# Microwave Assisted Extraction of Phenolics Compounds from Grape Vitis vinifera cv. País. A Comparison with Maceration

Estrella Aspe L., Raúl Fuentes U., and Katherina Fernández E.
Department of Chemical Engineering, University of Concepción, Concepción, Chile
Email: {easpe, raulfuentes, kfernandeze}@udec.cl

***Abstract*—Microwave assisted extraction (MAE) was applied for the extraction of phenolic compounds from grape seed and skin *Vitis vinifera L. cv. País*, and the results were compared with maceration. The goal was to optimize the phenolic compounds extraction from skin and seed, by MAE, studying the influence of extraction time and solid-liquid ratio (S/L). The MAE experiments were carried out according to Face-centered central composite design (CCD). The yield was modeled by a quadratic equation in a household microwave. Maceration was performed with an orbital shaker at 18 °C, to 180 rpm, for different times. The extracts were analyzed for total phenols content, means grade polymerization (mDP) and angiotensin-converting enzyme (ACE) inhibition. For skin and seed, optimum yield was determined to 180 s and S/L ratio equal to 1/11 for skin, and 1/14.9 for seed. In the experimental range used were obtained deviations were less than 5%. Differences were observed in extracts obtained by maceration and MAE. In MAE, for skin, less performance, more mdP and less ACE inhibition was obtained. Instead, seed presents lower performance, lower mDP and lower ACE inhibition. But, in maceration, kinetics is slower. So, it is necessary to study the energy cost before making scale up.**

***Index Terms*—MAE extraction, maceration, optimization, grapes, phenolic compounds.**

## I. Introduction

The cv. Pais grape is originated in Spain. It is a variety commonly found in the Biobio Region, Chile with low commercial value, and is mainly consumed as fresh fruit, but with a high amount of phenolic compounds.

Previous works [1]-[3] have demonstrated that cv Pais grape exhibits antioxidant capacity, contains high amounts of Proanthocyanidins (PAs) and the ability of inhibiting the angiotensin-converting enzyme (ACE).

Grape skin exhibits high antioxidant capacity, and there are high correlations between total phenols content and specific phenols (gallic acid, caffeic acid, and resveratrol) content and antioxidant capacity. The total phenols content and antioxidant capacity are higher in Chilean grapes vs. País, but minor that red Cabernet Sauvignon grape [1]

The PAs are located mainly in the skin and seeds of the grapes and they have phenolic compounds of high complexity. They are part of the flavonols family and are polymers composed by flavan-3-ol subunits connected by C-C bonds. The bioactive properties are determinated by molecular composition and size. PAs subunits are differenced by their substitutions and the stereochemistry of their structures. The most common monomers are (+)-Catechin (C), (-)-Epicatechin (EC), (-)-Epicatecuin-gallate (ECG) and (-)-Epigallocatequin (EGC) [2]

.PAs extracted from *V. vinifera* have demonstrated the ability of inhibiting the angiotensin-converting enzyme (ACE), decreasing the tension of blood vessels and blood volume, thus lowering blood pressure, proving it to be a potential treatment for hypertension and congestive heart failure [3]

Also, phenolic fractions have other bioactive properties, they present anti-mutagenic, anti-microbial, antitumor cardio protective and enzyme modulating properties, amongst many more, that are mostly due to their high metal quelating and antioxidant activities [4]. The mean degree of polymerization (mDP) of PAs, as well as the amount of aromatic hydroxyl groups determine these bioactive properties [5]. However, high molecule sizes can limit cellular adsorption of these compounds, thus limiting the effective bioactive properties mentioned and can isolate biologically active compounds [6]

The traditional methods as Soxhlet extraction, have been used for many decades, are very time-consuming and require relatively large quantities of solvents. In the last decade, there has been an increasing demand for new extraction techniques to shorten the extraction time, to reduce organic solvent consumption, and to prevent environmental pollution [7], [8]

Novel extraction methods including microwave assisted extraction (MAE), supercritical fluid extraction and pressurized solvent extraction have drawn significant research attention in the last decade. Microwave assisted extraction (MAE) is gaining interest as an advantageous method for the extraction of natural products because of its rapidity and low solvent consumption as compared to other extraction techniques.[9]-[14] It utilizes microwave energy to cause molecular movements and rotation of liquids with permanent or induced dipoles. When a biological material with favorable dielectric properties

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(plant material along with solvent for extraction) is placed in a microwave field, the molecules try to align with the oscillating electromagnetic field either by distortion or distribution of electron cloud within the molecule or by physical rotation of the molecular dipoles which leads to rapid heating of solvent and sample matrix [10]. Microwave assisted extraction is affected by a number of factors such as duration of microwave radiation (time extraction), power of microwave, type of solvent used, particle size of sample, temperature and solid/liquid ratio.[9]

The microwave extraction of phenolic compounds from grape seed was studied by several researchers [9]-[11]. Nevertheless, the phenolic compounds microwave extraction from grape skin has not been studied.

The objective of this work was to optimize the phenolic compounds extraction from skin and seed of grape *Vitis vinifera cv. Pais* by MAE, studying the influence of extraction time and solid-liquid ratio (S/L).

## II. Materials and Methods

### A. Materials

Pais grapes (*Vitis vinifera)* were harvested from Cerro Negro, Quillon, Chile, on March, 2012. They were frozen to -18 ℃.

All chemicals were of analytical or HPLC grade, obtained from Merck (Germany) or Sigma –Aldrich (U.S.A.)

### B. MAE and Maceration

The grapes were taken at room temperature. Skin and seeds of 62, 125, 200 or 375 grapes (according to experimental run S/L ratio) were manually separated. The skin and seeds obtained were weighed into a 250 ml Erlensmeyer-flask and put in contact with a water-acetone solution 2:1, v/v.

MAE was performed with the use of a household microwave (Daewoo, KOR-86 AH, Korea), without control temperature. For this reason, the process was performed by cycles with radiation (30 seconds to 900 W) and intercooling to achieve 20 °C (about 60 s).

Maceration (4 for skin and 3 for seed) was perforrned with an orbital shaker (New Brunswick Scientific G-24, USA) at room temperature (approximately 18 °C) with a stirring speed of 180 rpm, with extraction times of 3, 15, and 30 minutes, and 12 hours

After the extraction, the extracts were filtered under vacuum filtration in a Kitasato flask using filter paper (Xingxing Moderate 102, China). Then, the solvent was removed by evaporation at 35 ℃ and vacuum in the Bibby Rotary Evaporator (RE100, USA) and centrifuged in a Haraeus Sepatech Suprafuge 22 centrifuge for 10 minutes at 8000 rpm. The supernatants were taken to 100 mL and stored in glass flasks at -18 ℃, until their characterization.

### C. Experimental Design

According to previous publications on the phenolic compounds, the selection of time extraction and S/L ratio experimental ranges were performed by MAE. The extraction time was varied between 60 and 180 seconds and the S / L ratio between 1/25 and 1/6.7.

Optimization of MAE extraction condition of phenolic compounds was carried out using Face-centered central composite design (CCD). CCD is highly efficient and provides sufficient information on the effect of process variables for resourceful optimization with reduced number of total experimental runs [14]. The two independent factors studied were extraction time (60, 120 and 180 seconds) and S/L ratio (1/25.00, 1/10.00 and 1/6.67) and (1/50.00, 1/16.67 and 1/6.25) for skin and seed, respectively.

The CCD included 6 experimental runs for skin and 6, for seed, and was used to fit the second-order polynomial model [15]. Model and optimal conditions were validated by comparing them with results of three experimental runs for skin and seed (see Table I)

TABLE I. Model Validation Conditions for Experimental Run

| Solid –liquid ratio | | Extraction Time |
|---|---|---|
| Skin | Seed | [s ] |
| 1/3.33(**) | 1/5.56 | 120 |
| 1/6.67 | 1/16.25 (**) | 60 |
| O.C.(*) | O:C.(*) | 180 |
| | | |

(*): Optimal condition
(**): out experimental range point

### D. Chemical Analysis

The extracts were characterized using the following analysis: Total phenols, expressed as gallic acid equivalent (GAE), by the Folin-Ciocalteau modified method [16], [17], Phloroglucinolysis to estimate the mean degree of polymerization (mDP) [18] and the angiotensin I-converting enzyme (ACE ) activity was determined by monitoring the hydrolysis of HHL to produce HA, which was separated and quantified by HPLC. (Merck-Hitachi, LaChrom L7000 Series, Japan) [3].

## III. Results and Discussion

### A. Effect of Extraction Time on Yield Phenolic Compounds Liberated from Seed and Grape Skin by MAE

Experiments were performed to determine the effect of time on the total phenolic compounds yield extraction from seed and grape skin. (see Fig. 1 and Fig. 2)

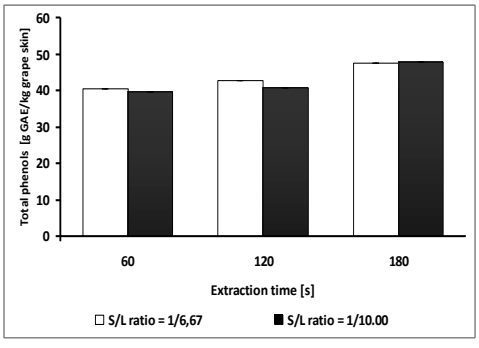


Figure 1. MAE extraction total phenols for grape skin cv Pais.

Fig. 1 and Fig. 2, for both skin and seed, it is observed that the extraction time increase produced an increment of the yield (expressed as total phenols concentration), which is consistent with the information provided by Reference [11] who studied the Silymarin extraction from *Sylibum marianum* and determined that at longer time the phenol extraction is favored.

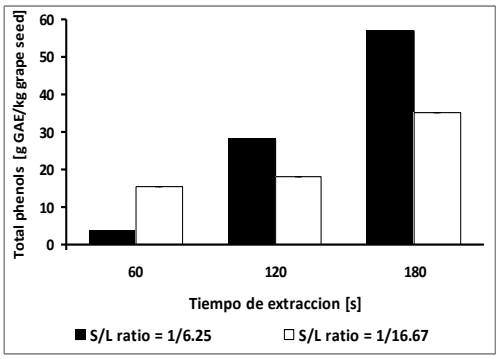


Figure 2. MAE extraction total phenols for grape seed cv Pais.

The phenols extraction through microwave from ground seeds of grapes (Pinot Noir) using a S/L ratio of 1/5 [g seed/mL of solvent] has been studied and also it has been reported that the total phenols extracted increased with the increment of time extraction, obtaining a total phenols concentration of 70 [g GAE/kg of seed] in 15 minutes time and 100 [g GAE/kg of seed] in 90 minutes time of extraction. Compared to the results obtained in the current work, with a maximum of 57 g GAE/kg of seed for a three-minute-extraction, a performance of 81% in 1/5 of the time performed by Reference [8] was observed.

From total phenols concentration increase, when increasing time for skin and seed, it is inferred that the extraction yield increases. Initially (in this study, from 60 to 120 seconds) changes and structural damage take place both in the skin and seeds; they promote the subsequent extraction of the phenolic compounds. The afore mentioned is reflected in images obtained by electronic microscopy (data not shown). Skin and seeds present different superficial features. In the skin, a greater surface area of contact was observed, therefore the matter transfer between the skin and the solvent takes place in a shorter time. This also explains what was observed in the experimental phase, where skin-solvent contact produced a fast coloration of it due to the rapid release of anthocyanin.

### B. *Effect of Solid/liquid (S/L) Ratio on Yield Phenolic Compounds Liberated from Seed and Grape Skin by MAE*

Fig. 3 and Fig. 4, for skin and seed, respectively, show to extraction time equal to 120 seconds the presence of maximum extraction for total phenols. The point of greater extraction for grape skin is obtained for a S/L ratio equal to 1/10.00 [g/mL] and for seed, this occurs on a S/L ratio equal to 1/16.67 [g/mL]. The presence of a maximum performance depending on the S/L ratio is consistent with what was reported by Reference [12] for extraction of phenolic compounds by MAE from *Geranium sibricum.*

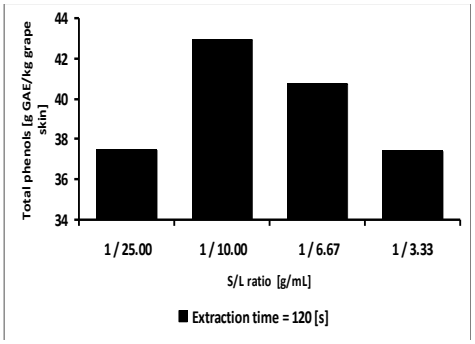


Figure 3. Effect of S/L ratio on total phenolic extraction yield from grape skin, .by MAE.

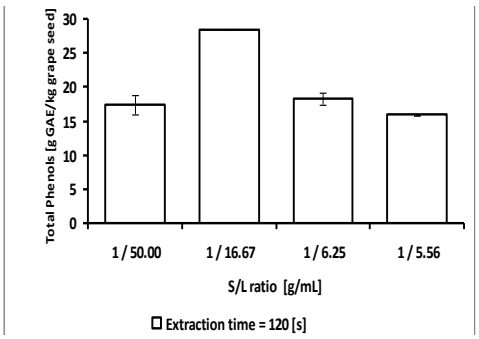


Figure 4. .Effect of S/L ratio on total phenolic extraction yield from grape seed .by MAE.

This can be explained due to at low S / L ratio, the solvent is capable of removing the small amount of phenol compounds present. If S / L ratio is increased, the amount of available phenols increases and also increases yield, until that the amount of solid is too high and the solid-liquid contact surface begins to decay, thus performance is decreased.

### C. *Factorial Design, Evaluation of the Response Surface for MAE Procedure*

With the experimental data shown in figures 1 to 4, the skin (equation 1) and seed (equation 2) performance was modeled. The model consisted of a quadratic equation.

$$F_{skin}\ [\text{g GAE/kg grape skin}] = -7.3130\ \mathbf{rsl^2} - 0.2179\ \mathbf{t'}^2 - 3.2195\ \mathbf{rsl*t'} + 4.7795\ \mathbf{rsl} + 6.4373\ \mathbf{t'} + 44.7215 \quad (1)$$

$$F_{seed}\ [\text{g GAE/kg grape seed}] = -18.2665\ \mathbf{rsl}^2 - 5.9166\ \mathbf{t'}^2 - 0.2130\ \mathbf{rsl*t'} + 3.4175\ \mathbf{rsl} + 25.5969\ \mathbf{t'} + 35.1922 \quad (2)$$

where **F,** is the total phenols yield. **rsl** and **t'** are linked with S/L ratio and extraction time by equations 3, 4 and 5.

$$\mathbf{rsl}_{\mathbf{skin}} = 30.03(S/L)\text{^}2+12.424\ /S/L) - 1.5455 \quad (3)$$

$$\mathbf{rsl}_{\mathbf{seed}} = -107.14\ (S/L)\text{^}2+33.571\ (S/L) - 1.6286 \quad (4)$$

$$\mathbf{t'} = 0.016667\ (t)-2 \quad (5)$$

where (S/L) is the solid-lquid ratio in [g/mL] and t is extraction time in [s]

Fig. 5 and Fig. 6 show a graphical representation of the model, for skin and seed, respectively. Both, equations 1 and 2 and figures 5 and 6, show that S / L ratio exhibits optimum value while the extraction time always favors

the extraction yield. These same relationships have been reported by Reference [11] for the silymarin extraction from *Silybum marianum*, by MAE. Thus, the optimum extraction time for both, skin and seed, is 180 seconds and the optimum solid-liquid ratio is 1/11.0 and 1/14.9 for skin and seed, respectively.

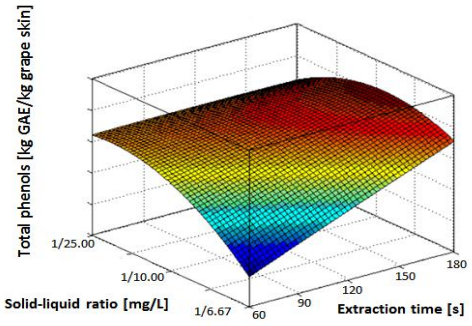


Figure 5. Response surface estimated from the factorial design plotting the quadratic model for total phenols extraction on grape skin (*Vitis vinifera cv Pais*) by MAE

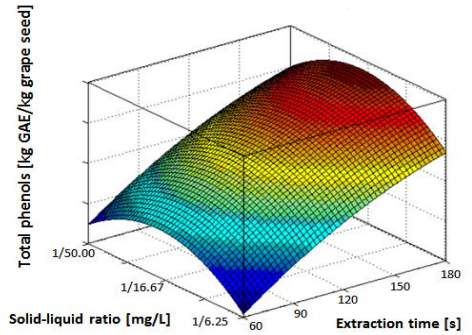


Figure 6. Response surface estimated from the factorial design plotting the quadratic model for total phenols extraction on grape seed (*Vitis vinifera cv Pais*) by MAE

### *D. Model Validation*

The model was validated considering the results of total phenols yields from experiments at points different from those used for obtaining the mathematical model. In Table II and Table III, the yields obtained for grape skin and seed respectively are shown.

Reference [9] has conducted studies about the microwave power effect, extraction time and solvent concentration measuring its effects in the total phenols extracted and in the antioxidant activity (DPPH) from grape seed (*Vitis vinifera*) These researchers approximated the total phenols content to a quadratic function obtaining good adjustments with a coefficient correlation $R^2$ equal to 0.9738[11]. Also, Reference [13] has obtained a high correlation between experimental data and second order polynomial model in the phenolic compounds extraction from *Pistachia vera*. In this work, both, skin and seed showed a coefficient correlation equal to 0.99.

### *E. Comparison between MAE and Maceration*

In Table IV, a comparison between the extraction yields, mDP and ECA inhibition of the extracts obtained by MAE is made.

TABLE II: MODEL VALIDATION FOR TOTAL PHENOLS EXTRACTION FROM GRAPE SKIN. COMPARISON BETWEEN EXPERIMENTAL DATA AND MODEL ESTIMATED VALUES

| S/L ratio [g/ml] | time [s] | Total phenols [g GAE/kg grape skin] | | % Deviation |
|---|---|---|---|---|
| | | Exp.value | Model value | |
| 1/3.33 | 120 | 37.4 ±0.5 | -13.216 | 383 |
| 1/6.67 | 60 | 39.6 ±0.6 | 41.046 | 3.52 |
| 1/11.00 | 180 | 48.5 ±0.4 | 51.035 | 4.95 |

TABLE III: MODEL VALIDATION FOR TOTAL PHENOLS EXTRACTION FROM GRAPE SEED. COMPARISON BETWEEN EXPERIMENTAL DATA AND MODEL ESTIMATED VALUES

| S/L ratio [g/ml] | time [s] | Total phenols [g GAE/kg grape seed] | | % Deviation |
|---|---|---|---|---|
| | | Exp.value | Model value | |
| 1/5.56 | 120 | 16.0 ±0.1 | 15.841 | 0.77 |
| 1/6.25 | 60 | 15.4 ±0.5 | -13.834 | 211.67 |
| 1/14.93 | 180 | 54.6 ±0.2 | 54.995 | 0.70 |

TABLE IV. COMPARISON OF CHEMICAL PROPERTIES OF EXTRACT FROM SKIN AND GRAPE SEED BY MAE AND MACERATION

| | Skin | | Seed | |
|---|---|---|---|---|
| | Mace-ration | MAE | Mace-ration | MAE |
| **S/L ratio [g/ml]** | 1/10 | 1/10 | 1/16.67 | 1/16.67 |
| **Extraction time** | 12 h | 180 s | 12 h | 180 s |
| **Total Phenols [g GAE/kg skin or seed]]** | 94.3 | 47.6 | 300.6 | 57.0 |
| **mDP** | 12.8 | 20.6 | 5.4 | 4.1 |
| **ACE Inhibition %** | 81.7 | 79.2 | 99.3 | 91.3 |

For Pais grape skin, through maceration, the greatest yield was obtained. The mDP for the skin extract obtained by maceration was a 37.5% less than the one obtained by microwave. The ECA inhibition was a 3,17% greater in the extraction by maceration.

For grape seed, the highest yield was obtained by maceration, but with an extraction time of 12 hours. The extract mDP of maceration is 32.11% higher than by microwave. ACE inhibition is 8.79% higher than the extract obtained by microwave.

Extraction by maceration and microwave, presents yields and chemical characteristics and different inhibition. Yields are higher for maceration, but much higher extraction times (12 hours compared to 3 minutes).

For skin, the lower mDP was obtained by maceration, while for the seed, using microwaves. ACE inhibition was high (over 75%) for both methods of extraction, being higher for maceration, but differences of 3.17% and 8.79%, for skin and seed, respectively.

Reference [14] optimized extraction conditions of phenolic compounds by MAE from oregano and

compared with conventional extraction method. The standardised MAE extraction conditions of time, temperature and wattage were observed to be 5 min, 100 ℃ and 200 W. The yield of extract is more in MAE than conventional method. Also MAE, showed a higher content of total polyphenols both lipophilic and hydrophilic.

Reference [14] suggest that this may be attributed to the better absorption of microwave energy, which increases temperature inside cells, resulting in the breaking of cell walls and releasing compounds into the surrounding solvent. In this work, this would explain the behavior of grape seeds.

## IV. Conclusions

Microwave assisted extracted phenolic extract of skin and grape seed was compared with conventional method of extraction. Experiments were conducted to determine the effect of time and S/L liquid on the extraction efficiency of microwave extraction and to model the same. Extracts obtained at different conditions were analyzed for yield, total phenols compounds, mDP, ACE inhibition and the means molecular weight. MAE was efficient and reliable, resulting in significant increase in the extraction efficiency when compared to conventional method, for grape seed.

All extracts obtained have favorable bioavailability. To define technology used on an industrial scale, it is necessary to conduct a study of the energy cost.

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**Estrella Aspé**, Date of Birth December 14, 1949. Civil Chemical Engineering (1974) University of Concepcion, Master in Science Engineering (1997) University of Concepcion.
She´s a full professor in the Chemical Engineering Department, Faculty of Engineering University of Concepcion.
Recent publications: "Heavy metals removal from influents to prevent mortality in salmon fry". Katherina Fernández, Marlene Roeckel, Estrella Aspé Acquacultural Engineering 58 (2014)103-106; "Simultaneous C and N removal from saline salmon filter effluents in anaerobic reactors Comprising anoxic-aerobic-Processes: effect of recycle ratio" Elisa Giustinianovich; Aspé Estrella; Huiliñir César, and Marlene Roeckel (2014). Journal of Environmental Science and Health, Part A. 49 (5), 584-592; "Effect of the bench scale extraction conditions on yield Pinus Radiata bark extract, antioxidant properties and composition" Valentina Ramos, Carlos Bocalandro, Sebastián Riquelme, Verónica Sanhueza, Estrella Aspé, Marlene Roeckel, Katherina Fernández (2013), Maderas, Ciencia y Tecnología, 15 (1): 31-44, 2013.

**Katherina Fernández**, Date of Birth April 25, 1977. Civil Chemical Engineer (2003) University of Santiago de Chile, Master in Science Engineering (2003) University of Santiago de Chile, Doctor of Science in Engineering (2007) Catholic University of Chile.
She´s an associated professor in the Chemical Engineering Department, Faculty of Engineering, University of Concepcion.
Recent publications: "Heavy metals removal from influentsto prevent mortality in salmon fry". Katherina Fernández, Marlene Roeckel, Estrella Aspé Acquacultural Engineering 58 (2014)103-106;

 

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**Raúl Fuentes.** Chemical Engineer (2013). University of Concepcion. Chile. Diplomated, environmental engineer (2013), University of Concepcion. He's recently graduated and his interests include software application on engineering, process simulations, environmental engineering