absorbance was obtained at 465 nm. Buffer solutions of 2.65, 3.94, 5.15 and 6.89 were prepared in order to determine the color of the initial concentration.

2.5. Chemiluminescence

Chemilumenescence measurements were carried out on a Fluorimeter-Luminometer, keeping the light off and using only the photomultiplier of the apparatus. The following solutions were used for all chemiluminescent measurements and were prepared weekly:

1. Boric acid buffer (0.05 M): 3.1g of boric acid was weighted and dissolved into 1L of dieonised water using a volumetric flask. pH of the solution was adjusted to 9 using a NaOH solution (1M).

2. Luminol solution: ($5.6xl0^{-4}$): 25mg of luminol was weighted and dissolved into 250ml of the above borate buffer (pH=9) using a volumetric flask which was kept in dark flask.
3. Co (II)/EDTA in borate buffer: 20 mg of $CoCl_2.6H_2O$, ($8.4x10^{-4}$M) was weighed and mixed with lg of EDTA ($2.63xl0^{-3}$) and then dissolved into 100ml of borate buffer in a volumetric flask.
4. Hydrogen peroxide ($5.4xl0^{-3}$): 11ml of the 30% (W/W) stock solution (9.8M) was diluted to 100ml with deionised water in a volumetric flask (1.08M). 5ml of this solution was diluted to 100ml with deionised water in a volumetric flask ($5.4xl0^{-2}$M). 5ml of the last solution was diluted to 50ml with deionised water in a volumetric flask. The solution should be prepared new each week because it is degraded.

2.6. Extraction of polyphenols

The extraction was done by lowering the pH of 30 ml of OOMW to 2 by using HCl (1N). Afterward further extractions were done by using a mixture of two solvents, ethyl acetate and the diethyl ether (8:2); the extractions were done 3 times successively by taking 40ml of the solvent mixture. Sodium sulfate was used in the mixture in order to eliminate traces of water. Finally the solvents were evaporated in a rotary evaporator and the residue was dissolved in ethanol (4ml) and stored in the refrigerator at -20°C.

2.7. High Performance Liquid Chromatography (HPLC) Analyses

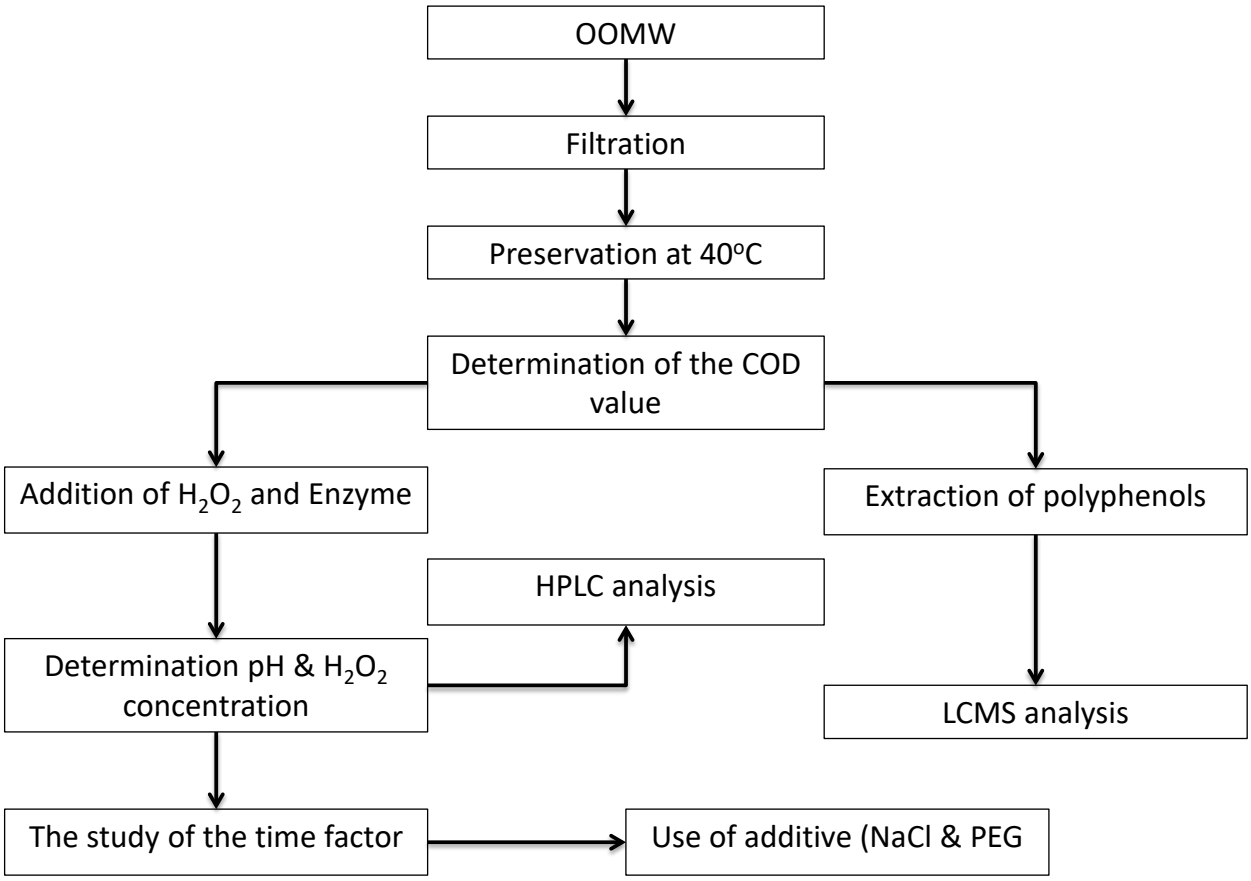
The equipment utilized was an HP 1090, series II liquid chromatograph, coupled with a diode array detector and controlled by Agilent ChemStation software. The column was a LiChrosphore PR18, 5µm, 250 x 4mm (Merck, protected by a guard volume packed with the same material). Both columns were maintained at 40°C. Eluent (A) and eluent (B) were 0.5 % formic acid and

MeCN, respectively. The flow rate was 1 mL min^{-1}, and the elution program used as follows: 0-5 min, 5% B, 5-4 min, 100% B, 45-55min, 100% B. Monitoring of the eluate was performed at 275 and 320nm.

2.8. Measure of the COD value

The closed reflux colorimetric method was used to determine COD value. Each time the appropriate amount of sample was introduced into commercially available digestion solution (Hach, Europe, Belgium), and the mixture was then incubated for 120 mn in a COD reactor (Model 45600-Hach Company, USA). The COD concentration was measured colorimetrically using a DR/2010 Spectrophotometer (Hach Company, USA).

2.9. Experimental Overview



3. Results and Discussion

The results showed a pH optimum of around 2.65, where the highest removal of the total phenols was obtained, in comparison to the initial phenol concentration. Furthermore, by increasing the concentration of H_2O_2 from 0.258 mM to 5.15mM the removal efficiency of the total phenols and o-diphenols increased but it was optimal at around 2.58 mM. Maximum phenolic removal was around 48% of the initial phenolic content.

Color intensity was decreased gradually from 0.63 and it showed the highest color decrease at a pH of around 2.65 and at a concentration of H_2O_2 of approximately 2.58 mM, where the intensity reached was 60% less than the initial one.

From the results given in Figure (2), it was clearly emerged that phenolic compounds present in the olive oil wastewater have an antioxidant activity. The non-treated wastewater showed an antioxidant activity of around 40.1; after the treatment the activity changed with the use of a different pH. At a pH of 2.65, the antioxidant activity was around 51.2 and it was the highest activity in comparison to a pH of 3.94 and a pH of 5.15. At 6.89, the activity was also high.

Results showed clearly that the dilution 1 over 20 could have an effect on:

• The efficiency of total phenol removal,

• The decrease in the color intensity of the olive oil wastewater, and

• The antioxidant activity.

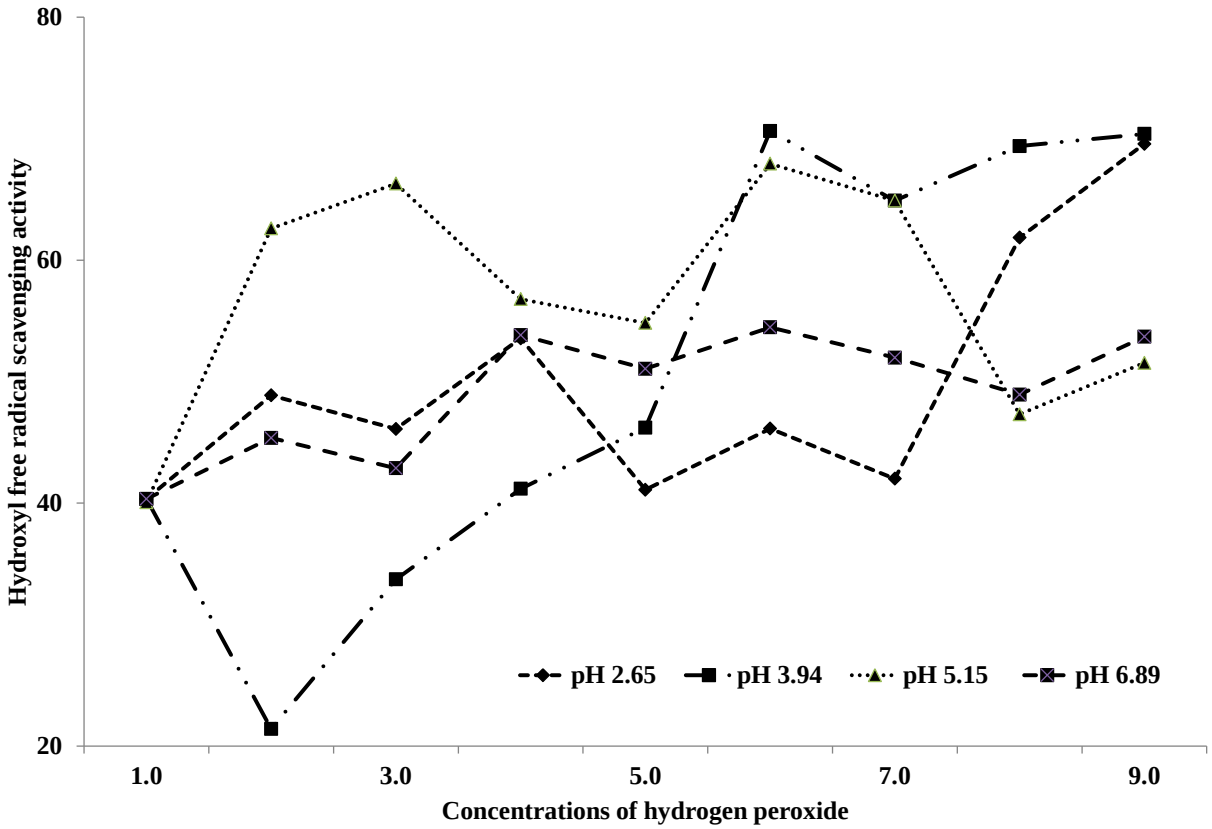


Figure 2. The antioxidant activity of the treated olive oil wastewater by crude peroxidase homogenate with the use of different ranges of pH and different concentrations of hydrogen peroxide. The treatment was allowed to proceed for 3h with H_20_2 concentrations: 0.258, 0.515, 0.7, 1.03, 1.3, 2.58, 3.4, and 5.15 mM).

The concentration of total phenols was reduced to 48% with the use of 1/20 dilution of the OMWW, as shown in Figure (3). The concentration of o-diphenol started to decrease after starting the dilution of the olive oil wastewater, but an unusual increase was illustrated at a dilution of 1/5, 1/10 and 1/20, as shown in Figure (4). Dilution 1/20 showed the highest antioxidant activity in comparison with the other dilutions as shown in Figure (5).

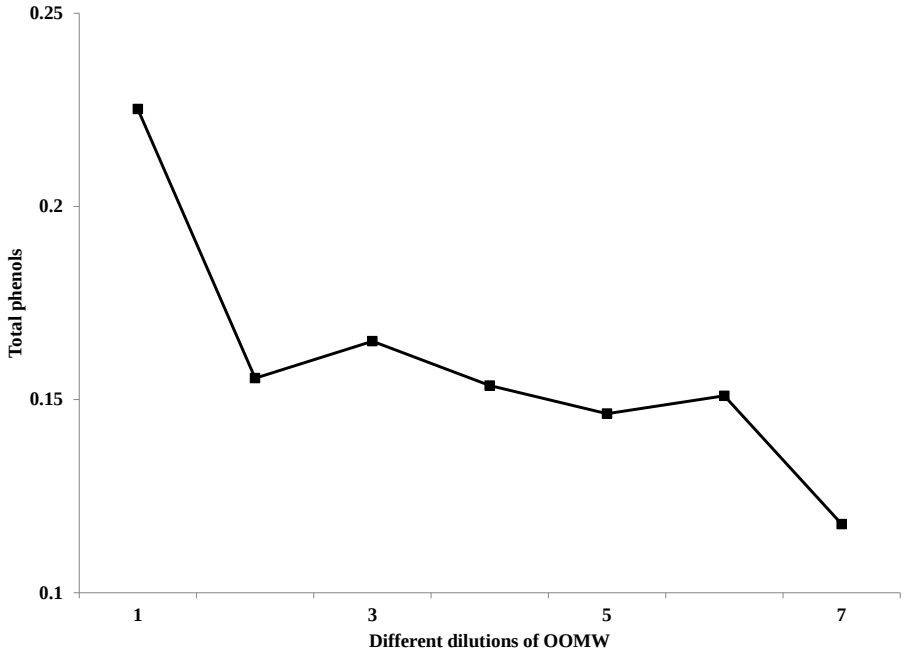


Figure 3. Removal of total phenols concentration from the olive oil wastewater treated by the use of crude peroxidase homogenate, a pH of 2.65 and a concentration of hydrogen peroxide of 2.58 mM. Wastewater was diluted in ranges of 1/2, 1/3, 1/5,1/8,1/10 and 1/20.

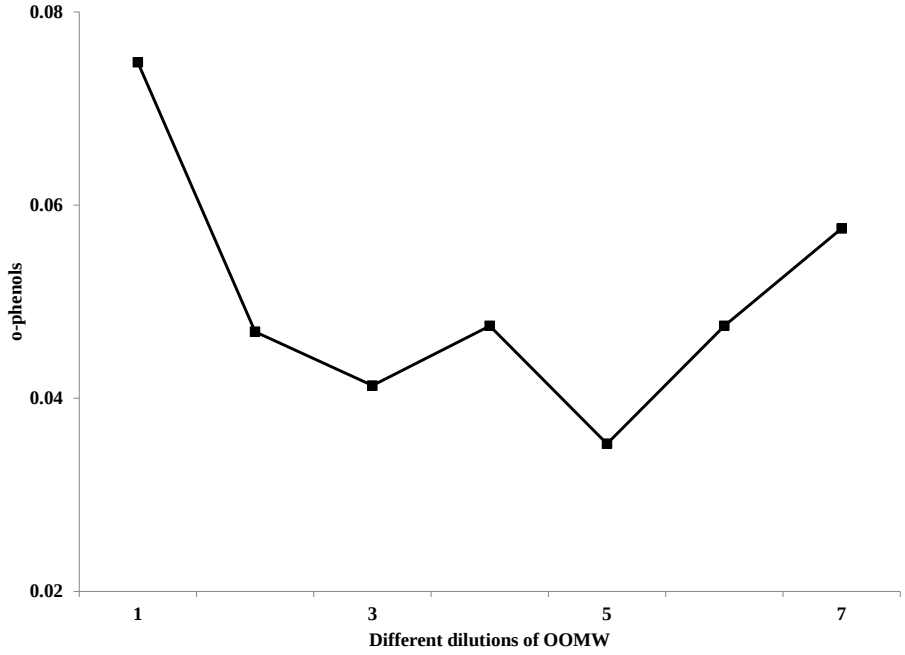


Figure 4. Removal of o-phenols concentration from the olive oil wastewater treated by the use of crude peroxidase homogenate, a pH of 2.65 and a concentration of hydrogen peroxide of 2.58 mM. Wastewater was diluted in ranges of 1/2, 1/3, 1/5,1/8,1/10 and 1/20.

The color intensity increased after half dilution of the olive oil wastewater. At a dilution of 1/3 and 1/5, the color intensity started to decrease, but it was higher than the initial value. The color intensity at the dilutions 1/8, 1/10 and 1/20 was 0.7, 0.6 and 0.25 respectively, where a large decrease at the last dilution around 60% was observed as shown in Figure (6).

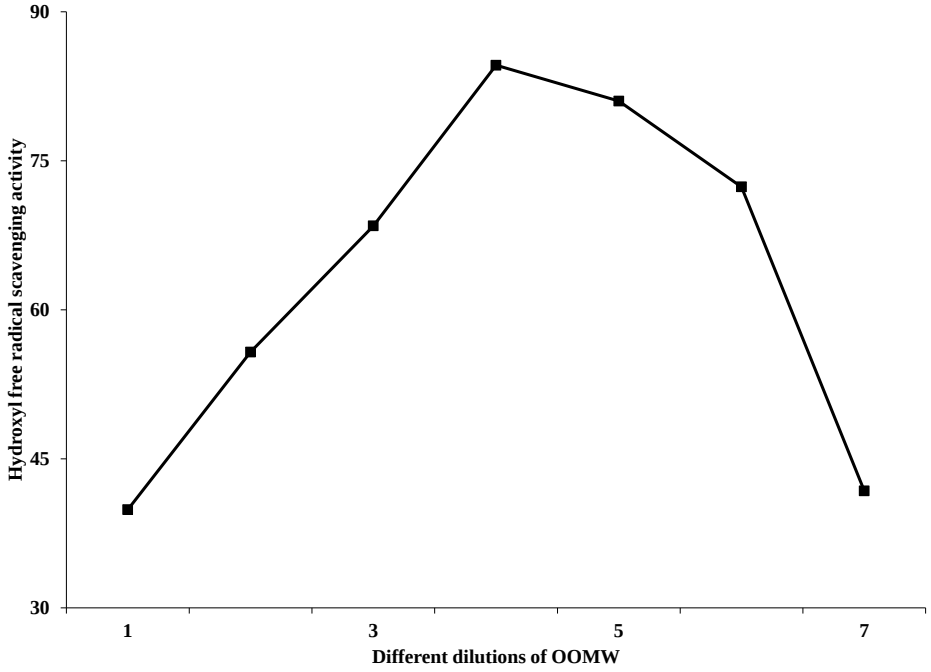


Figure 5. Antioxidant activity of the olive oil wastewater treated by the use of crude peroxidase homogenate, a pH of 2.65 and a concentration of hydrogen peroxide of 2.58 mM. Wastewater was diluted in ranges of 1/2, 1/3, 1/5,1/8,1/10 and 1/20.

Treatment was carried out using the same optimal conditions for the pH and the concentration of hydrogen peroxide for three hours before starting the treatment, NaCl was added at concentrations of 0.5, 1, 2, 3, 4 and 5%. Results showed that 2 and 3% of NaCl could improve the efficiency of total phenols and o-diphenols removal. The color intensity decreased from 0.63, which was the initial value, to around 0.3, i.e. a decrease of approximately 56%. It stayed low except for at 4% NaCl where there was a small increase up to 0.4, as shown in Figure (7). The antioxidant activity was higher with the use of 4% NaCl, as shown in Figure (8).

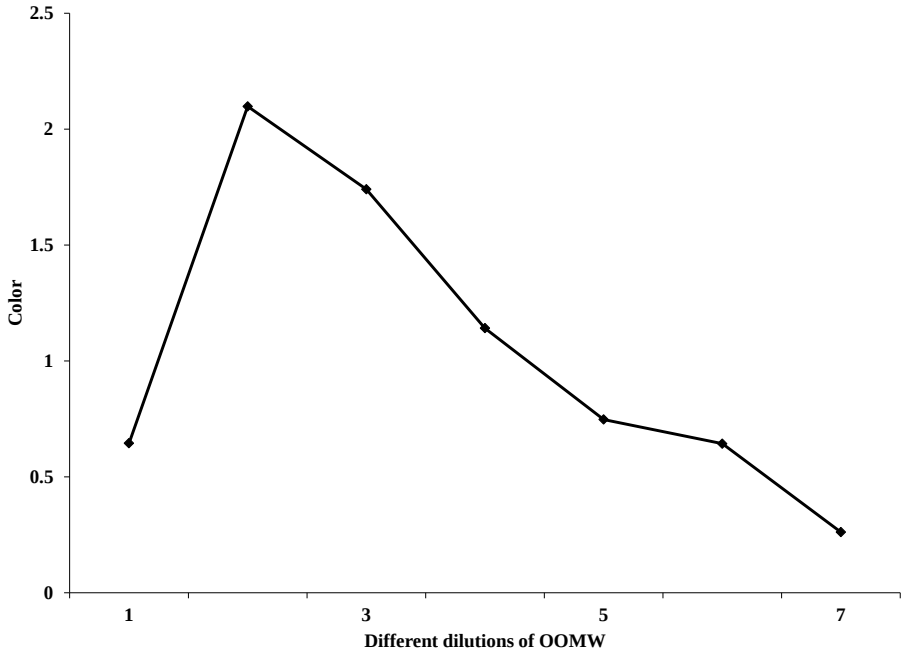


Figure 6. The color change of the olive oil wastewater treated by the use of crude peroxidase homogenate, a pH of 2.65 and a concentration of hydrogen peroxide of 2.58 mM. Wastewater was diluted in ranges of 1/2, 1/3, 1/5,1/8,1/10 and 1/20.

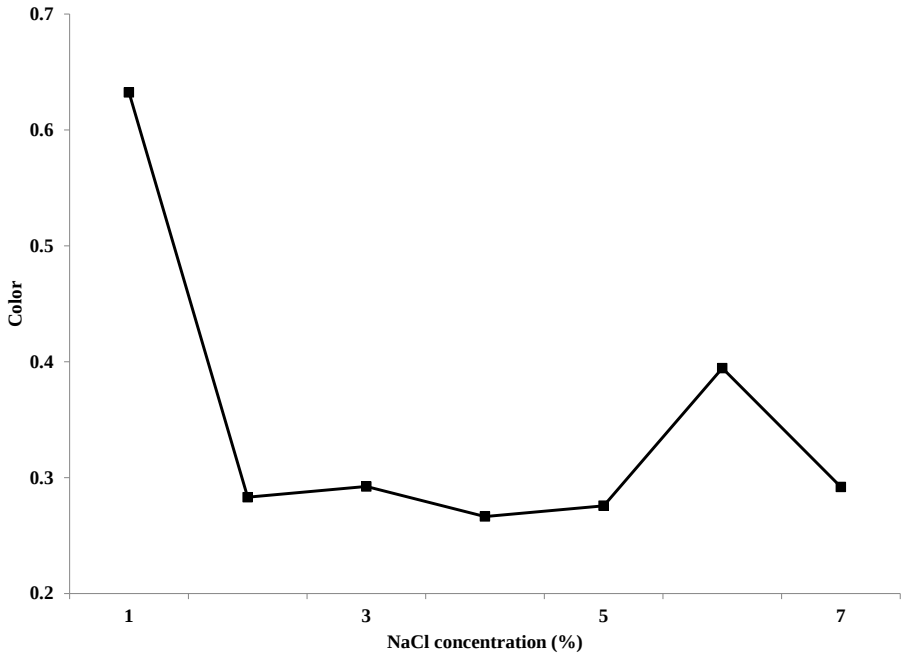


Figure 7. The color changes of the olive oil wastewater treated by the use of crude peroxidase homogenate a pH of 2.65, a concentration of hydrogen peroxide of 2.58 mM and the use of NaCl as an additive with the consequent percentages 0.5,1,2,3,4 and 5 %.

Polyethylene glycol could be used to protect the enzyme from inactivation during phenol removal. Thus, its presence could increase the lifetime of the enzyme and therefore increase the potential economic feasibility of the enzymic process. A decrease in the concentration of total phenols and o-diphenols as well as in the color intensity was observed with the use of polyethylene glycol in the treatment (Katsoyannos et al., 2006).

Total phenol concentration decreased gradually with an increase in the PEG concentration, where the highest decrease was around 48% at a concentration of 4000 mg/1 of PEG. A decrease in the concentration of o-diphenols was observed, as shown in Figure (9). The decrease in the color intensity was not as great as in the other treatments; in fact it decreased around 36%, as shown in Figure (10). It should be mentioned that the lowest concentration for the total phenol concentration was observed in this treatment at a concentration of 4000 mg/1 of PEG.

Each enzyme has an optimal pH that helps maintain its three-dimensional shape. Changes in pH may denature enzymes by altering the enzyme's charge. The combined effect of pH and concentration of hydrogen peroxide was studied in the treatment of OOMW with onion peroxidase (Moon et al., 2006). Experiments were performed using different buffer solutions to determine the optimal pH and concentration of H_20_2.

The removal efficiency of total phenols and o-diphenols was optimal at a relatively low pH (2.65). It increased slightly with the increase of H_20_2 concentration and reached the maximal value with almost 2.58mM. The gradual addition of the H_20_2 could lead to the possibility of enzyme inactivation (Fernández-Agulló et al., 2013).

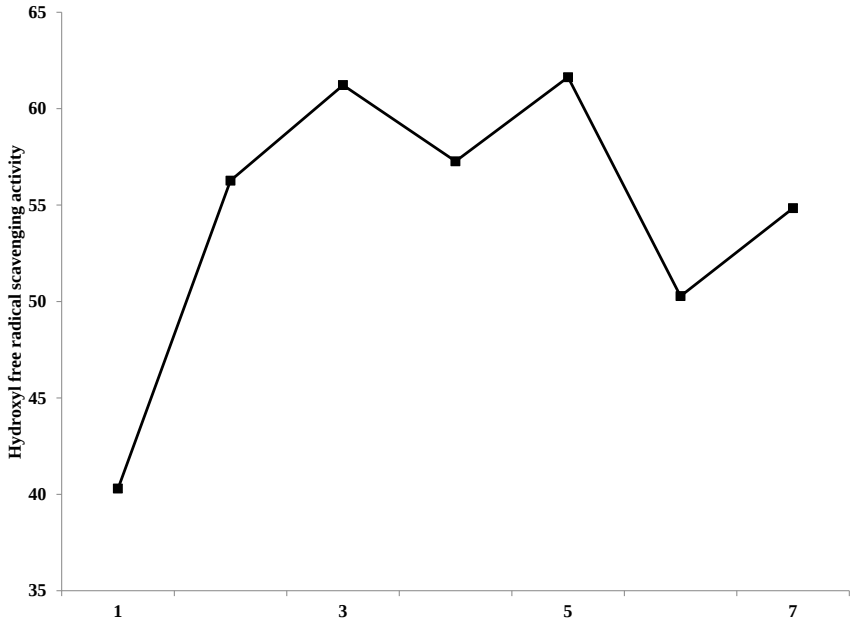


Figure 8. The antioxidant activity of the olive oil wastewater treated by the use of crude peroxidase homogenate, a pH of 2.65, a concentration of hydrogen peroxide of 2.58 mM and the use of NaCl as an additive with the consequent percentages 0.5,1,2,3,4 and 5 %.

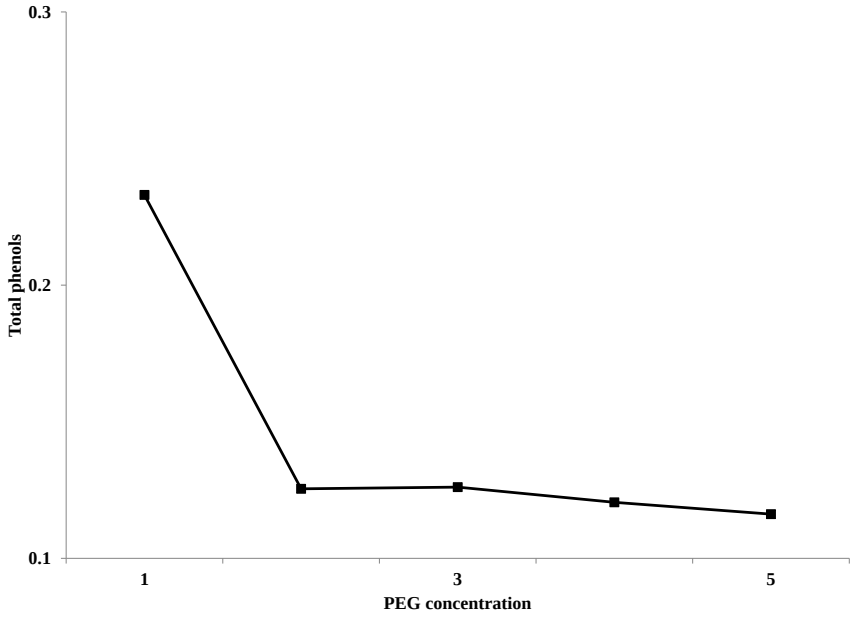


Figure 9. Removal of total phenols from the olive oil wastewater treated by the use of crude peroxidase homogenate,, a pH of 2.65, a concentration of hydrogen peroxide of 2.58 mM and the use of PEG as an additive with the consequent concentrations 1500, 2000, 3000 and 4000 mg/1.

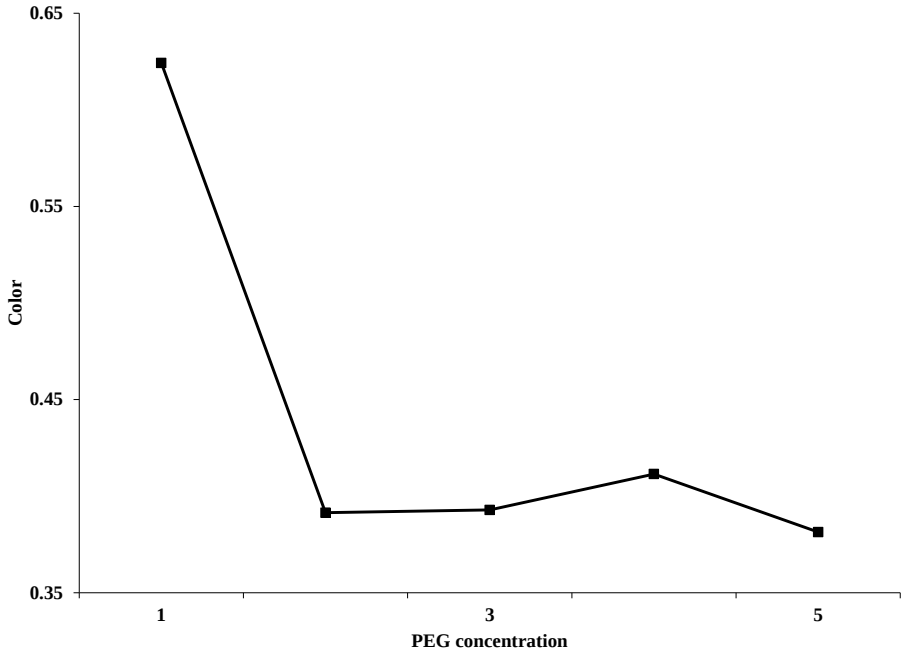


Figure 10. The color changes in the olive oil wastewater treated by the use of crude peroxidase homogenate, a pH of 2.65, a concentration of hydrogen peroxide of 2.58 mM and the use of PEG as an additive.

Based on the experimental results, the following arguments could be drawn: Total phenols and o-diphenols seem to be highly removed at a relatively low pH. It may be assumed that peroxidase oxidizes numerous phenols in the presence of hydrogen peroxide, generating corresponding phenoxy radicals and forming substances that are much less water soluble than the original substrate. These insoluble polymers then precipitate out of solution and can be separated by simple filtration or centrifugation (Tong et al., 1997).

HPLC analyses of the non-treated and treated OOMW at relatively optimum conditions regarding pH and the concentration of hydrogen peroxide were performed. Phenolic compounds were not identified, but results showed that some of these phenolics were degraded after the treatment (Kauffmann et al., 1999, Aparicio and Harwood, 2013).

The removal decreased at higher pH values as well as at high H_20_2 concentrations. High concentrations of hydrogen peroxide can lead to the deactivation of the enzyme and, as a result, to the decrease in the total phenols and o-diphenols concentration (Cannac et al., 2009). A high pH can result in a low enzyme activity. Most of the organic pollutants are soluble at neutral or alkaline pH. Their solubility reduces in acidic pH; this may also explain the removal of most of the phenols at pH 2.65.

Moreover, in the case of different time courses, removal was evident after half and one hour, when most of the enzymes were still highly active. After a period of two and three hours, the removal reaction was followed by a smaller removal process. This slowdown could be attributed to the simultaneous decrease in the concentration of all the reacting species (phenol or H_2O_2), or to the small decrease in the enzyme activity. Another explanation also could be the resolubilization of the polymerized phenols (Qin et al., 2004). However, total phenols removal after four and five hours can be explained by an oxidation process which lead to the decrease in the total phenols concentration and finally it can be assumed that because of the long stirring, resolubilization of the polymerized phenols occurred after six hours of treatment (Cheng et al., 2006).

The results regarding the decomposition of total phenols showed numerous fluctuations in comparison to the o- diphenols, which showed a high decomposition after 3 hours of stirring. The decomposition was seen to be stable for all the time courses. It can be concluded that o-diphenols can be totally decomposed in a short period of time, in comparison to the total phenols (Ko and Chen, 2008).

Dilution of the olive oil wastewater showed that the more the enzyme was diluted the greater the decomposition of the total phenols. An obvious decrease in the o-diphenols was achieved by increasing the dilution factor, even if many fluctuations were observed. This could have two explanations:

Some substances reduce or even stop the catalytic activity of enzymes in biochemical reactions. They block or distort the active site. When the olive oil wastewater is undiluted or a little diluted, phenols or o-diphenols highly concentrated in the olive oil wastewater could have an inhibitory effect on the onion solid waste homogenate (Masella et al., 2004).

At a constant enzyme concentration and at lower concentrations of substrates, the substrate concentration is the limiting factor. As the substrate concentration increases, the enzyme reaction rate increases. However, at very high substrate concentrations, the enzymes become saturated with substrate and a higher concentration of substrate does not increase the reaction rate (Beauchamp et al., 2005).

PEG has a greater affinity with the polymer products than the enzyme; most of the polymers were preferentially coupled with PEG so that the enzyme was prevented from being adhered and entrapped by precipitation products. As for both total phenols and o-diphenols, the removal efficiencies were low in the absence of PEG (Pugazhenthi and Kumar, 2004). However, they were significantly enhanced with an increase in the amount of PEG added. The further addition of PEG would result in a slight reduction in the phenol and o-diphenols. The relationships indicated that the PEG requirements were directly linked to the total amount of phenol in the solution. The concentration of phenols being high, the amount of phenoxy radicals and polymers

increases and the addition of PEG should be increased enough to disperse them (Cheng et al., 2006).

Each enzyme has an optimal salt concentration. If the salt concentration is close to zero, the charged amino acid side chains of the enzyme molecules will attract to each other. The enzyme will denature and form an inactive precipitate (Visioli et al., 2002). If, on the other hand, the salt concentration is too high, normal interaction of charged groups will be blocked, new interactions will occur, and again the enzyme will precipitate. An intermediate salt concentration is the optimum for many enzymes. This can explain the results obtained regarding the removal of total phenols and o-diphenols after the addition of different concentrations of salt (Kumar et al., 2015).

The addition of 0.5 and 1% seemed to be low, but on the other hand 4 and 5% seemed to be very high and might cause enzyme precipitation. The use of intermediate NaCl concentration (2 and 3%) however, seemed to improve the efficiency of total phenols and o-diphenols removal (Najafian et al., 2009, Bonatsou et al., 2018).

Real industrial wastewater generally contains a number of different pollutants. Hence, even if just a few of them are easily precipitated by onion peroxidase, they will facilitate the removal of the others by the enzyme and hydrogen peroxide. Such removal will induce the decrease in the color intensity. Upon initiation of the reaction using H_2O_2 and peroxidase, reaction mixtures changed color, indicating the formation of phenolic polymers (Njuma et al., 2017). Treatment was carried out for 3 hours, after which a significant decrease in the color intensity was observed by increasing the concentration of hydrogen peroxide. This can be explained by the oxidation followed by the precipitation of total phenols. A small increase in the color intensity was

observed at a high concentration of hydrogen peroxide, which can be attributed by either to the deactivation of the enzyme due to the high concentration of hydrogen peroxide or to the resolubilization of the polymerized phenols (Antenucci et al., 2016).

However excess stirring can help in the resolubilisation of such pollutants, which is why, an increase in the color was noticed after 4 and 5 hours of stirring in comparison to the treatments carried out within a shorter time course. Dilutions of the olive oil wastewater resulted in a further decrease in the color intensity (Esfandyari et al., 2015, Wu et al., 2017) . The results showed that the more the olive waste is diluted the more the solution becomes colorless. The use of additives (salt and polyethylene glycol) had a positive effect on the decrease in the color intensity of the olive oil wastewater due mostely to the decrease in the concentration of total phenols and odiphenols(Mazzeu et al., 2015).

Much of the benefit of olive oil consumption has been attributed to the presence of natural antioxidant compounds, several of which have been isolated. Among the antioxidant compounds that have been isolated from olive oil are various phenolic compounds, including hydroxytyrosol, tyrosol, p-hydroxybenzoic acid, vanillic acid, caffeic acid, oleuropein and other phenolic compounds (Della Pelle et al., 2015, Rosello-Soto et al., 2015) . During the treatment the enzyme passes through the catalytic cycle consuming H_2O_2 and phenolic substrate generating phenoxy radicals(Liu et al., 2015) .

The highest antioxidant activity was attributed to the OOMW before the treatment; after the treatment it decreased but it was high when the pH was lowered to 2.65 and when NaCl was used as an additive in the treatment. The change in the antioxidant activity can be attributed to the change in the total phenols concentration.

4. Conclusions

In this study it can be concluded that peroxidase extracted from onions possesses greater activity under a low pH and concentration of hydrogen peroxide in order to remove total phenols and o-diphenols in the olive oil mill wastewater. Many factors can affect the degree of degradation, such as the dilution of the Olive Oil Mill Wastewater and the time factor. However it was shown that the more we diluted the Olive Oil Mill Wastewater the greater the removal; another conclusion drawn was that o-diphenols can easily be oxidized, in comparison to the total phenol. The possibility- of using seawater for dilution of the olive oil wastewater may be an appealing option, because when using a medium concentration of salt, degradation was enhanced. Another interesting finding was when Poly Ethylene Glycol was used; Olive Oil Mill Wastewater color as well as the antioxidant activity changed with a change in the concentration of phenols and o-diphenols. The results obtained in this study strongly support the concept of using enzymes for decontamination of the environment, although a few important problems must be addressed in future investigations. The toxicity of the Olive Oil Mill Wastewater can be minimized, and the environment therefore protected, by using onion peroxidase.

Acknowledgment

This article was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah. The authors, therefore, acknowledged with thanks DSR for technical and financial support.

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