

# Effect of Phenolic Acid on Antioxidant Activity of Wine and Inhibition of Pectin Methyl Esterase

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## ABSTRACT

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Antioxidant activity, malolactic fermentation and sensory evaluation of the grape must after fermentation in the presence of gallic acid and coumaric acid, as well as the inhibitory mechanism of gallic acid and coumaric acid on pectin methyl esterase (PME), were investigated. The content of malic acid and lactic acid increased 40.4% and 36.9% compared to the control when commercial pectic enzyme (CPE) was used. The increase in malic acid content was enhanced by 64.8% and 83.4%, compared to the control in the presence of CPE + Gallic acid and CPE + Coumaric acid respectively. Ferric reducing/antioxidant power (FRAP) increased in the samples with added CPE. In addition to an increase in the FRAP, antioxidant capacity was enhanced in the CPE + Gallic acid and CPE + Coumaric acid samples. No significant differences were found in the content of total anthocyanin and in the value of sensory characteristics. The content of total flavanols increased significantly in the samples with added CPE. Lineweaver-Burk plots of PME, with gallic acid or coumaric acid, indicated that gallic acid and coumaric acid were mixed inhibitors of PME.

**Key words:** Commercial pectic enzyme, coumaric acid, gallic acid, pectin methyl esterase, wine.

**Abbreviations:** PME: pectin methyl esterase, PAL: pectate lyase enzyme, PG: polygalacturonase, CPE: commercial pectic enzyme, DE: degree of esterification, EGCG: epigallocatechin gallate, DPPH: diphenyl- $\beta$ -picrylhydrazyl, FRAP: ferric reducing/antioxidant power assay, TA: total anthocyanins, HM-CL-AIS: highly methoxylated cross-linked alcohol-insoluble solid column, EGCG: epigallocatechin gallate, PME: PME inhibitor.

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## INTRODUCTION

Phenolic substances, which present in large amounts in plant derived products, are important components of the human diet. Phenolic acids are plant metabolites reported to act as major bioactive compounds useful in the prevention of chronic diseases and the promotion of health benefits. For example, they possess cytoprotective ability in the prevention of diabetic neuropathy complications<sup>16</sup>. Consumption of products rich in phenolic acids, such as wine and the “Mediterranean diet”, has been reported to be correlated with a reduced risk of cardiovascular disease<sup>11,31</sup>.

The methanol content of the freshly squeezed juices of fruits and vegetables increases during storage. This increase in methanol is positively associated with activities of pectin methyl esterase (PME) and pectate lyase (PAL) enzymes in the fruit juices. It is also positively associated with PME activity, but is negatively associated with polygalacturonase (PG) activity in vegetable juices<sup>14</sup>. The addition of commercial pectic enzyme (CPE) plays an important role in the process of wine-making for extraction, clarification, and filtration of fruit juice and wine puree in order to increase the yield and quality (e.g., pigment, flavor, transmittance, and viscosity)<sup>20,30</sup>. However, methanol is produced in large quantities after the enzymatic degradation of internal natural pectins by PME in CPE during mashing, fermentation, and the aging stages of the wine-making process. Hence, the use of CPE suffers the major drawback of high methanol content levels, especially when the levels are above the given safety limits in some wine products<sup>25,28,34,35</sup>. Blocking harmful PME activity, as well as removing PME activity in CPE, to avoid methanol levels in excess of the given safety limit is recommended<sup>15,34,35</sup>.

Hou et al.<sup>15</sup> revealed that either the addition of gallic acid or coumaric acid in wine-making caused a decrease in methanol content and an increase in the healthy wine compounds. No related studies were found in the area of kinetics of phenolic acids on PME inhibition or on the antioxidant activity of wine enhanced by phenolic acids after fermentation. With the above facts in mind, this study aimed to investigate the inhibitory mechanism of gallic acid and coumaric acid on PME; the influence of gallic acid and coumaric acid on antioxidant activity; and malolactic fermentation and the sensory evaluation of grape must after fermentation.

## MATERIALS AND METHODS

### Materials

Fresh Black Queen grapes (*Vitis vinifera* × *V. labrusca*) were purchased from a local supermarket in Taichung County, Taiwan. The commercial liquid pectic enzyme was obtained from a microbial source (Peclyve CP) and the commercial wine yeast (RA-17 *Saccharomyces cerevisiae*) was purchased from Lallemand Australia Pty. Ltd. (North Adelaide, Australia). Citrus pectin, with a degree of esterification (DE) of 90–93%, and methanol, were both purchased from Sigma (St. Louis, MO, USA). Citrus pectin with a DE of 60–66%, D-galacturonic acid, and polygalacturonic acid were purchased from Fluka (Buchs, Switzerland). Ethanol (95%) was purchased from the Taiwan Tobacco and Liquor Corporation, Taiwan.

### Preparation of the wine

The red grapes were cleaned with distilled water and crushed into grape puree. Sucrose and Na-pyrosulfite were added to the grape puree to reach 25°Brix and 100 ppm respectively. The grape puree was divided into the following four groups before yeast inoculation: (1) control group: without external enzyme added, (2) CPE group: with commercial pectic enzyme 0.2 mL/L CPE containing 10 units of PE, 0.39 units of PG, and 70 units of PL, (3) CPE + Gallic acid group: with 0.2 mg/L gallic acid and 0.2 mL/L CPE, (4) CPE + Coumaric acid group: with 0.2 mg/L coumaric acid and 0.2 mL/L CPE.

Commercial wine yeast RA-17 (0.25 g), previously activated in 25 mL warm water (40–43°C) for 15 min to make a suspension was added to 1 kg of the grape puree. Fermentations were conducted at room temperature (25 ± 2°C) for 15 days. During fermentation, sampling was conducted every 3 days to determine the changes in physico-chemical properties. Samples were centrifuged at 13,000 × *g* for 20 min at 4°C.

### The α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging activity analysis was performed according to the procedure reported by Yamaguchi et al.<sup>36</sup> The sample solution (0.2 mL) was mixed with 0.1 M Tris-HCl buffer (pH 7.4, 0.8 mL) and then added to 1 mL of 0.125 mM DPPH in ethanol. This solution was incubated at room temperature for 20 min in the dark and the absorbance was measured at 517 nm by DPPH.

### The ferric reducing/antioxidant power (FRAP) assay

This assay was conducted according to the procedure reported by Benzie and Strain<sup>4</sup>. The working FRAP reagent was produced by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine solution and 20 mM FeCl<sub>3</sub>·6H<sub>2</sub>O in a 10:1:1 ratio prior to use and heated to 37°C in water bath. A total of 3.0 mL FRAP reagent was added to a test tube and a blank reading was taken at 593 nm using a spectrophotometer. A total of 100 μL of wine and 300 μL of distilled water was added. After addition of the sample to the FRAP reagent, a second

reading at 593 nm was performed after 90 min of incubation in a 37°C in water bath. The changes in absorbance after 90 min, from the initial blank reading, were compared to the standard curve of FeSO<sub>4</sub>.

### Determination of total anthocyanins

Total anthocyanins was determined according to Arnous et al.<sup>2</sup> In brief, wine (0.02 mL) was mixed with 20% sodium metabisulphite solution (0.02 mL) and the absorbance was measured at 520 nm ( $A_{520}^{SO_2}$ ). Further, wine was mixed with 0.98 mL 1 N HCl solution and the absorbance of HCl was measured ( $A_{520}^{HCl}$ ) after 180 min. The concentration of total anthocyanins (TA) was calculated as follows:

$$TA \text{ (mg/L)} = 20 \times [A_{520}^{HCl} - (5/3) \times A_{520}^{SO_2}]$$

### Determination of total flavanols

Total flavanol content was estimated according to the procedure of Arnous et al.<sup>2</sup> Wine (0.2 mL) was mixed with 1 mL 0.1% *p*-dimethylaminocinnamaldehyde solution. The absorbance at 640 nm was measured after 10 min. The total flavanol concentration was estimated from a calibration curve constructed using catechin.

### Purification of pectin methyl esterase

PME was purified from a commercial liquid pectic enzyme (North Adelaide, Australia) according to the procedure of Wu et al.<sup>34</sup> with some modifications. Commercial pectic enzyme (0.2 mL) was applied on highly methoxylated cross-linked alcohol-insoluble solid (HM-CL-AIS) column (2.5 cm × 20.0 cm; flow rate, 40 mL/h) for separation. The column was equilibrated with 0.01 M citric acid buffer (pH 4.5) and then eluted with the same buffer at a 0–1 M NaCl gradient. The 3 mL/tube fractions were collected and assayed for PME activity. The PME containing fraction was collected, dialyzed, and further purified by gel filtration through a Sephacryl S-100 column (1.6 cm × 60.0 cm; flow rate, 40 mL/h), equilibrated with 0.01 M citric acid buffer (pH 4.5) containing 0.1 M NaCl. The purified PME was then concentrated and stored at –20°C until required.

### Determination of pectin methyl esterase activity

PME activity was determined using a 0.1 M NaCl / 0.5% citrus pectin (DE 60–66%) solution, with a pH adjustment according to the method described by Jiang et al.<sup>18</sup> One unit of enzyme activity was defined as one μmol of free carboxyl group formation from pectin per min. Michaelis constants (*K<sub>m</sub>*) and the maximum velocity (*V<sub>max</sub>*) of PME were determined using the Lineweaver-Burk double reciprocal plot, in which the reciprocals of the initial rates of the PME activity were plotted against the reciprocals of the pectin concentrations. In addition, the inhibition kinetics of gallic acid and coumaric acid were analyzed using Lineweaver-Burk plots.

### Protein determination

Protein concentrations were determined by the BIO-RAD protein assay (Bio-Rad, USA), using bovine serum albumin as the standard.

## Statistical analysis

Statistical analysis was accomplished using SAS Statistical Software, Version 9.1 (SAS Institute). Triplicate samples were each analyzed twice in this study. The difference between the means was analyzed using Duncan's multiple range test.

## RESULTS AND DISCUSSION

### Effect of phenolic acids on malolactic fermentation

The content of malic acid decreased from 72.1 to 46.0 mg/L (control) in grape must after 15 days fermentation. It increased in the CPE added groups as compared to the control group (Table I). The content of malic acid increased from 46.0 mg/L (control) to 75.9 and 84.8 mg/L in the presence of gallic acid and coumaric acid respectively.

The content of lactic acid decreased from 11.0 to 8.4 mg/L (control) in grape must after 15 days fermentation. It increased in the CPE added groups as compared to the control group, however, the increase in lactic acid content was inhibited in the presence of gallic acid. No relationship between the CPE activity and the level of malolactic fermentation was detected, even though the content of malic acid and lactic acid increased 40.4% and 36.9% of the control group by CPE. The increase of malic acid content was enhanced to 64.8% and 83.4% of the control group in the presence of gallic acid and coumaric acid respectively.

The components or metabolic byproducts in wines produced during alcoholic fermentation (by wine yeast) and malolactic fermentation (by lactic acid bacteria) or aging are important for wine quality. Malolactic fermentation, which consists of the decarboxylation of L-malic acid to L-lactic acid, causes acid reduction, flavor modification and has a significant impact on the stability and organoleptic character of quality wines<sup>5,32</sup>. It is believed that this fermentation helps to improve the sensory complexity of wine by producing compounds with important flavor profile contributions<sup>10</sup>. The utilization of CPE in wine-making is beneficial to increase the yield and quality of pigment, and for flavor, transmittance and viscosity of wine<sup>20,30</sup>. An increase in malic acid can contribute to the improvement of acidity of wines produced in hot climates with low acidity grape must<sup>7</sup>. Wines from high acid musts can also benefit from malolactic fermentation. Therefore, the increase of malic acid in CPE, CPE + gallic

acid and CPE + coumaric acid added groups suggests that adding CPE and phenolic acids in the wine-making process might also be beneficial to the quality of wines, especially for the low acidity grape musts produced in hot climates.

There were no significant differences found in the value of sensory characteristics of wine such as, color intensity, flavor complexity, mouthfeel complexity, flavor rich endurance, tannins, acidity, and balance (data not shown). In the presence of gallic acid or coumaric acid with CPE, the values of lightness, red content, yellow content, total pigment, and total phenolic acid increased significantly<sup>15</sup>. During the wine-making process, the malic acid concentration was found to be useful in controlling the progress of the malolactic fermentation. As for the quality of total acidity, taste and flavor characteristics of wine, these have been found to positively depend on the quantity of malic acid<sup>1</sup>.

The effects of CPE, gallic acid and coumaric acid on the taste and quality of wine, from the point of sensory evaluation, were not changed by the spontaneous malolactic fermentation in the current study. Malolactic fermentation can be induced by a starter culture (lactic acid bacteria), hence the higher amount of malic acid with the CPE added groups, especially in the presence of gallic acid and coumaric acid, which suggests that the addition of gallic acid and coumaric acid could benefit malolactic fermentation and the quality of wine.

### Effect of phenolic acids on antioxidant activity

The effect of gallic acid or coumaric acid on the antioxidant activity of wine is shown in Table II. The DPPH scavenging effect and FRAP capacity of grape must increased after fermenting into wine (Control group). They were enhanced in the presence of CPE (CPE added groups). No significant ( $p > 0.05$ ) increase in DPPH scavenging effect was observed in the CPE + Gallic acid and CPE + Coumaric acid groups as compared to the CPE group. Nevertheless, the FRAP capacity of the CPE + Gallic acid and CPE + Coumaric acid group was higher than the CPE group.

The content of total flavanols increased from 74.9 to 203.5 (control group) and 244–253 mg/L (CPE added group) in grape must after 15 days fermentation (Table III), while the content of total anthocyanin increased from 16.7 to 46.7–47.7 mg/L (control and CPE adding groups) in the grape must. Compared to the control group, there was no significant ( $p > 0.05$ ) difference in the content of

**Table I.** Effect of gallic acid or coumaric acid on the content of malic acid and lactic acid in wine.

Condition*	Malic acid (mg/L)	Lactic acid (mg/L)
Grape must	72.0 ± 7.8 <sup>ab</sup>	11.0 ± 0.6 <sup>a</sup>
Control	46.0 ± 3.7 <sup>d</sup>	8.4 ± 0.6 <sup>b</sup>
CPE	64.6 ± 1.7 <sup>c</sup>	11.5 ± 0.2 <sup>a</sup>
CPE + Gallic acid	75.8 ± 2.2 <sup>b</sup>	9.2 ± 0.5 <sup>b</sup>
CPE + Coumaric acid	84.8 ± 9.2 <sup>a</sup>	11.0 ± 1.8 <sup>ab</sup>

\* Grape must: without fermentation and no PME added; control: fermented and no PME added. Means in each column with the same letter are not significantly different ( $p > 0.05$ ).

**Table II.** Effect of gallic acid or coumaric acid on the antioxidant activity of wine.

Condition*	DPPH scavenging effect (%)	FRAP capacity (mM Fe <sup>2+</sup> )
Grape must	32.8 ± 1.0 <sup>d</sup>	1.80 ± 0.04 <sup>d</sup>
Control	40.6 ± 2.8 <sup>c</sup>	3.88 ± 0.07 <sup>c</sup>
CPE	50.5 ± 5.9 <sup>ab</sup>	4.61 ± 0.07 <sup>b</sup>
CPE + Gallic acid	53.4 ± 8.1 <sup>a</sup>	4.77 ± 0.08 <sup>a</sup>
CPE + Coumaric acid	48.3 ± 2.1 <sup>ab</sup>	4.78 ± 0.03 <sup>a</sup>

\* Grape must: without fermentation and no PME added; control: fermented and no PME added. Means in each column with the same letter are not significantly different ( $p > 0.05$ ).

total anthocyanin in the CPE added group; however, an increase in the content of total flavanols in the presence of CPE was detected. Thus, the increase of FRAP capacity in the presence of gallic acid and coumaric acid was not related to total flavanols and total anthocyanin.

The antioxidant activity of wines is related to the total phenolics and total flavonoids and is considered to be highly beneficial to health<sup>13</sup>. A strong correlation among total phenolic and flavonoid levels and antioxidant activities (using the DPPH and FRAP methods) has also been reported by Lamien-Meda et al.<sup>19</sup> According to a report by Cai et al.<sup>6</sup>, different categories of phenolic compounds (including flavanols, flavonols, chalcones, flavones, flavanones, isoflavones, tannins, stilbenes, curcuminoids, phenolic acids, coumarins, lignans, and quinones) from traditional Chinese medicinal plants express different radical scavenging activity. Flavonoids, the most ubiquitous polyphenols, are classified into flavanols, flavones, flavonols, flavanones, isoflavones, and anthocyanidins<sup>33</sup>. There was no correlation observed between antioxidant activity and total flavanols or total anthocyanin in this study, i.e., the increase of FRAP capacity caused by the addition of gallic acid and coumaric acid was not flavanol and anthocyanin related.

### Inhibition kinetics of phenolic acids on pectin methyl esterase

The affinity constant of the substrate to the binding site ( $K_m$ ) and the maximum velocity ( $V_{max}$ ) of PME were estimated against PME, which was purified from microbial CPE by an affinity HM-CL-AIS column and a Sephacryl S-100 column. The values of  $K_m$  (x-intercept) and  $V_{max}$  (y-intercept) of microbial PME were 5734.67 mM and 16.67 U/mg protein, respectively (Table IV). In order to gain more insight to the reaction of gallic acid and coumaric acid on the demethoxylation of pectin, purified PME was used for inhibition kinetics. Lineweaver-Burk plots of PME with gallic acid and coumaric acid (Fig. 1) indicated that gallic acid and coumaric acid were

capable of binding to both PME and to the PME-pectin complex and showed as mixed-type inhibition against PME<sup>27</sup>.

The inhibition type of polysaccharides (about 200 kDa) exhibited in potato was a noncompetitive inhibition to potato PME<sup>26</sup>, whereas methanol acted as a noncompetitive inhibitor and polygalacturonic acid acted as a competitive inhibitor to PME from *Aspergillus niger*<sup>8</sup>. Additionally, a proteinaceous PME inhibitor from kiwi to banana and strawberry PME, followed a noncompetitive pattern, whereas carrot PME and kiwi PME followed a competitive pattern<sup>3,24</sup>.

Epigallocatechin gallate (EGCG) is reported to act as a competitive inhibitor to tomato PME, and the inhibitory interaction occurs at the catalytic binding site of PME via a hydrophobic interaction<sup>23</sup>. In this study, gallic acid and coumaric acid exhibited a mixed inhibition pattern on the PME from *S. cerevisiae*.

The estimated  $K_i$  increased from 9.6 mM to 22.3 mM in the presence of 0.1% and 0.5% gallic acid respectively. It increased from 17.8 mM to 21.0 mM in the presence of 0.1% and 0.5% coumaric acid respectively (Table IV).  $K_i$  values of EGCG and PME inhibitor (PMEI) from kiwi to tomato PME were 420  $\mu$ M (measured by cyano-acetate substrate) and 0.053  $\mu$ M (using citrus pectin as the substrate) respectively<sup>9,23</sup> and to kiwi PME with an  $K_i$  of 0.22  $\mu$ M (using citrus pectin as the substrate)<sup>3</sup>. The  $K_i$  values of gallic acid and coumaric acid were higher than that of EGCG and kiwi PMEI.

Small molecule gallic acid and coumaric acid for PME inhibition both provide a lower cost method for enzymatic activity control of PME, during isolated life stages and tissues, as well as for food processing. The various inhibition patterns of phenolic acids (gallic acid and coumaric acid) indicate that the chemical structure and the hydrophobicity of phenolic acids might play an important role in inhibitory strength on PME.

High intake of antioxidant food is found to lower the risk of incidental lifestyle-related diseases<sup>12,29</sup>. More and more evidence is accumulating that the consumption of grape and grape extracts and/or grape products rich in polyphenols, such as those found in red wine, is beneficial for the prevention of chronic degenerative diseases such as cardiovascular disease<sup>22</sup>. Lee et al.<sup>21</sup> have investigated the antioxidant action mechanism of *p*-coumaric acid in stressed bovine aortic endothelial cells and revealed that pretreatment with *p*-coumaric acid causes an induction of peroxidase and prevents lipid peroxidation and cell death caused by high glucose plus arachidonic acid exposure. Caffeic acid and cinnamic acid are reported to promote the insulin receptor tyrosyl phosphorylation, which up-regulates the expression of the insulin signal associated proteins, increases the uptake of glucose, and alleviates insulin resistance in cells as a consequence<sup>17</sup>. Although plant phenolic compounds display antioxidant effects in biological systems, the action mechanism remains controversial.

A decrease in methanol content and an increase in total phenols (2330–2700 fold of the added amount of gallic acid and coumaric acid) were observed. Thus the addition of gallic acid and coumaric acid in the wine-making process could potentially reduce methanol content, while in-

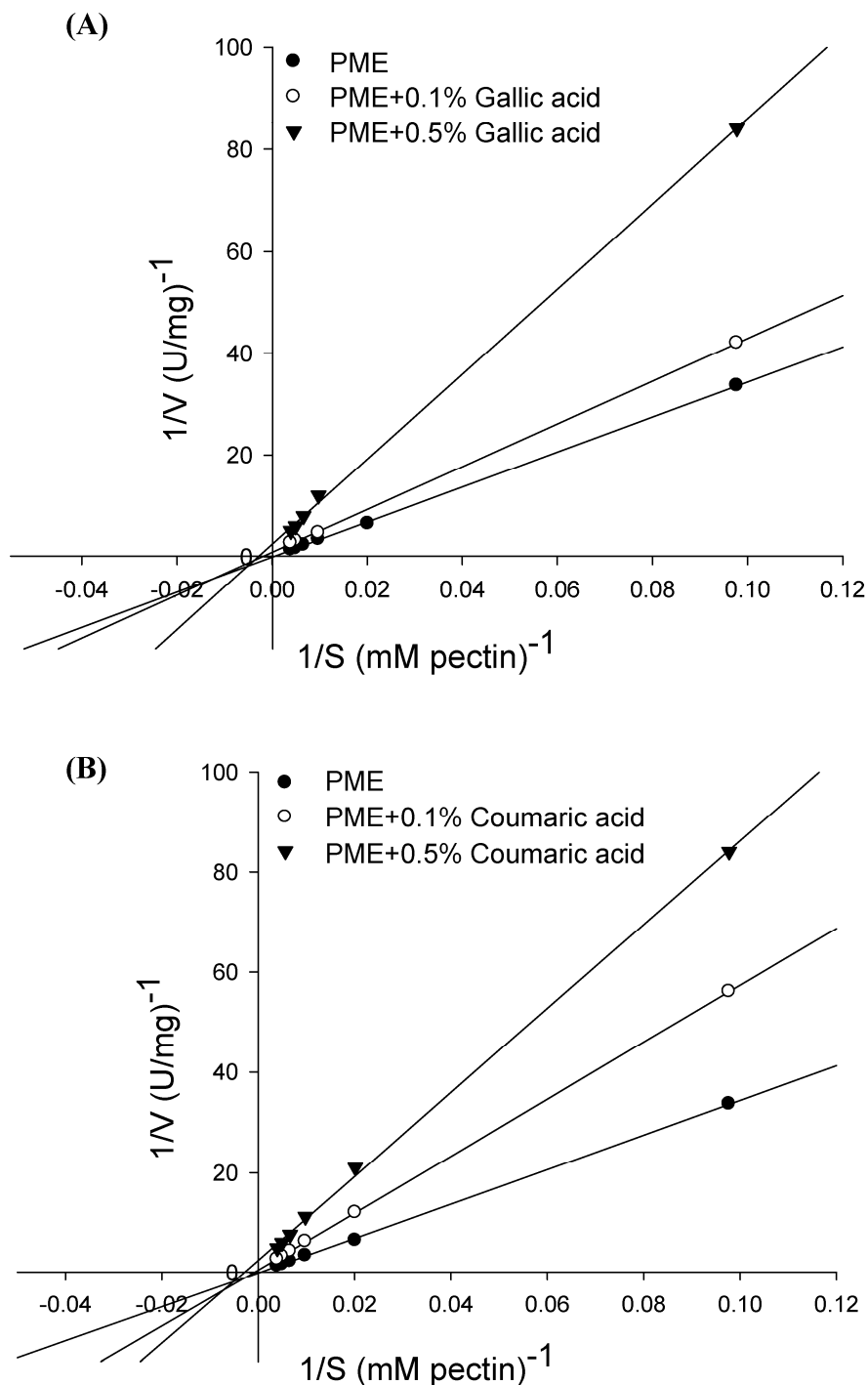
**Table III.** Effects of gallic acid or coumaric acid on total anthocyanins and flavanols of wine.

Condition*	Total anthocyanin (mg/L)	Total flavanols (mg/L)
Grape must	16.7 ± 0.9 <sup>b</sup>	74.8 ± 22.2 <sup>c</sup>
Control	47.7 ± 0.2 <sup>a</sup>	203.7 ± 8.8 <sup>b</sup>
CPE	46.7 ± 0.3 <sup>a</sup>	252.9 ± 9.6 <sup>a</sup>
CPE + Gallic acid	46.8 ± 0.2 <sup>a</sup>	244.3 ± 5.4 <sup>a</sup>
CPE + Coumaric acid	46.8 ± 0.5 <sup>a</sup>	244.4 ± 14.1 <sup>a</sup>

\* Grape must: without fermentation and no PME added; control: fermented and no PME added. Means in each column with the same letter are not significantly different ( $p > 0.05$ ).

**Table IV.** Comparison of the catalytic parameters of PME in the presence of gallic acid or coumaric acid.

Condition	Apparent $V_{max}$ (U/mg)	Apparent $K_m$ (mM)	$K_i$ (mM)
Control	16.6 ± 5.6	5698.0 ± 1927.0	--
+ 0.1% gallic acid	1.9 ± 0.3	1063.3 ± 172.8	9.6 ± 0.3
+ 0.5% gallic acid	0.4 ± 0.0	333.6 ± 11.1	22.3 ± 0.3
+ 0.1% coumaric acid	1.0 ± 0.0	487.7 ± 21.8	17.8 ± 2.3
+ 0.5% coumaric acid	0.3 ± 0.0	254.4 ± 7.6	21.0 ± 1.0



**Fig. 1.** Lineweaver-Burk plot of PME in the presence of gallic acid (A) and coumaric acid (B).

creasing the release of malic acid, total phenolic acids and FRAP capacity and at the same time lowering the risk of disease. This health benefit could be applicable in wine marketing promotions.

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#### REFERENCES

1. Antonelli, M. L., Spadaro, C. and Tornelli, R. F., A microcalorimetric sensor for food and cosmetic analyses: L-Malic acid determination. *Talanta*, 2008, **74**, 1450-1454.
2. Arnous, A., Makris, D. P. and Kefalas, P., Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece. *J. Food Comp. Anal.*, 2002, **15**, 655-665.
3. Balestrieri, C., Castaldo, D., Giovane, A., Quagliuolo, L. and Servillo, L., A glycoprotein inhibitor of pectin methylesterase in

- kiwi fruit (*Actinidia chinensis*). *Eur. J. Biochem.* 1990, **193**, 183-187.
4. Benzie, I. F. F. and Strain, J. J., The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal. Biochem.*, 1996, **239**, 70-76.
  5. Bloem, A., Lonvaud-Funel, A. and de Revel, G., Hydrolysis of glycosidically bound flavour compounds from oak wood by *Oenococcus oeni*. *Food Microbiol.*, 2008, **25**, 99-104.
  6. Cai, Y. Z., Mei, S., Jie, X., Luo, Q. and Corke, H., Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. *Life Sci.*, 2006, **78**, 2872-2888.
  7. Castellari, L., Ferruzzi, A., Magrini, A., Giudici, P., Passarelli, P. and Zambonelli, C., Unbalanced wine fermentation by cryotolerant vs. non cryotolerant *Saccharomyces* strains. *Vitis*, 1994, **33**, 49-52.
  8. Christgau, S., Kofod, L. V., Halkier, T., Andersen, L. N., Hockauf, M., Dörreich, K., Dalbøge, H. and Kauppinen, S., Pectin methyl esterase from *Aspergillus aculeatus*: expression cloning in yeast and characterization of the recombinant enzyme. *Biochem. J.*, 1996, **319**, 705-712.
  9. D'Avino, R., Camardella, L., Christensen, T., Giovane, A. and Servillo, L., Tomato pectin methylesterase: modeling, fluorescence, and inhibitor interaction studies-comparison with the bacterial (*Erwinia chrysanthemi*) enzyme. *Proteins*, 2003, **53**, 830-839.
  10. Davis, R. C., Wibowo, D., Eschenbruch, R., Lee, T. H. and Fleet, G. H., Practical implications of malolactic fermentation. *Am. J. Enol. Vitic.* 1985, **36**, 290-301.
  11. De Gaetano, G., Castelnovo, A. D., Rotondo, S., Iacoviello, L. and Donati, M. B., A meta-analysis of studies on wine and beer and cardiovascular disease. *Pathophysiol. Haemost. Thromb.*, 2002, **32**, 353-355.
  12. Engelhart, M. J., Geerlings, M. I., Ruitenbergh, A., Van Swieten, J. C., Hofman, A., Witteman, J. C. M. and Breteler, M. M. B., Dietary intake of antioxidants and risk of Alzheimer disease. *J. Am. Med. Assoc.*, 2002, **287**, 3223-3229.
  13. Giovanelli, G., Evaluation of the antioxidant activity of red wines in relationship to their phenolic content. *Ital. J. Food Sci.*, 2005, **17**, 381-383.
  14. Hou, C. Y., Lin, Y. S., Wang, Y. T., Jiang, C. M. and Wu, M. C., Effect of storage conditions on methanol content of fruit and vegetable juices. *J. Food. Compost. Anal.*, 2008, **21**, 410-415.
  15. Hou, C. Y., Lin, Y. S., Wang, Y. T., Jiang, C. M., Lin, K. T. and Wu, M. C., Addition of phenolic acids on the reduction of methanol content in wine. *J. Food Sci.*, 2008, **73**, C432-C437.
  16. Huang, S. M., Chuang, H. C., Wu, C. H. and Yen, G. C., Cytoprotective effects of phenolic acids on methylglyoxal-induced apoptosis in neuro-2A cells. *Mol. Nutr. Food Res.*, 2008, **52**, 940-949.
  17. Huang, D. W., Shen, S. C. and Wu, J. S. B., Effects of caffeic acid and cinnamic acid on glucose uptake in insulin-resistant mouse. *J. Agric. Food Chem.*, 2009, **57**, 7687-7692.
  18. Jiang, C. M., Li, C. P., Chang, J. C. and Chang, H. M., Characterization of pectinesterase inhibitor in jelly fig (*Ficus awkeotsang* Makino) achenes. *J. Agric. Food Chem.*, 2002, **50**, 4890-4894.
  19. Lamien-Meda, A., Lamien, C. E., Compaoré, M. M., Meda, R. N., Kiendrebeogo, M., Zeba, B., Millogo, J. F. and Nacoulma, O. G., Polyphenol content and antioxidant activity of fourteen wild edible fruits from Burkina Faso. *Molecules*, 2008, **13**, 581-594.
  20. Lao, C. and Lopez-Tamames, E., Grape pectic enzyme treatment effect on white musts and wines composition. *J. Food Sci.*, **61**, 1996, 553-556.
  21. Lee, S. J., Mun, G. I., An, S. M. and Boo, Y. C., Evidence for the association of peroxidases with the antioxidant effect of p-coumaric acid in endothelial cells exposed to high glucose plus arachidonic acid. *BMB Rep.*, 2009, **42**, 561-567.
  22. Leifert, W. R. and Abeywardena, M. Y., Cardioprotective actions of grape polyphenols. *Nutr. Res.*, 2008, **28**, 729-737.
  23. Lewis, K. C., Selzer, T., Shahar, C., Udi, Y., Tworowski, D. and Sagi, I., Inhibition of pectin methyl esterase activity by green tea catechins. *Phytochemistry*, 2008, **69**, 2586-2592.
  24. Ly-Nguyen, B., Van Loey, A. M., Smout, C., Verlent, I., Duvetter, T. and Hendrickx, M. E., Effect of intrinsic and extrinsic factors on the interaction of plant pectin methylesterase and its proteinaceous inhibitor from kiwi fruit. *J. Agric. Food Chem.* 2004, **52**, 8144-8150.
  25. Massiot, P., Le Quééré, J. M. and Drilleau, J. F., Biochemical characteristics of apple juices and fermented products from musts obtained enzymatically. *Fruit Processing*, 1994, **4**, 108-113.
  26. McMillan, G. P. and Pérombelon, M. C. M., Purification and characterization of a high pI pectin methylesterase isozyme and its inhibitor from tubers of *Solanum tuberosum* subsp. *tuberosum* cv. Katahdin. *Physiol. Mol. Plant Pathol.* 1995, **46**, 413-427.
  27. Nelson, D. L. and Cox, M. M., Lehninger Principles of Biochemistry, 5th edition. W. H. Freeman and Co: New York, USA, 2008.
  28. Revilla, I. and Gonzalez-SanJose, M. L., Methanol release during fermentation of red grapes treated with pectolytic enzymes. *Food Chem.*, 1998, **63**, 307-312.
  29. Seddon, J. M., Ajani, U. A., Sperduto, R. D., Hiller, R., Blair, N., Burton, T. C., Farber, M. D. and Willett, W., Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *J. Am. Med. Assoc.*, 1994, **272**, 1413-1420.
  30. Soufleros, E. H., Irini, P., Petridis, D., Lygerakis, M., Mermelas, K., Boukouvalas, G. and Tsimitakis, E., Instrumental analysis of volatile and other compounds of Greek kiwi wine, sensory evaluation and optimization of its composition. *Food Chem.*, 2001, **75**, 487-500.
  31. Trichopoulou, A., Bamia, C. and Trichopoulos, D., Mediterranean diet and survival among patients with coronary heart disease in Greece. *Arch. Intern. Med.*, 2005, **165**, 929-935.
  32. Volschenk, H., Viljoen-Bloom, M., Subden, R. E. and van Vuuren, H. J., Malo-ethanolic fermentation in grape must by recombinant strains of *Saccharomyces cerevisiae*. *Yeast*, 2001, **18**, 963-970.
  33. Wang, Y. and Ho, C. T., Polyphenolic chemistry of tea and coffee: a century of progress. *J. Agric. Food Chem.*, 2009, **57**, 8109-8114.
  34. Wu, M. C., Jiang, C. M., Huang, P. H., Wu, M. Y. and Wang, Y. T., Separation and utilization of pectin lyase from commercial pectic enzyme via highly methoxylated cross-linked alcohol-insoluble solid chromatography for wine methanol reduction. *J. Agric. Food Chem.*, 2007, **55**, 1557-1562.
  35. Wu, J. S., Wu, M. C., Jiang, C. M., Hwang, Y. P., Shen, S. C. and Chang, H. M., Pectinesterase inhibitor from jelly-fig (*Ficus awkeotsang* Makino) achenes reduces methanol content in carambola wine. *J. Agric. Food Chem.*, 2005, **53**, 9506-9511.
  36. Yamaguchi, T. H., Takamura, T., Matoba, J. and Terao, J., HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci. Biotech. Biochem.*, 1998, **62**, 1201-1204.

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