# A NOVEL FERULOYL ESTERASE FROM *OENOCOCCUS OENI* FOR THE IMPROVEMENT OF WINE QUALITY

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## INTRODUCTION

Although malolactic fermentation [1] is the main transformation carried out by *Oenococcus oeni* during vinification, it is not the only one. There are other metabolic reactions that can have positive effects on quality of wine as a myriad of other changes occur to complete the transformation of grape juice to wine. Many of these processes involved the action of enzymes. Such enzymes can be originated from several sources that included the grape microbiota, the inoculated microbes, or microbes associated with winery equipment. To improve wine quality and complexity, efforts have often been centered on desirable enzymatic activities, such as esterases from microorganisms encountered during vinification [2]. Esters are flavour compounds that are extremely important for the flavour profile of wines, e.g. esters are responsible for the desirable, fruity aroma of young wines.
*O. oeni* is frequently utilized as a starter culture to promote malolactic conversion. Therefore *O. oeni* enzymes are highly relevant on wine aroma.

## OBJETIVE

The aim of this study was to genetically identify and biochemically characterize a feruloyl esterase from *Oenococcus oeni*

## MATERIALS AND METHODS

*Gene cloning, protein production and purification*
A gene from *O.oeni* was cloned in the expression vector pURI3-Cter by using a strategy of ligation independent of cloning (LIC) [2]. This vector produced a recombinant protein with a His-tag on its C-terminal. *O. oeni* protein was hyperproduced in *Escherichia coli* BL21 (DE3) together with another expression vector, p-GRO 7, which encodes a chaperone system (Takara). Purification was carried out by one -step IMAC chromatography.

*Biochemical characterization*.
Esterase activity characterization was performed by a spectrophotometric method using *p*-nitrophenyl derivates as substrates and following absorbance at 348nm. Substrate specifity was determined by a colorimetric method using an ester library and *p*-nitrophenyl as pH indicator. In addition optimum temperature and pH, thermal stability and effect of additives were studied [2].

## RESULTS

### *O. oeni* esterase: hyperproduction and purification

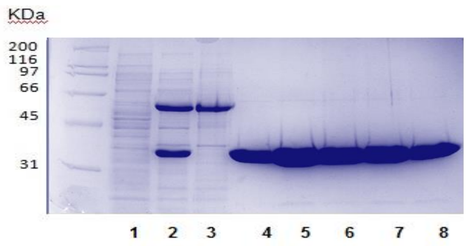


**Figure 1**. SDS-PAGE analysis. 1, soluble protein extract from *E. coli* BL21 (DE3) (pURI3-Cter) cells; 2, soluble protein extract from *E. coli* BL21 (DE3) (pURI3-Cter-*oeoe*) cells; 3, fraction not retained in the column; 4 – 8 eluted OEOE protein fractions.

### *O. oeni*: substrate specificity

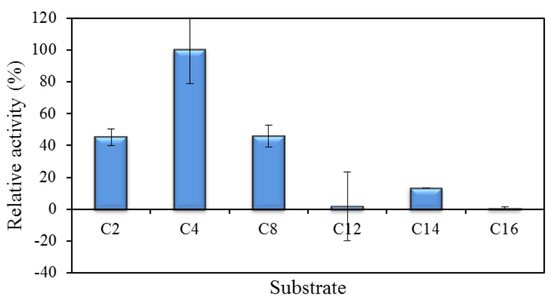


**Figure 2.** Substrate specificity assay. C2, (*p*- nitrophenyl acetate); C4, (*p*-nitrophenyl butirate); C8, (*p*- nitrophenyl caprilate); C12, (*p*-nitrophenyl laurate); C14, (*p*- nitrophenyl miristate); C16, (*p*- nitrophenyl palmitate).

### Biochemical characterization

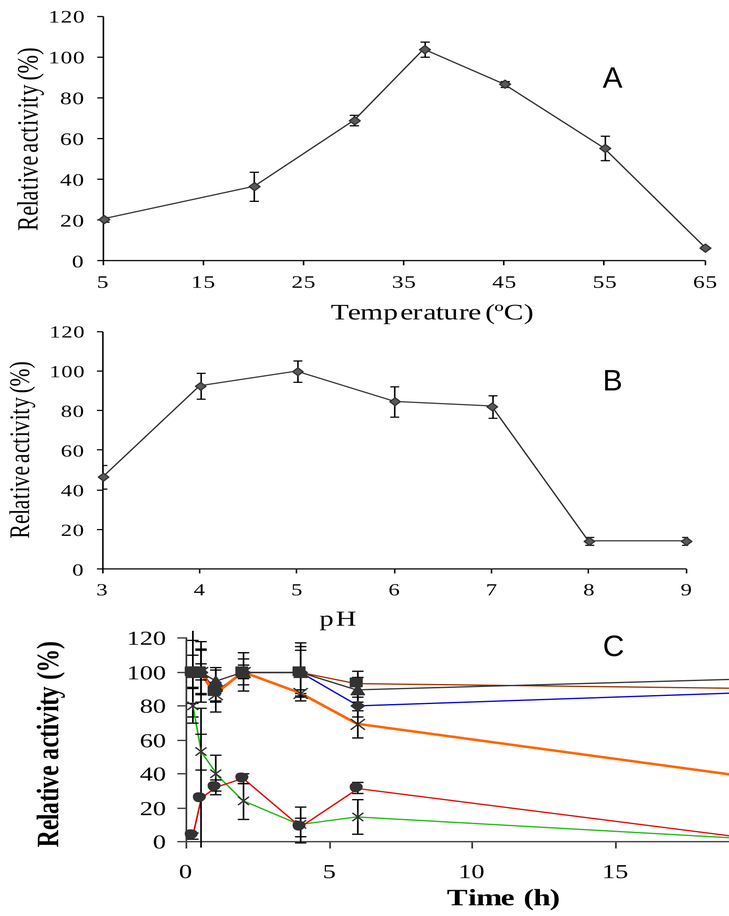


**Figure 3.** Biochemical properties of OEOE esterase (A) Activity *vs* temperatures. (B) Activity *vs* pH. (C) Thermal stability after preincubation at 20 ºC, (blue), 30 ºC (brown), 37 ºC (black), 45 ºC (orange) 55 ºC (red) and 65 ºC (green).

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**Table I**. Esterase activity on different esters

| Substrate | Activity |
|---|---|
| Methyl caffeate | (+) |
| Methyl p-coumarate | (+) |
| Methyl sinapinate | (+) |
| Methyl salicylate | (+) |
| Methyl benzoate | (+) |
| Methyl ferulate | (+) |
| Ethyl ferulate | (+) |
| Methyl gallate | (+) |
| Ethyl gallate | (+) |
| Propyl gallate | (+) |
| Lauryl gallate | (-) |
| Methyl-2,4-dihydroxybenzoate | (+) |
| Methyl-2,5-dihydroxybenzoate | (+) |
| Methyl-4-hydroxybenzoate | (+) |
| Ethyl-4-hydroxybenzoate | (+) |
| Ethyl-3,4-dihydroxybenzoate | (+) |
| Methyl vainillate | (+) |
| Chlorogenic acid | (-) |
| Quercetin | (-) |
| Catechin | (-) |
| Epicatechin gallate | (+) |
| Tannic acid | (-) |
| Ellagic acid | (-) |
| Rosmarinic acid | (-) |
| Oleuropein | (-) |

**Table II**. Additive effect on esterase activity

| Additive | Relative activity (%) |
|---|---|
| Control | 100,00 |
| KCl | 115,96 |
| $HgCl_2$ | 1,77 |
| $CaCl_2$ | 139,48 |
| $MgCl_2$ | 149,58 |
| $ZnCl_2$ | 120,43 |
| $CuCl_2$ | 122,67 |
| $NiCl_2$ | 153,08 |
| $MnCl_2$ | 129,49 |
| Tween 20 | 167,73 |
| Tween 80 | 180,72 |
| Triton-X-100 | 189,44 |
| SDS | 0,73 |
| Urea | 120,74 |
| DMSO | 155,05 |
| Cysteine | 107,38 |
| DTT | 115,00 |
| *β*-mercaptoethanol | 128,37 |
| EDTA | 120,16 |
| PMSF | 44,57 |
| DEPC | 144,53 |

## CONCLUSIONS

The protein showed esterase activity on a large number esters derived from phenolic acids. It showed feruloyl esterase activity as well as esterase activity on esters derived from hydroxybenzoic acids (e.g., gallic acid). The protein exhibited optimum temperature and pH for activity at 40 ºC and 5.0, respectively. Furthermore, the protein kept 80% of its maximal activity after 20 h incubation at 20 ºC, 30 ºC and 37 ºC. The obtained results indicated that the esterase from *O. oeni* could be an adequate esterase enzyme to be used during vinification to flavor enhancement of wine.

## REFERENCES

(1) Sumby, K.M, Grbin, P.R and Jiranek, V. (2014) Implications of new research and technologies for malolactic fermentation in wine. Appl Microbiol Biotechnol 98:8111–8132

(2) Esteban-Torres M, Landete JM, Reverón I, Santamaría L, de Las Rivas B, Muñoz R. (2015) A *Lactobacillus plantarum* esterase active on a broad range of phenolic esters. Appl Environ Microbiol 81:3235–3242

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