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Traceability of Phytosanitary Products in the Production of a Sherry Wine Vinegar

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In the present work, the monitoring of the evolution of the different phytosanitary products employed in the production of a Sherry wine vinegar has been carried out. The study covers the complete process, from the grape ripening to the vinegar fermentation. For the liquid sample analysis, a method based on SBSE (stir bar sorptive extraction) coupled to GC−MS and previously developed was used. For the grape samples, the use of two different extraction methods (ultrasound assisted extraction and microwave assisted extraction) was considered. Both methods were correctly optimized by means of factorial designs and were finally compared to each other. Considering the obtained results, the ultrasound extraction method was chosen to make the extraction of the solid samples. After the extraction process, the different extracts were analyzed by means of SBSE−GC−MS. The achieved results show the decrease of the phytosanitary product residues during the grape ripening, most of them being removed completely before the final product.

KEYWORDS: SBSE; Sherry wine vinegar; phytosanitary products; pesticides; traceability

INTRODUCTION

Phytosanitary products, or most commonly called pesticides, are chemical compounds usually used on agriculture in order to prevent the possible diseases originated by insect plagues, fungi or other pests to crops such as grapes. In the frame of Jerez, the most common families of pesticides used belong to the group of organophosphorous compounds, thiocarbamates, phthalimides, benzimidazoles, dicarboximides, triazines and phenylureas, each one used for a different disease (*1*).

A quantity of pesticide remains deposited on the grape and decreases progressively over time, until the grapes are harvested, when minimal residues can persist till the final product such as wine (*2*) or wine vinegar. The appearance of these pesticide residues into the final vinegar depends on several factors, as many as steps exist in the production process from the grape to the vinegar, including the intermediate must and wine (*3*). Depending on the pesticide and the production process, the evolution will be different. The chemical degradation is the essential way of elimination and logically depends on the chemical stability of the molecule (*4*). Sometimes, more toxic degradation products than the original substance are produced such as methyl paraoxon derived from methyl parathion (*5*). The reduction of these residue contents can be caused by adsorption onto the solid matter and subsequent elimination with the marc, by the action of yeasts and enzymes, the addition of fining agents, acid hydrolysis, or acetic fermentation, among other processes (*6*−*8*). But even with all these processes, some pesticides can persist till the final vinegar (*9*).

Hazard Analysis and Critical Control Points (HACCP) is a systematic preventive approach that addresses physical, chemical, and biological hazards as a means of prevention rather than finished product inspection. HACCP is used in the food industry to identify potential food safety hazards, so that key actions, known as Critical Control Points (CCPs), can be taken to reduce or eliminate the risk of the hazards being realized. In the case of vinegar production, phytosanitary product residues can be considered a CCP, so methodologies for their analysis, vigilance and correction must be proposed, in order to guarantee the traceability and food safety of vinegars from grapes treated with this kind of product. On the other hand, due to a progressive change in consumers, the number of products with the "ecologic" or "organic" label is increasing on the market. The use of phytosanitary products in the elaboration of these foods is forbidden. However, in the case of "organic wines" Andrey and Amstutz (*10*) revealed that 61% of 83 labeled "organic wines" found in Swiss marketplaces contained pesticide residues. So for vinegar, not only those companies producing "ecologic" vinegar but also those interested in the food safety of their products should monitor the complete vinegar production process.

Stir bar sorptive extraction (SBSE) is a fast, simple and relatively newly developed technique where a magnetic stirring bar of polydimethylsiloxane (PDMS) is added to the sample to

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Table 1. Factor levels for the extraction condition optimisation. UAE: Ultrasound assisted extraction; MAE: Microwave assisted extraction

	UAE				MAE			
values	sample (g)	solvent (mL)	extraction time (min)	centrifugation time	sample (g)	solvent (mL)	extraction time (min)	extraction *T* (°C)
low (−)	10	10	10	5	2	10	5	75
high (+)	30	50	20	15	10	30	20	125
center	20	30	15	10	6	20	12	100

promote the transfer of analytes to the polymer coating, and after a predetermined extraction period, the analytes are thermally desorbed in the GC injector. It has been successfully used in the analysis of pesticides on food samples such as vegetables (*15*), grapes (*16*), juices (*17*), wine (*18, 19*), and vinegar (*9*). On the other hand, the evolution of different pesticides from the processed grapes through the winemaking process to the final wine product has been studied (*8, 11*−*14*) but to date, a complete study of vinegar production traceability has never been carried out. In the present work, the monitoring of the evolution of the different phytosanitary products employed in the production of a Sherry wine vinegar covering the complete process, from the grape ripening to the vinegar fermentation, has been carried out. For grape samples, the use of two different extraction methods (ultrasound assisted extraction and microwave assisted extraction) was considered. Both methods were correctly optimized by means of 2^4 factorial designs, and finally were compared with each other. After the extraction process, the different extracts were analyzed by means of SBSE−GC−MS. For the liquid sample analysis such as musts, wines and vinegars, a method based on SBSE−GC−MS and previously developed was used (*9*).

MATERIALS AND METHODS

Chemicals. Pesticides, comprising Pyrimethanil, flufenoxuron, chlorpyrifos-methyl, vinclozolin, metalaxyl, fenitrothion, malathion, dicofol, chlorpyrifos, cyprodinil, triadimenol, procymidon, hexythiazox, folpet, fludioxonil, iprodion, benalaxyl, and fenhexamid were supplied by Sigma-Aldrich (PESTANAL, Riedel-de Haën, Seelze, Germany). A global stock standard solution was prepared by accurately weighing 5−10 mg of each individual pesticide standard into a 50 mL volumetric flask, dissolving with acetone and diluting to volume with ethanol. Working samples used in the analytical process development were prepared by spiking different amounts of the global standard solution on a quantity of clean crushed grape, homogenizing carefully prior to the extraction method.

The standard solution was stored at 4 °C.

Heptachlor epoxide, supplied by Sigma-Aldrich, was employed as internal standard.

Taking into account its poor stability (*19*), Iprodion was quantified by means of its degradation product (3,5-dichlorophenyl)hydantoin.

Sampling and Fermentation Conditions. All the grape samples (Palomino Fino variety) were taken from a local vineyard (southern of Spain) during the ripening process, from June to September of 2007. The sampling was carried out periodically every week, covering the studied field as much as possible. The number of bunches collected on each sampling was from 25 to 30. These bunches were subsequently mixed and homogenized, and were stored at −20 °C until they were analyzed.

The must and wine samples came from the studied grapes, and the sampling was carried out also weekly from September of 2007 to June of 2008, when the vinegar was made. During the alcoholic fermentation (two weeks), the sampling was carried out in a more exhaustive way, in order to achieve a better control of the process. In the same way, during the vinegar production process, which involved three days, samples were taken twice a day until the end of the process (7.92° acidity and 1.60° Baumè). Both processes, alcoholic and acetic fermentation, were carried out in industrial scale in the conventional way (*20*). Briefly, for alcoholic fermentation, the pH of the must (obtained by means of 1.5 kg/cm^2 pneumatic press) was adjusted to 3.25 with tartaric acid; 100 mg/L SO_2 equivalent (potassium metabisulfite) were also added; the fermentation temperature was set at 25 °C, employing *Saccharomyces cerevisiae* as fermentation yeast. In the case of the acetic fermentation, the process was carried out in a cylindrical stainless steel reactor with concave bottom (submerged cultivation type) with a capacity of 33000 L, containing, at the start of the process, a load of vinegar to which the base wine is added. The process was subjected to a system of aeration from the bottom of the tank (70 m^3/h). The temperature was set at 32 °C.

The treatment employed in the vineyard was the following: fungicides Vimar Combi (folpet and metalaxil) on April 2007 and Dimenol (triadimenol) on May 2007, both employed against *Oidium*; insecticide chlorpyrifos on June 2007, employed against *Lobesia botrana* (responsible for *Botrytis cinerea*).

Sample Preparation. The solid samples (grapes) were subjected to consecutive extraction processes. Ten grams of crushed sample was submitted either to ultrasound assisted extraction using 10 mL of methanol as a solvent, during 10 min, or to microwave assisted extraction using 10 mL of methanol as a solvent, during 5 min and reaching a temperature of 125 °C. After this, the extracts were centrifuged at 4000 rpm for 5 min. After centrifugation, 0.5 mL of the liquid was taken and added to 10 mL of Milli-Q water into a 100 mL Erlenmeyer flask in order to carry out the SBSE extraction. 20 mm × 0.5 mm (length × film thickness) PDMS commercial stir bars, supplied by Gerstel (Mülheim a/d Ruhr, Germany), were used. Each sample was spiked with 10 μL of a solution of heptachlor epoxide (342 μg/L in acetone). The Erlenmeyer flask was placed on a 15 position magnetic stirrer (Mülheim a/d Ruhr, Germany) and stirred at 1000 rpm at 25 °C for 150 min. After the extraction was finished, the stir bar was washed in distilled water and dried with a lint-free tissue. Finally, thermal desorption was carried out.

The liquid samples (musts, wines and vinegars) were extracted using a methodology developed in a previous work (*9*).

After each analysis, a cleaning up procedure was performed (300 °C during 15 min). After this treatment, the stir bars did not show any measurable signal of pesticides.

Apparatus. During the microwave assisted extraction optimization process, the microwave system used was the ETHOS-1600 (Milestone, Shelton, CT) closed vessel oven system equipped with 10 perfluoro alkoxy (PFA) vessels. Extractions were performed at 500 W. The system employed to carry out the ultrasound assisted extractions was supplied by Selecta (Barcelona, Spain).

In relation to the SBSE extraction, the coated stir bars were thermally desorbed using a commercial TDS-2 thermal desorption unit (Gerstel) connected to a programmed-temperature vaporization (PTV) injector CIS-4 (Gerstel) by a heated transfer line. The PTV injector was installed in an Agilent 6890 GC-5973 MS system (Agilent Technologies, Palo Alto, CA). The thermodesorption unit was equipped with a MPS 2 L autosampler (Gerstel) capable of handling the program for 98 coated stir bars. The desorption temperature was programmed in the following way: from 30 to 300 °C (held for 10 min) at 60 °C/min under a helium flow (75 mL/min). Cryofocusing temperature: −150 °C using liquid nitrogen. Final heating from −150 to 300 °C (held for 5 min) at 10 °C/s for analysis by GC−MS. Capillary GC−MS analyses in the electron impact mode were performed on an Agilent 6890 GC-5973N MS system (Agilent, Little Falls, DE). The column used was a HP-5 capillary column (J&W Scientific, Folsom, CA), 30 m × 0.25 mm i.d., with a 0.25 μm coating, and the carrier gas was helium at 1.0 mL/min.

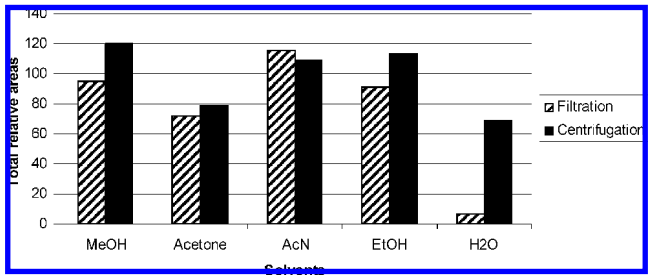

Figure 1. Ultrasound assisted extraction. Total relative areas obtained using different solvents with filtering or centrifuging. Studied solvents: methanol (MeOH); acetone; acetonitrile (AcN); ethanol (EtOH); water (H_2O).

The GC oven was programmed as follows: 70 °C for 2.5 min, then arriving to 150 at 25 °C/min; subsequently to 200 at 3 °C/min, and to 300 at 8 °C/min, being held for 10 min. The mass detector was operated in the EI+ mode at 70 eV. Selected ion monitoring mode, choosing for each compound one quantifying ion and two or three qualifying ions, was employed.

Experimental Design. The Statgraphics Statistical Computer Package "Statgraphics Plus 5.1" for Windows was used for data treatment.

Both studied extraction processes, ultrasound assisted extraction (UAE) and microwave assisted extraction (MAE), were optimized by means of 2^4 factorial designs. **Table 1** gives the different considered analytical parameters and their maximum (+) and minimum (−) values for each optimization process.

RESULTS AND DISCUSSION

Development of the Extraction Method. Two extraction methods (ultrasound and microwave) were developed by means of statistical approaches. For each case, 2^4 factorial designs were employed involving 17 experiments in duplicate (a centerpoint for each factor was considered). Total chromatographic area of the quantifying ions corresponding to all the pesticides studied was selected as the experimental response for optimizing. After that, both methods were finally compared to each other in order to choose the optimal one for pesticide extraction from grapes.

Ultrasound Assisted Extraction. Previous Studies. The starting point was a method on ultrasound extraction (*15*) which seemed to be adequate to analyze pesticides in grapes, and from that, the possibility of using different solvents in the extraction method was checked. Several tests were carried out in order to select the optimun solvent and the cleaning step. A total amount of clean grape was weighed, crushed, spiked with a known quantity of pesticides and mixed during 5 min. Fifteen grams of the mix was employed to carry out each step, using 30 mL of methanol, acetone, acetonitrile, ethanol and water, and filtering (0.45 μm with prefilter of 0.1 μm) or centrifuging (4000 rpm during 6 min). All analyses were made in triplicate. After that, the extracts were analyzed by means of SBSE. The total relative areas obtained using different solvents (with filtration and centrifugation) are shown in **Figure 1**. As can be seen, the highest response was obtained using methanol and centrifugation. In general, better responses were obtained after centrifugation of the extracts. Therefore, methanol was selected as solvent and centrifugation was chosen as cleaning step prior to SBSE.

Ultrasound Assisted Method. Extraction Condition Optimization. Sample amount, volume of solvent, extraction time and centrifugation time were evaluated to achieve the best overall analytical conditions. The data obtained were evaluated by ANOVA at the 5% significance level. These results are shown in bar chart format with the effects sorted out in rank order

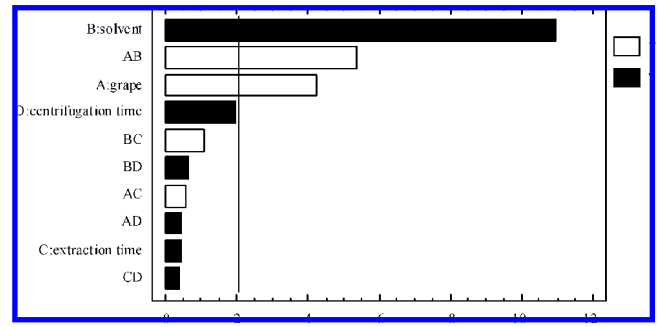

Figure 2. Ultrasound assisted extraction. Pareto chart of the main effects for the studied pesticides.

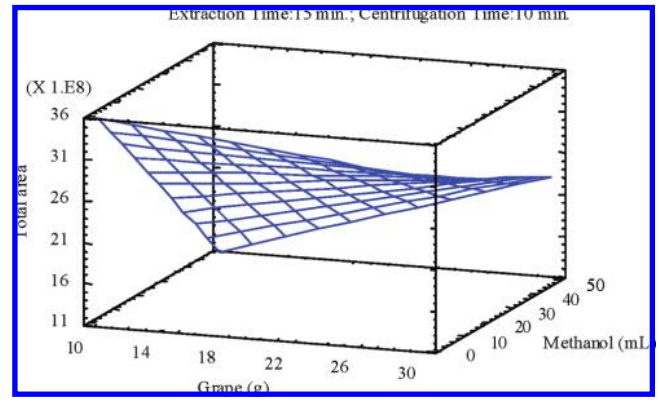

Figure 3. Ultrasound assisted extraction. Estimated response surface obtained by plotting sample amount vs volume of solvent.

(**Figure 2**). Volume of methanol had the higher significant effect, showing a negative sign. Sample amount was also a significant parameter, showing a positive sign, whereas centrifugation time and extraction time had not a significant effect (at $p < 0.05$). The figure also shows a positive interaction between sample amount and volume of solvent. **Figure 3** represents the estimated response surface taking into account the sample amount and volume of solvent as variables. The mentioned interaction can be seen, because when the employed volume of methanol is low, higher response is achieved with minor sample amounts, whereas when a high volume of methanol is employed, the obtained response practically does not change with the used sample amount. Therefore, the final selected conditions for the ultrasound assisted extraction were as follows: sample, 10 g; methanol, 10 mL; time extraction, 10 min; and time of centrifugation, 5 min.

Microwave Assisted Extraction. Previous Studies. Mean conditions were used as a starting point (*21, 22*), to compare different solvents usually employed in this kind of extraction. Five grams of sample was extracted with 20 mL of acetone, acetonitrile, ethanol, methanol and water successively, reaching to 100 °C during 10 min with a stirring of 30%. The refrigeration time used was 20 min, and all the extracts were centrifuged. **Figure 4** shows the mean values ($n = 3$) of relative areas of all the studied pesticides opposite the employed solvent. As can be seen, methanol acts as the best extractant; therefore it was selected as solvent for microwave assisted extraction.

Microwave Assisted Method. Extraction Condition Optimization. Sample amount, volume of solvent, extraction temperature and extraction time were evaluated to achieve the best overall analytical conditions. The values considered for each analytical parameter are listed in **Table 1**. The data obtained were evaluated by ANOVA ($p < 0.05$). These results can be seen in **Figure 5**. Sample amount was the most influential variable,

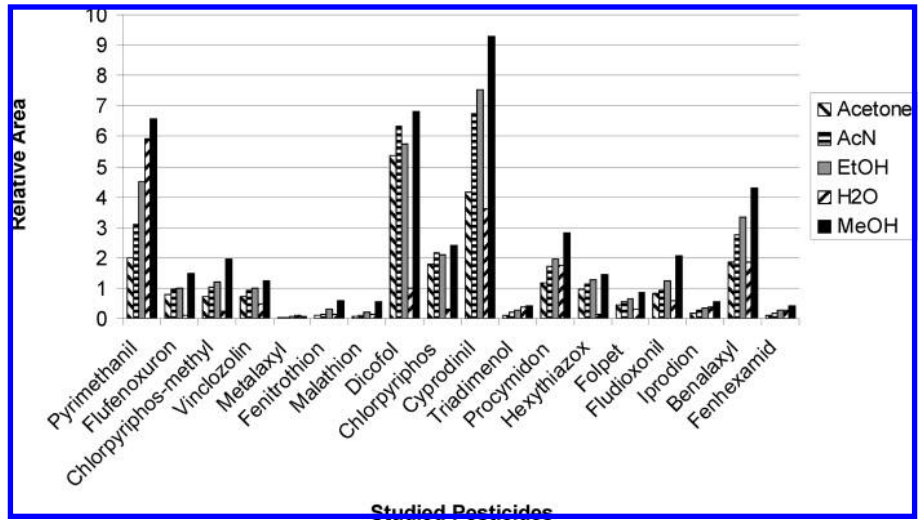

Figure 4. Microwave assisted extraction. Mean responses obtained for all studied pesticides using different solvents. Studied solvents: acetone; acetonitrile (AcN); ethanol (EtOH); water (H_2O); methanol (MeOH).

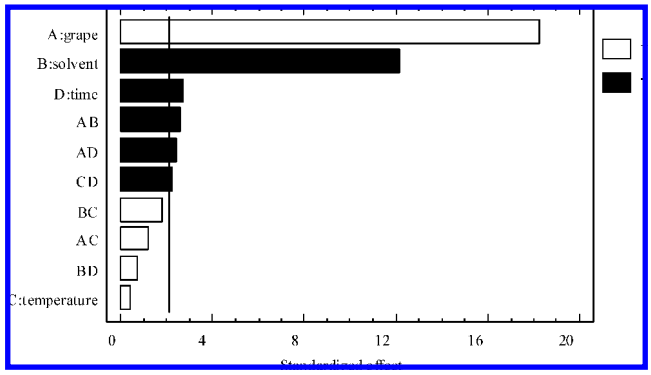

Figure 5. Microwave assisted extraction. Pareto chart of the main effects for the studied pesticides.

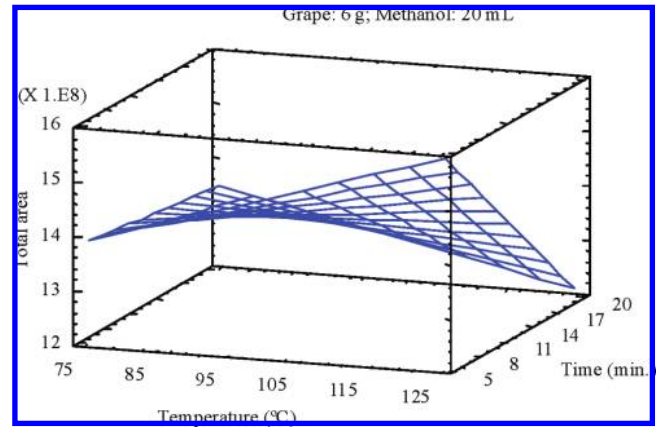

Figure 6. Microwave assisted extraction. Estimated response surface obtained by plotting extraction temperature vs extraction time.

showing a positive sign. Volume of solvent was also influential but showed a negative sign. Extraction time and extraction temperature had a very low or insignificant effect (at $p < 0.05$). There are several significant interactions between the variables, but the most interesting is the interaction extraction temperature−extraction time (**Figure 6**). When shorter extraction times were used, higher values of extraction temperature allowed higher values of analytical response, whereas for longer extraction times and higher extraction temperatures, lower responses were obtained. Therefore, the final selected conditions for the microwave assisted extraction were 10 g of sample; 10 mL of methanol; 5 min of extraction time at 125 °C.

Comparison of the Optimized Methods. After optimizing both methods, 5 extractions of the same sample were carried out using each one and the results were compared. The obtained results (**Figure 7**) pointed out little differences in favor of the ultrasound method. Taking into account, in addition, its higher operational capacity, such us the possibility of doing more extractions at the same time or the possibility of avoiding the heating of the sample, the final selected method for the extraction of pesticides in grapes was the ultrasound assisted method.

Performance Characteristics. *Calibration, Linearity and Detection and Quantitation Limits.* Amounts of clean crushed grape were spiked with pesticides in eight levels of concentration in triplicate, and the complete extraction process was carried out. The [pesticide/internal standard] molecular ion peak area ratio for the identified pesticide was used for each compound. The correlation coefficients obtained for each compound (**Table 2**) were good ($r^2 > 0.99$). The range of linearity studied for each compound also appears in **Table 2** and covered the concentration ranges expected for the various pesticides. This was also corroborated by the "on-line linearity (LOL) = 100 − RSD(b)", with values higher than 96% (**Table 2**). RSD(b) is the relative standard deviation of the slope (expressed as a percentage).

The detection and quantitation limits were estimated by extrapolating to zero concentration from the calibration curves constructed for each pesticide, using the relative standard deviation of the analytical signal corresponding to a zero concentration value. In this way, these limits were calculated as three and ten times, respectively, the relative standard deviation of the analytical blank values obtained from the calibration curve. The values obtained (**Table 3**) are much lower than those permitted by the Spanish legislation (*23*).

Accuracy. Recovery and Repeatability. Two different known concentrations of pesticides were spiked to different grape samples (A and B) with different contents of water, in order to see the possible effect of variation of water during the grapes' ripening. The complete extraction process was carried out, and both the concentrations before and after the additions were determined. The percentage of recovery was calculated for each studied compound on the evidence of these concentrations (**Table 3**). All the experiments were made in triplicate. As can be seen, the values of recovery range from 72% to 122%, acceptable values taking into account that the samples have been submitted to two successive processes of extraction (ultrasound

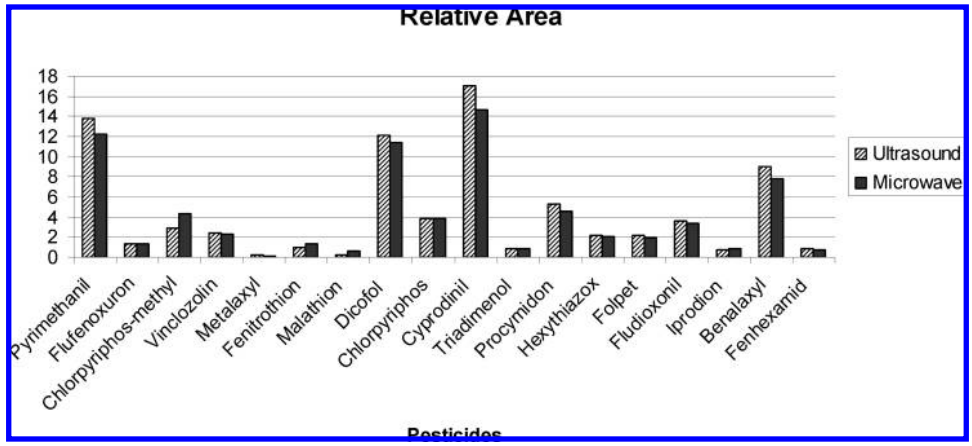

Figure 7. Mean results ($n = 5$) for all studied pesticides obtained from the comparison between ultrasound assisted extraction and microwave assisted extraction.

Table 2. Characteristics of the Calibration Curves

compound	linear range (μg/L)	regression coefficient	linearity (LOL, %)	slope	intercept
pyrimethanil	10.8−1080	0.9948	97.44	0.0168	−0.4904
flufenoxuron	10.8−540	0.9932	96.28	0.0017	0.0265
chlorpyrifos-methyl	10.2−510	0.9915	96.22	0.0026	−0.0449
vinclozolin	9.2−920	0.9957	97.51	0.0034	0.0089
metalaxyl	21−2100	0.9930	97.34	0.0001	0.0034
fenitrothion	10−500	0.9947	96.73	0.0009	−0.0217
malathion	16−800	0.9946	96.98	6×10^{-5}	−0.0005
dicofol	9.4−940	0.9926	97.12	0.0183	−0.0851
chlorpyrifos	8.6−860	0.9993	98.98	0.0045	−0.0305
cyprodinil	11−825	0.9958	97.83	0.0292	−0.3763
triadimenol	20.2−2020	0.9929	97.32	0.0007	−0.0064
procymidon	9.8−735	0.9964	97.87	0.0111	−0.1523
hexythiazox	10−1000	0.9942	97.45	0.0032	−0.0839
folpet	50−1000	0.9973	97.66	0.0005	−0.0351
fludioxonil	13−1300	0.9930	97.20	0.0045	−0.0902
iprodion[a]	21.8−1635	0.9966	97.78	0.0007	−0.0325
benalaxyl	9.6−960	0.9957	97.81	0.0126	0.1232
fenhexamid	18−1800	0.9912	97.02	0.0008	0.0333

[a] Degradation product: (3,5-dichlorophenyl)hydantoin.

Table 3. Performance Characteristic

compound	detection limit (LOD, μg/L)	quantitation limit (LOQ, μg/L)	recovery (%) grape A	recovery (%) grape B	repeatability (RSD, %)
pyrimethanil	7.34	10.34	98.74	112.52	3.81
flufenoxuron	8.14	11.43	74.96	80.13	19.93
chlorpyrifos-methyl	7.88	9.98	84.35	101.87	6.53
vinclozolin	7.99	9.21	94.6	99.58	2.67
metalaxyl	17.54	18.99	91.22	89.61	5.25
fenitrothion	8.87	10.12	87.39	115.13	8.42
malathion	10.34	15.87	72.81	112.09	2.95
dicofol	7.87	9.09	82.8	82.65	8.21
chlorpyrifos	6.70	8.78	102.14	118.73	5.65
cyprodinil	8.99	10.56	112.86	121.96	6.02
triadimenol	12.35	16.04	104.6	111.93	7.83
procymidon	6.76	8.34	83.22	78.08	4.80
hexythiazox	7.77	10.12	74.5	82.17	11.60
folpet	39.96	49.12	96.73	101.23	6.68
fludioxonil	10.03	13.45	82.48	120.73	5.92
iprodion[a]	17.45	20.56	84.39	120.34	10.14
benalaxyl	6.99	9.04	93.87	106.19	9.80
fenhexamid	14.76	19.34	86.17	107.99	5.87

[a] Degradation product: (3.5-dichlorophenyl)hydantoin.

assisted extraction and stir bar sorptive extraction). No interference due to the different contents of water was also corroborated in view of these results.

In addition, both the polyphenolic content of the grape and its sugar content change during the ripening process. This fact can affect our extraction processes, therefore two studies, in reference

Table 4. Influence of the Polyphenolic and Sugar Content in the Pesticides' Extraction

compound	polyphenolic influence study (RSD, %)	sugar influence study (RSD, %)
pyrimethanil	10.92	11.17
flufenoxuron	25.21	22.34
chlorpyrifos-methyl	11.12	7.15
vinclozolin	8.53	9.37
metalaxyl	13.81	16.58
fenitrothion	14.80	7.83
malathion	12.14	8.74
dicofol	9.51	6.59
chlorpyrifos	10.00	8.97
cyprodinil	9.75	7.91
triadimenol	13.96	12.29
procymidon	10.06	12.72
hexythiazox	9.88	5.35
folpet	8.09	7.57
fludioxonil	13.60	9.81
iprodion[a]	14.41	10.79
benalaxyl	13.36	9.36
fenhexamid	10.23	9.91

[a] Degradation product: (3.5-dichlorophenyl)hydantoin.

to this, have been carried out. On the one hand, various polyphenolic compounds were added in five different levels of concentration and in triplicate (gallic acid and *p*-coumaric acid from 12 mg/L to 0.12 mg/L; vanillic acid, protocatechuic acid and ferulic acid from 4 mg/L to 0.04 mg/L; caffeic acid from 20 mg/L to 0.2 mg/L) to a pesticide-spiked sample and then, the complete extraction process was carried out. On the other hand, and separately, different amounts of glucose were added in five levels of concentration and in triplicate (0 g/L to 150 g/L) to a pesticide-spiked sample in order to be subsequently subjected to the complete extraction process. The RSD values obtained can be seen in **Table 4**. Almost all values (except flufenoxuron) are lower than 15%. These results inform us that both the polyphenolic and the sugar content do not affect significantly the pesticide extraction process, and therefore, the optimized method can be applied to the complete ripening process.

The repeatability was calculated by means of five different ultrasound assisted extractions of five samples with a known amount of pesticides added, using after that five different stir bars. The relative standard deviations are showed in **Table 3** and are, in general, under 12%, which confirms the accuracy of the method. Only flufenoxuron shows a higher RSD (19.93%).

Monitoring of Pesticides during the Sherry Wine Vinegar Production. After optimizing and validating the grape extraction

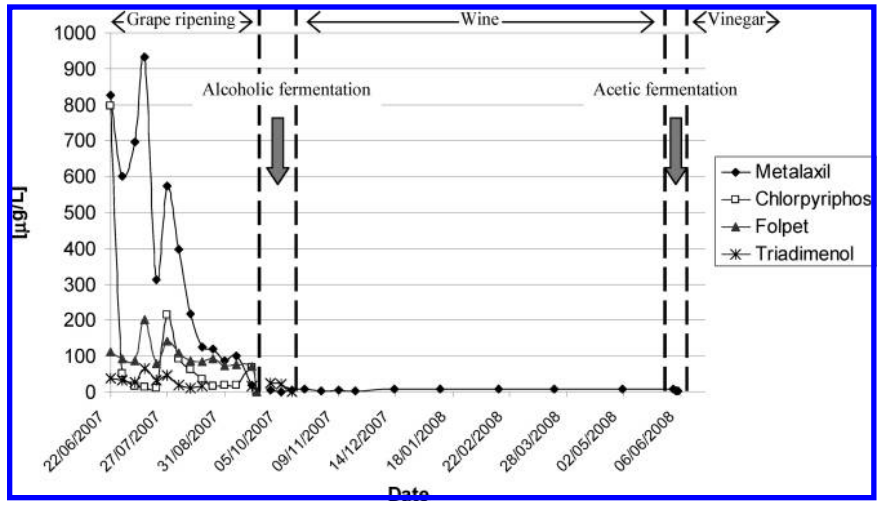

Figure 8. Evolution of four pesticides during the production process of a typical Sherry wine vinegar.

method, a monitoring of the pesticides during the complete process of production of a typical Sherry wine vinegar has been carried out. The traceability of this product involved the analysis of the grapes during the ripening; the analysis of the subsequent must during the fermentation process; the analysis of the produced wine till it was finally used to produce the vinegar; and the analysis during the acetic fermentation process. All the analyses were made in triplicate. Four pesticides were found during the traceability of the vinegar: metalaxil, chlorpyrifos, folpet and triadimenol, being those which were used during the ripening process. The evolution of these compounds during the process is shown in **Figure 8**. As can be seen, in general terms, all of them decrease and in some cases, drastically, during the period of ripening. Metalaxil goes from 931.91 to 19.52 μg/L (97.90% of decrease), whereas chlorpyrifos started with values of 791.37 μg/L arriving at the end of the ripening to values of 19.20 μg/L (97.57%). The rest decreased but in a more moderate way. Folpet fell 64.33% (from 201.06 to 71.72 μg/L) and triadimenol decreased 74.96% (66.42−16.63 μg/L). Several authors have reported similar decreases of these pesticides due to their degradation and transformation in other products (*4*, *24*, *25*). Some irregular rises can be seen in the tendency of the pesticide degradation. This could be explained taking into account that the various grapes in the field were possibly not affected in the same way by the phytosanitary treatments. Although the sampling was made as much representative as possible, a low percentage of error always exists, but even with this experimental error, the trend of diminution of the pesticides is clearly shown. Chlorpyrifos and folpet disappeared just with the beginning of the fermentation process, whereas triadimenol remained during a pair of weeks, being completely degraded during the course of the fermentation process. Metalaxil decreased with the fermentation process but did not disappear, ranging from 7.81 to 3.83 μg/L.

Of these four pesticides, metalaxil and triadimenol show a certain water solubility (8400 mg/L and 95 mg/L respectively) whereas chlorpyrifos and folpet are practically insoluble in water, so this fact could explain the losses observed at the beginning of the alcoholic fermentation. These pesticides would be eliminated together with the solids derived from the fermentation process (dead yeasts, skins, seeds, stems). Hydrolysis phenomena could also explain these decreases. No data are available about the possibility of an enzymatic degradation of these compounds in must and wines.

Metalaxil did not disappear with the acetic fermentation either, finding lower values of 3.68−1.93 μg/L. Although this pesticide presents a high persistence, also reported by other authors (*26*), the found values of the residues are low enough to consume this product without any kind of health risk (*23*).

As can be seen, it is important to have reliable and sensitive methods which allow determining the concentration of the residues of phytosanitary products, due to the high persistence of some of them. This is indispensable when dealing with products labeled as "ecologic", where the use of this kind of phytosanitary products is completely forbidden by the corresponding legislation. In this work, a method of ultrasound assisted extraction for the determination of pesticides in grapes prior to SBSE−GC−MS analysis has been developed. It has been applied to study the traceability of the phytosanitary products employed during the production of a typical Sherry wine vinegar. Chlorpyrifos, triadimenol and folpet are completely eliminated during the vinegar production process whereas metalaxil remains in the final product, but in a very low concentration, safe for human consumption.

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