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**Artigo**

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# Extraction of bioactive polyphenols from grape marc by a matrix solid-phase dispersion method

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**Abstract** A matrix solid-phase dispersion process to extract polyphenols from grape marc, a winemaking byproduct, has been optimized by Response Surface Methodology. The dependent variables evaluated were the total polyphenols content, flavanols, hydrocynnamates, and flavonoids. The performance of the extraction method in terms of linearity and precision has also been assessed. The optimized MSPD method provides good results to extract polyphenols from white winemaking byproducts, much less studied than those from red wines, requiring low solvent consumption and being low cost and fast (15 min). The analytical instrumentation is available almost everywhere, thus becoming a ready-to-use methodology for virtually any control or winery laboratory. The optimized method has been applied to a set of bagasse samples from Albariño grapes (*Vitis vinifera* sp) cultivated in Galicia (NW Spain) and coming from five different types of grapevine training techniques. The vine training factor was significant on the basis of the content of the different groups of polyphenols determined in the MSPD extracts.

**Keywords** Grape pomace; Matrix solid-phase dispersion; Polyphenols; Vine training techniques; Winemaking byproducts.

## Introduction

There is an increasing interest, supported by environmental and economic reasons, to recover and exploit wastes from the food industry, because such residues can be used as a source of natural bioactive compounds which could in turn be used in pharmaceutical, cosmetics or back in the food industry. Polyphenols are a group of such compounds and they can be found, among others, in grape marc, the byproduct generated during the winemaking process (Alonso et al. 2002). Winery byproducts are of particular interest since grape (*Vitis vinifera*) is amongst the world's largest fruit crop, being the 6$^{th}$ in Europe with 26.8·10$^{6}$ MT (data from 2010) (FAO 2011). The antioxidant, antiradical and antifungal activities of bagasse or grape marc, which resides mainly in its polyphenolic content, have been the subject of recent research (Alonso et al. 2002; Baydar et al. 2004; Kammerer et al. 2004; Sagdic et al. 2011; Yilmaz & Toledo 2003) as well as its use as source of bioactive compounds. Polyphenols have been pointed out as some of the more interesting bioactive phytochemicals with important nutritional and health benefits (El Gharras 2009; Quideau et al. 2011) and useful as functional ingredients in the food processing industry (Gonzalez et al. 2010).

There is no uniform or official extraction procedures for grape and wine research, and the influence of extraction methodology on grape composition values has been already demonstrated (Lee & Rennaker 2011). Conventional extraction of polyphenols is generally performed by maceration (Virot et al. 2010) and in this context, different solvent systems have been tested to potentially provide the basis for a sustainable process of integrated exploitation of vinification by-products (Makris et al. 2007b). Nevertheless, this technique is not always interesting for an industrial extraction and/or production while the process is very slow and demands costly technological aids (Virot et al. 2010). Other techniques employed in the extraction of polyphenols from semi-solid and solid samples of different plant origin have been critically reviewed very recently (Ignat et al. 2010), but the matrix solid-phase dispersion (MSPD) approach proposed here has not been considered. Nevertheless, in a more general previous review (Liu et al. 2008) a few sample preparation approaches involving MSPD to extract polyphenols are included, which are briefly discussed below.

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MSPD (Barker et al. 1989) is a process for conducting simultaneous disruption and extraction of solid and semi-solid samples. The MSPD has a number of advantages over the classical procedures of sample treatment: simpler and faster analytical procedures; impossibility of forming emulsions; substantially reduced solvent consumption; improved extraction efficiency, because the entire sample is exposed to the extractant (Bogialli & Di Corcia 2007); and the option of performing extraction and clean-up at the same time reducing the possibilities of sample contamination and decreasing even more the amount of organic solvent required in the process (Barker 2000; Kristenson et al. 2006). The first applications of MSPD to the extraction of polyphenols were in the field of medicinal plants, specifically phenolic acids (Ziaková et al. 2003) and isoflavonoids (Xiao et al. 2004). Later, Manhita et al. (Manhita et al. 2006) applied the so-called sample disruption methods in the extraction of anthocyanins from vegetable samples, in what appears to be the first application of these techniques in *Vitis vinifera* (red grapes). These authors established differences depending on the nature of the dispersant material, naming MSPD the technique that uses silica substituted with C18 and proposing sea sand disruption method (SSDM) when the abrasive material was washed sea sand. While originally the MSPD involved the use of dispersants with a base of silica (Barker et al. 1989), the term has been adopted even when using alternative materials, and hence, SSDM has not been used any more.

Most applications of MSPD to grapes and related products have to do with the determination of pesticide residues (Albero et al. 2004; Fernández et al. 2000; Lagunas-Allue et al. 2010; Lian et al. 2010; Montes et al. 2009; Ramos et al. 2009). Recently, MSPD has been used to extract separately phenolic compounds and organic acids from white grapes in just one step (Dopico-García et al. 2007) trying to simplify a solid-liquid extraction followed by a solid-phase extraction (SL-SPE) method previously developed also by themselves. In that approach, the authors just used C18-based sorbents and concluded that MSPD was simpler and faster than SL-SPE, but the quantitative performance by SL-SPE was the best, especially for organic acids. The only previous work on the optimization of a MSPD procedure for the extraction of phenolic compounds is from Minuti and Pellegrino (Minuti & Pellegrino 2008), but its application is to red wines. However, a MSPD method is proposed for the first time here to the extraction of polyphenols from white winemaking byproducts

In the present work, we have optimized a MSPD method to extract polyphenolic compounds from the bagasse obtained as byproduct after the white winemaking, confining first the experimental domain with a set of preliminary experiments and then using Response Surface Methodology (RSM) for the fine tuning of the most influential factors. The dependent variables to be evaluated for each set of experimental conditions are robust and well established spectrophotometric indexes; namely the total polyphenols (TP), the total flavanols (TF), the total hydrocynnamates (THC), and the total flavonoids (TFC) contents. It has already been shown (Makris et al. 2007a) that these indexes pertaining to the polyphenolic composition are excellent parameters to assess the value of winery byproducts, among other food plant wastes, in comparative terms. The performance of the extraction method in terms of linearity and precision (inter and intra-day) have also been evaluated.

The optimized method was applied to a set of bagasse samples from Albariño grapes (*Vitis vinifera* sp) cultivated in Galicia (NW Spain) and used for the production of high quality white wines under the Protected Designation of Origin *Rias Baixas*. The set of grape marc samples comes from five different types of grapevine training techniques. The content of the different groups of polyphenols was determined in the corresponding MSPD extracts and used to evaluate the significance of the vine training factor.

## Materials and Methods

### Chemicals

Materials used as dispersant phases were: washed sea sand (200-300 µm, Scharlau); florisil (60-100 mesh, Supelco), $C_{18}$ (9-12% carbon, Aldrich), alumina (50-70 mesh, Sigma Aldrich) and silica gel 60 (230-240 mesh, Merk KGaA).

Extraction solvents used were methanol HPLC grade, supplied by Panreac (Castellar del Vallès, Barcelona, Spain); ethanol, acetone and ethyl acetate analytical grade provided by Merck (Darmstadt, Germany). Ultrapure water was produced in the laboratory with a Milli-Q gradient system (Millipore, Bedford, MA, USA). Hydrochloric acid (35%) was supplied by BDH, Aristar.

The Folin&Ciocalteau phenol reagent was obtained from Sigma. Other chemicals that are needed to determine the spectrophotometric indexes were DMACA (p-dimethylamino-cinnamaldehyde, Sigma), sodium hydroxide (NaOH, Merck), sodium nitrite ($NaNO_2$, PRO-BVS), sodium carbonate ($Na_2CO_3$, Panreac) and aluminum trichloride ($AlCl_3$, Merck).

Pure polyphenolic standards were used to build the calibration curves to determine the equivalencies for the spectrophotometric indexes: Gallic acid 99 % (CAS 149-91-7), Catechin 99 % (CAS 154-23-4) and Chlorogenic acid 98 % (CAS 327-97-9) and they were all supplied by Sigma-Aldrich (Steinheim, Germany).

### Grape Marc Samples

In order to advance the robustness of the experimental results, preliminary experiments and chemometrically designed experiments were carried out with a control bagasse sample (CB) built from a pool of Albariño grape marcs from different wineries and subzones of the Protected Designation of Origin *Rias Baixas* (Galicia, NW Spain). All grape marc samples were placed into plastic freezer bags, sealed and frozen immediately after the

pressing process (-20 ºC). To calculate the moisture content of the samples, 3 g of bagasse were dried in an oven at 105 °C. The sample was weighed before and after the dryness step. This operation was carried out in triplicate. All data were expressed on dry weight (dw).

Bagasse grape samples from the five different training techniques (VTT) studied were kindly donated by *Bodegas Martin Codax, S.A.*, one of the most outstanding Galician wineries (NW Spain), after the winemaking process. All grapes have been grown in the subzone O Salnés from the P.D.O. Rías Baixas. Two bagasse samples from grapes grown under each different VT were extracted twice using the developed MSPD method (n = 4). Following is a brief description of the available VTTs:

Vine arbor (VA): Traditional vine training technique with a “cane and spur” pruning system.

*Geneva Double Curtain (GDC):* Improves grape quality by reducing shade within a dense canopy, by dividing the mass of foliage into two “curtains”, enhancing exposure to light, quality of fruit and yield.

*Arched (A):* It is a conventional espalier or trellis based on a double-arched Alsace Style

*Espalier (E):* It is also a conventional espalier but with narrow lanes and with a pruning system Double Guyot.

*Scott Henry (SH):* It is essentially a variation on the Double Guyot system that produces a single, high curtain of vine easy to be mechanically harvesting and also with benefits concerning yield and quality.

The use of VTT in viticulture is intended principally to find equilibrium between the quantity of foliage and the quantity, quality and health of the grapes, besides to control the yield of the vineyard and to facilitate mechanization of certain agricultural tasks. The type of vine training system to use will be also determined by the climate conditions (mainly sunlight, humidity and wind). Some of the considered VTT, such as the Geneva Double Curtain (GDC) or the Scott Henry (SH), constitute a novelty in Galicia (NW of Spain).

Besides, the use of smaller initial particle sizes is a general trend in recent years in sample-preparation methods from any polyphenols-containing solid matrix (Liu et al. 2008). Sample particle size is an important parameter to ensure the success of the extraction procedure. Thus, to evaluate this aspect, four different apparatus have been used: three technical laboratory mills (tangential Restch MM400, orbital Retsch PM100 and ultra-centrifugal Restch ZM200) and a conventional electric coffee grinder Moulinex.

## MSPD procedure

One g grinded bagasse sample was gently blended with 2 g of the dispersing phase (sea sand, florisil, C18, alumina or silica gel) into a glass mortar using a glass pestle until a homogeneous mixture was obtained (ca. 5 min). Thus, once the blending process was complete, the mixture was transferred into a MSPD column filled with 0.5 g of washed sea sand at the bottom and provided with a polypropylene frit (IST, 16mm/20 µm). A second frit was placed on top of the sample before compression with a syringe plunger.

Elution was made by gravity flow using ethyl acetate, ethanol, methanol (MeOH) or different mixtures of MeOH:water, acidified with HCl to pH=1 in some of the experiences. The selected elution volume was between 5 and 15 mL, depending on the experiment, and the eluent was collected into a graduated conical tube. Five mL of the extract were concentrated under a nitrogen stream (VLM EC1 Sample Concentrator) to 0.5 mL.

## Preliminary Experiments

To properly define and restrict the experimental domain for optimizing the MSPD extraction procedure, some experiments related to the pretreatment of the bagasse (homogenization and particle size), the selection of the dispersing agent and the eluting solvent, as well as different extract cleaning procedures, were carried out. All experiments were performed in duplicate and each of the obtained extracts was submitted twice to the analytical procedure. The response was evaluated in terms of TP content.

Looking for the best homogenization of the sample, three different technical mills (tangential, orbital and ultra-centrifugal) and a conventional electric coffee grinder have been compared. For these tests, the samples must be dried and therefore remained in an oven at 40 °C for 24 hours. Two additional tests were also made with frozen and lyophilized bagasse samples using the coffee grinder.

## Determinations

*Total Polyphenols (TP):* The amount of total polyphenols in grape bagasse extracts was determined according to the Folin-Ciocalteau (FC) colorimetric method(Singleton & Rossi 1965). The reaction mixture was prepared by mixing 5 mL of water solution of extract, 100 µL of the FC reagent and 1 mL of an aqueous solution of sodium carbonate (20% $Na_2CO_3$). After vortexing, the reaction mixture was kept 30 min in the dark at ambient temperature, time enough for the reduction of the FC reagent by the polyphenolic compounds under alkaline conditions, resulting in the development of a blue color recorded at 760 nm (Spectrophotometer Shimazdu, UVmini-1240, Tokyo-Japan) and measured against a blank prepared with Milli-Q water. TP were quantified from a calibration curve prepared with gallic acid standard solutions in concentrations ranging from 3 to 20 $mg.L^{-1}$ ($R^2$=0.9982) and expressed as mg of gallic acid equivalents in the liquid extract ($mg.L^{-1}$ GAE). TP sample concentrations were expressed as mg gallic acid per g of dry weight of bagasse (mg gallic $g^{-1}$ dw).

*Total Flavanols (TF):* The measurement of total flavanols were adapted from Psarra *et al.* for white wines (Psarra et al. 2002). A 0.4 mL aliquot of the extract properly diluted in methanol were placed directly in the cuvette. Then 2 mL of the derivatization reagent DMACA solution were added (0.1 % in 1 M HCl in MeOH). The mixture was vortexed and then

the reaction kinetics was followed in the spectrophotometer, getting the maximum absorbance at 640 nm, which is reached at about 3 minutes. Methanol was used as blank. TF concentration was estimated from a calibration curve of catechin, obtained plotting known concentrations of the standard in methanol (1-16 mg.L$^{-1}$) against $A_{640}$ ($R^2$ = 0,9991). Results in the extract were expressed at catechin equivalents (mg. L$^{-1}$ CTE). Final concentrations were expressed as mg catechin per g of dry weight bagasse (mg catechin/g dw).

*Total Hydrocynnamates (THC):* The analysis of hydroxycinnamates in the MSPD extracts was also adapted from Psarra *et al (Psarra et al. 2002)* by measuring the $A_{320}$ value of the samples properly diluted in methanol in a quartz cuvette, using methanol as blank. Results were expressed as chlorogenic acid equivalents (mg. L$^{-1}$ CGAE), using the linear regression equation obtained by plotting known concentrations of CGA (5-30 mg.L$^{-1}$ CGAE) against $A_{320}$ ($R^2$ = 0,9968). Final concentrations were expressed as mg chlorogenic acid per g of dry weight bagasse (mg GCA g$^{-1}$ dw).

*Total Flavonoids Content (TFC):* Total flavonoids were measured according to the colorimetric assay used by Kim *et al (Kim et al. 2003)*. A 1 mL aliquot of appropriately water diluted extract was added to a 10 mL volumetric flask containing 4 mL of Milli-Q water. At zero time, 0.3 mL of an aqueous solution of $NaNO_2$ (5%) was added to the flask. After 5 min, 0.3 mL of 10% $AlCl_3$ was added. One min after, 2 mL of 1 M NaOH were added to the mixture. Immediately, the volume of the reaction flask was leveled with Milli-Q water and thoroughly mixed. The $A_{510}$ value of the mixture was determined against a water blank. TF concentration was estimated from a calibration curve of catechin, obtained plotting known concentrations of catechin in water (5-200 mg.L$^{-1}$) against $A_{520}$ ($R^2$ = 0,995). Results in the extract were expressed as catechin equivalents (mg. L$^{-1}$ CTE). Final concentrations were expressed as mg catechin per g of dry weight bagasse (mg catechin/g dw).

*Statistical analysis:* The experimental design and the data analysis were performed using Statgraphics Plus v5.1 software Manugistics, Rockville, MD, USA. Response Surface Methodology (RSM) was used for setting up the experimental design, with two factors (concentration and volume of the methanol in the extractant solution) and four dependent variables. The results obtained, both in RSM and in the evaluation of the different vine training techniques, were evaluated by analysis of variance (ANOVA), which measures whether a factor contributes significantly to the variance of the response. A comparison of means based on LSD Fisher test was used to determine significant differences between the levels within each factor.

## Results and discussion

### Optimization of the extraction process

No significant differences between the various types of mill or between the different sample pretreatment procedures (drying, freeze-drying, and freezing) were detected in terms of TP (data not shown). Since bagasse grape samples are stored frozen after collection in the wineries, the direct milling process with the coffee grinder has been selected, greatly simplifying the tasks prior to extraction, besides being economical and practical.

Regarding the nature of the dispersant, sea sand, florisil, C18, alumina and silica gel, were preliminarily assessed. These set of extractions were carried out keeping the dispersant:sample relationship in 2:1, one of the most common ratios (Barker 2007); and using a control solvent system consisted of 0,1% HCl in MeOH:$H_2O$ (70:30) (Figure 1). In the selected experimental conditions, the dispersant significantly affected the yield of TP extracted ($P_{0.05}$ = 0.2347). Florisil, C18 and alumina gave the lower responses, in terms of TP, for the extraction of polyphenols from white winemaking byproducts; while both sea sand and silica gel yielded the best results. Since differences between these both dispersants were not significant and due to the substantial difference in the price of both dispersing phases, sea sand was finally selected as dispersant agent for MSPD. The use of sand as dispersant has an additional advantage because the resistance to the elution is lower than with any other solid support material utilized.

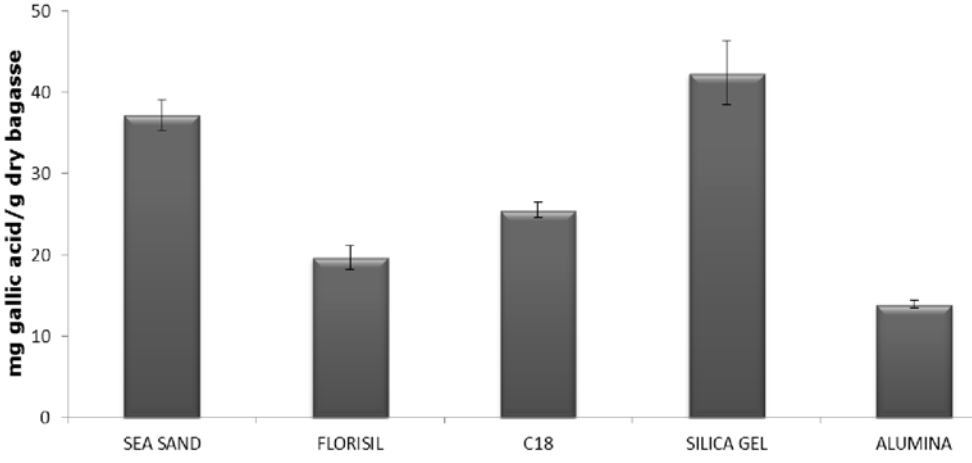


**Figure 1**.- Influence of the nature of the dispersant in the total polyphenols extraction from a control grape marc sample (n=3; confidence interval in terms of standard deviation)

Polyphenolic extracts of grapes and grape byproducts are normally obtained with water_alcohol mixtures in different proportions, acidified or not (Kammerer et al. 2004; Makris et al. 2007b; Ziaková et al. 2003). Thus, ethanol and methanol were tested as eluents. Ethyl acetate has also been used with this purpose (Kammerer et al. 2004) and thus it was included in the preliminary assays, too. Figure 2 graphically summarizes the outcomes. Acidified methanol-water mixtures gave the best results (pH 1 better than pH 2,5) showing nevertheless differences in function of the organic solvent percentage; so we decided to use acidified methanol as the organic solvent in the process of fine tuning by means of response surface methodology (RSM).

Several clean-up strategies have been considered in order to avoid or decrease possible matrix interferences in the analysis of the extracts: C18 at the bottom of the MSPD column; C18 added to the dispersant mixture, and a step of solvent clean-up with hexane:dichloromethane previous to the elution solvent. In all cases, 1 g of the cleaning agent was used. The obtained TP data, expressed as mg gallic acid.g$^{-1}$ dry bagasse, were compared with those obtained for a control without clean-up and showed significant differences at the expense of cleaning procedures: C18 bottom (13.81±0.081); C18 added (14.42±0.048); solvent clean-up (10.14±0.030) and CB (14.86±0.023); thus the next experiments were carried out without clean-up step.

Once defined the experimental domain, the influence of the organic solvent in the eluting solvent mixture and the influence of the eluting solvent volume were fine-tuned using the response surface methodology (RSM). The objective was to simultaneously optimize the levels of these variables to attain the best system performance. A face-centered central composite design 2^2 was chosen, so that the axial distance is equal to 1 and the values of percentage and volume originated in the matrix of experiments can be easily measured (Figure 3). Two central points were also added in order to increase the degrees of freedom to evaluate the experimental error. The final experiment number was ten and the correspondent factors and levels considered in the experimental design are also shown in Figure 3. Several dependent variables have been evaluated for each set of experimental conditions, namely the total polyphenols content (TP), the total flavanols content (TF), the total hydroxycinnamates content (THC) and the total flavonoids content (TFC).

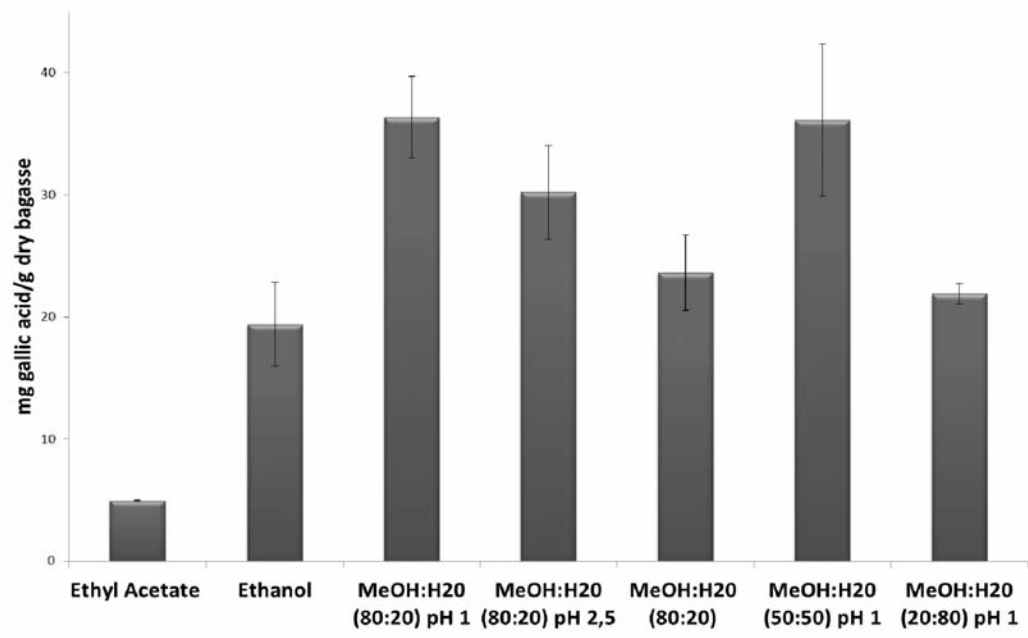


**Figure 2**.- Influence of the eluting solvent in the total polyphenols extraction from a control grape marc sample (n=4; confidence interval in terms of standard deviation)

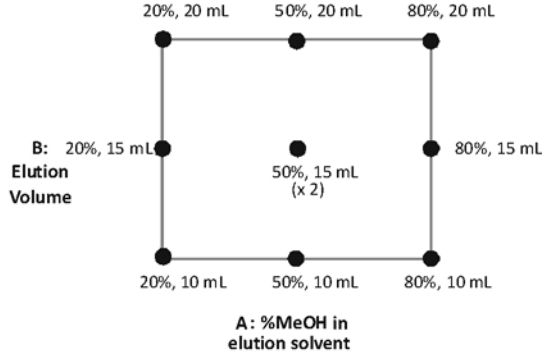


**Figure 3**.- Scheme of the experimental design for being tested with the Response Surface Methodology. The face-centered central composite design 2^2 with a replicated center point is showed. Factor A: level -1 (20 %), level 1 (80 %). Factor B: level -1 (10 mL), level 1 (20 mL); (α=1). The matrix of experiments was randomly generated

Results of ANOVA are shown in Table 1. The results showed that the percentage of methanol in the solvent system was the most important factor affecting extraction efficiency, and this factor was significant for all the dependent variables studied. On the other hand, nor the elution volume neither the interaction among factors were statistically significant in any case (Table 1). The main effect plots depicted in figure 4 (A-D) for all the dependent variables are valuable graphical tools for the interpretation of experimental design outcomes, showing the main effects with a line drawn between the low and the high level of the corresponding factors. The length of the line is proportional to the effect magnitude of each factor in the extraction process, and the slope sign indicates the level of the factor producing the highest response. The effect of the significant factor (percentage of methanol) was positive for every dependent variable (see fig. 4 A to D) meaning that higher responses were obtained at the highest percentage considered (80 %). As discussed above, the elution volume was not significant in any case (fig.4 A-D), and thus the intermediate value of 15 mL was selected.

We validated the methodology with a series of intra- and interday assays, by using a representative grape marc sample (the bagasse coming from the vine arbor training technique). The method was found to be precise with RSD values within 6, 9, 10 and 4 % for TP, TF, THC and TFC respectively (intraday assay, n=3). Interday RSDs were 7, 9, 10 and 4 % for the same indexes. Day-to-day variation was assessed by analyzing replicates on three separate days.

The final proposed MSPD extraction method derived from the optimization process is as follows: 1 g (fresh weight) of freshly frozen (-20ºC) bagasse was finely ground with a conventional electric coffee grinder, blended with 2 g of washed sea sand as dispersing phase, and transferred into a MSPD column. The elution of the polyphenolic fraction was carried out with 15 mL of a MeOH:$H_2O$ mixture, 80:20, acidified with HCl to pH 1. The obtained extracts were submitted to the different spectrophotometric determinations (TP, TF, THC and TFC) without further clean-up.

| | **Factors** | | | | **Interaction** | |
|---|---|---|---|---|---|---|
| | **A: MeOH (%)** | | **B: Volume (mL)** | | **AB** | |
| **Dependent Variables** | *F*-ratio | *p*-value | *F*-ratio | *p*-value | *F*-ratio | *p*-value |
| TP | 164.00 | *0.0002* | 5.06 | 0.0877 | 2.37 | 0.1982 |
| TF | 66.36 | *0.0012* | 0.08 | 0.7860 | 0.83 | 0.4129 |
| THC | 69.32 | *0.0011* | 0.93 | 0.3902 | 0.20 | 0.6807 |
| TFC | 12.35 | *0.0246* | 0.01 | 0.9255 | 0.19 | 0.6886 |

**Table 1**.- ANOVA results showing the significance of main effects and corresponding P values. *Statistically significant results are shown in italics*

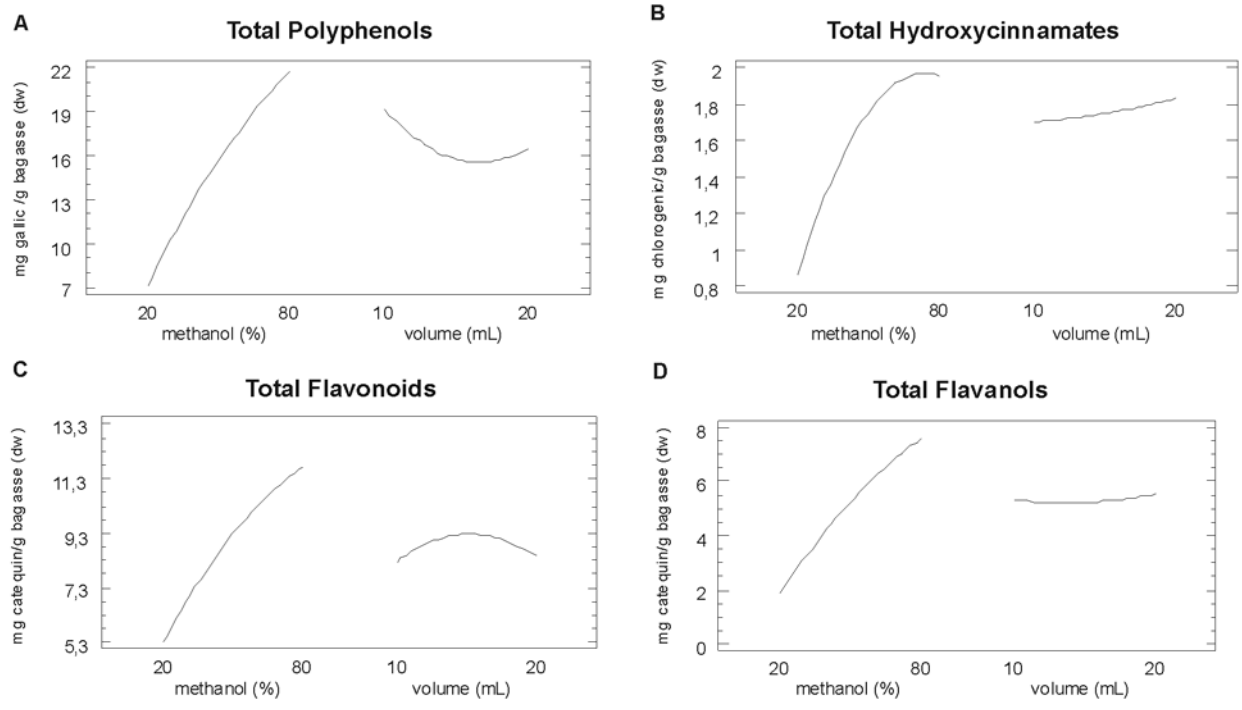


**Figure 4**.- Plots of the main effects (methanol concentration and volume of the elution solvent) for the four phenolic indexes determined in bagasse: A = TP; B = TF; C = THC; D = TFC

### Application of the method to bagasse samples: comparative study of different Vine Training Techniques (VTT).

The influence of the VTT in the polyphenols content of the bagasse MSPD extracts was here assessed by ANOVA; the corresponding F-ratios and p-values obtained indicate that the way of how grapes are grown affects the polyphenol content for all the families of compounds studied ($F_{TP}$ =10.56; $F_{TF}$ =3.51; $F_{THC}$ = 34.15; $F_{TFC}$ = 15.61; $p \leq 0.005$ for all of them). Figure 5 (A-D) shows the corresponding graphic output, where different letters above the error bars in the mean plots indicate significant differences at P<0.05 (LSD Fisher test). Regarding the total polyphenols (TP, fig. 5A) and the total flavonoids contents (TFC, fig. 5D), the GDC and the SH training techniques gave the highest values, and both systems belong also to the group that yields the maximum values for both total flavanols (TF, fig. 5B) and total hydroxycinnamates (THC, fig. 5C). The vine arbor system (VA) produces bagasse with the lowest values for all polyphenols families, followed by the crop in an espalier (E). The bagasse from grapes cultivated by the arched training system (A) gave intermediate values with a tendency towards higher. It should be recalled that these data are observations of bagasse samples from a single grape harvest, vintage 2010. Data from more than one vintage would be needed in order to identify more clear trends and to properly establish relationships between the content of the different families of polyphenols and the vine training techniques.

It is important to highlight that, besides being the first known application of the MSPD to the extraction of polyphenols from white wine grape marc, one of the most interesting findings of this study is that the proposed methodology can easily be applied in the control laboratories of any winery. The proposed MSPD method does not require expensive reagents and the determination of the spectrophotometric indexes only require a simple spectrophotometer available in virtually any laboratory. This methodology will allow wineries technicians to evaluate the results of any viticulture or enology experiment based on the spectrophotometric determination of such indexes in extracts that will be obtained quickly (15 minutes on average) and cheaply (less than 0.3 € per extract). This is also significant because, given the seasonality of vine crops, it is important to compare data between consecutive vintages, which with this methodology can be easily obtained.

## Conclusions

This study assesses the potential of the MSPD approach for the extraction of polyphenols from bagasse produced in the high quality white winemaking process, using RSM as the optimization tool. The optimized MSPD method provide good results, requiring low solvent consumption and short time (15 min) when compared to classical methods (about 24 hours macerations); each extract is very cheap and the required analytical instrumentation is available almost everywhere.

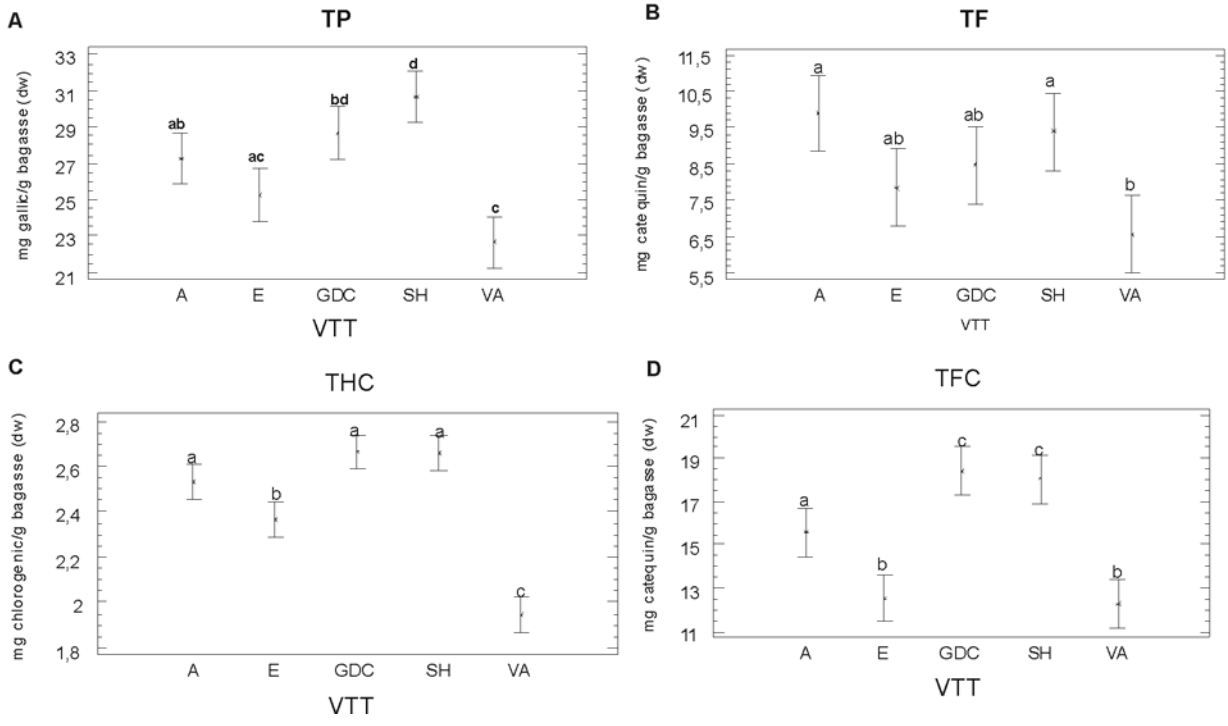


**Figure 5**.- Values (mean ± pooled standard error) of the four phenolic indexes in the bagasse samples from diverse VTT. Different letters above the error bars in the mean plots indicate significant differences at P<0.05 (LSD Fisher test)

The methodology was applied to albariño grape marc samples obtained from grapes grown using different VTTs. ANOVA data from a single vintage indicate that GDC and SH produced bagasse richer in polyphenols.

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