Research Article

Determination of Lactones in Wines by Headspace Solid-Phase Microextraction and Gas Chromatography Coupled with Mass Spectrometry

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Application of headspace solid-phase microextraction (HS-SPME) coupled with high-resolution gas chromatographic (HRGC) analysis was studied for determining lactones in wines. Six different SPME fibers were tested, and the influence of different factors such as temperature and time of desorption, ionic strength, time of extraction, content of sugar, ethanol, tannins and anthocyanins, and pH and influence of SO₂ were studied. The proposed HS-SPME-GC method is an appropriate technique for the quantitative analysis of γ-butyrolactone, γ-hexalactone, trans-whiskey lactone, γ-octalactone, cis-whiskey lactone, γ-nonalactone, γ-decalactone, δ-decalactone, and γ-undecalactone in wines. Method reproducibility and repeatability ranged between 0.6 and 5.2% for all compounds. Detection limit for γ-butyrolactone was 0.17 mg/L and a few μg/L for the rest of the compounds. The optimized method has been applied to several wine samples.

1. Introduction

Among volatile components of wine, lactones and particularly the γ-lactones and whiskey lactones play an important role in terms of their contribution to the aroma.

γ-Lactones series, δ-lactones, and whiskey lactones are the most abundant lactones in wines and the more sensory important lactones.

Lactone smell is usually described as “fruity” or “coconut-like, fruity” (γ-hexalactone); “coconut-like” (γ-octalactone); “peach-like, milky” (γ-decalactone), or “fruity, sweet floral” (γ-dodecalactone). These compounds are formed by cyclisation of the corresponding γ-hydroxycarboxylic acids [1].

Lactones are among the most important compounds contributing to the sensory characteristics of wines aged in oak wood. Whiskey lactones and some volatile phenols coming directly from wood have been recognized as important odor active compounds in Madeira wines [1, 2].

They are already present in natural oak and their content increases due to ageing. From an organoleptic point of view, they are the most important lactones extractable from oak casks. Furthermore, they have been reported as potential aging markers in Madeira wines [3, 4].

Oak species, geographical origin, silvicultural treatment of tree, and processing of wood have influence on volatile composition of barrel wood. These volatile compounds are susceptible to migrate from oak wood to wine. Although the volatile composition of wine undergoes an evolution during bottle aging, this is carried out in such a way that the most important characteristics spread into wine from wood and remain until the end of bottle aging. These conclusions emphasize the importance of species and geographical origin of oak wood in the volatile composition of wines during aging [5].

Other less studied lactones such as pantolactone and the 4-carbethoxy-γ-butyrolactone have been found in samples submitted to oxidative ageing specially in barrel-aged wine [6].

Chemical structure defines their sensorial and chemical properties [6, 7]. Lactones aromatic descriptors are influenced by the type of aromatic ring, functional groups,
and substituent length chain [8]. Synergic effects can exist due to smell similarities [9].

cis-Whiskey-lactone isomer is a stronger odorant than the trans-isomer reflected by its olfaction threshold and is an important contribution to wine aroma [10]. The rest of lactones can influence wines like Zinfandel, Pinot Noir, Merlot, and Cabernet Sauvignon, particularly, γ-nonalactone and δ-decalactone with smell values higher than threshold [8].

γ-Butyrolactone is considered as an inductor of physical and psychic addictions and has been classified as psychotropic by FDA (US Food and Drug Administration) [11].

Different methods have been proposed for extraction of wine. Analytical methods for gas chromatography determination of lactones need a previous concentration step due to the low concentrations existing in wine [6, 10, 12]. However, these methodologies are not free from artefacts [13], and none of them has been optimized for a wide group of lactones. A great number of wine aroma compounds have been characterised as lactones, many of them extracted by SPME. Published works account for particular compounds like diacetyl [14], sotolon [15], families of compounds such as sulphides and disulphides [16, 17], aldehydes [18–20], esters [21], alcohols [22], butylin compounds [23], terpenes [24, 25], acetics [26], fatty acids [27, 28], or wide sets of different volatile compounds [29, 30].

The aim of this work was to apply the GC-MS technique combined with automatic headspace (HS) SPME to develop a new method to determine a set of lactones in wine (γ-butyrolactone, γ-hexalactone, γ-octalactone, whiskey-lactone, γ-nonalactone, γ-decalactone, δ-decalactone, and γ-undecalactone) and to apply the method to determine lactone content in white and red wines samples.

2. Experimental

2.1. Chemicals and Reagents. The following lactones were studied (CAS number in brackets): γ-butyrolactone [96-48-0], γ-hexalactone [695-06-7], γ-octalactone [104-50-7], cis- and trans-whiskey-lactones [39212-23-2], γ-nonalactone [104-61-0], γ-decalactone [706-14-9], δ-decalactone [705-86-2], and γ-undecalactone [104-67-6], γ-Heptalactone [105-21-5] and 3,4-dimethylphenol [95-65-8] were used as internal standards (IS). These standards with purity above 99% were supplied by Aldrich (Steinheim, Germany, and Milwaukee, WI, USA) and Fluka (Buchs, Switzerland). Sodium chloride [7647-14-5] supplied by Merck was used to control ionic strength. Ethanol (analytical reagent grade; Merck, Darmstadt, Germany) [64-17-5] and Milli-Q water (Millipore, Bedford, USA) were used as solvents. Commercial tannins and anthocyanins were purchased from Agrovin (Alcázar de San Juan, Spain); sucrose [57-50-1], tartaric acid [87-69-4], potassium disulfite [16731-55-8], and sodium hydroxide [1310-73-2] (analytical reagent grade) were purchased from Panreac (Barcelona, Spain).

Individual stock standard solution in ethanol of γ-butyrolactone, γ-hexalactone, γ-heptalactone, γ-octalactone, γ-nonalactone, γ-decalactone, δ-decalactone, and γ-undecalactone and 3,4-dimethylphenol (5000 mg/L) and whiskey-lactone (10000 mg/L) were prepared. Concentrated synthetic wine solution containing L(+)-tartaric acid (87-69-4) (11 g/L) and ethanol (13%) was prepared and adjusted to pH 3.2 with sodium hydroxide and was used to prepare all synthetic test solutions. Stock solution of potassium disulfite (5.55 g/L) in water and tannins (25.39 g/L) with anthocyanins (12700 g/L) in ethanol (Agrovin, Ciudad Real, Spain) were also prepared. Individual internal standard stock solution containing γ-heptalactone (13.12 mg/L) and 3,4-dimethylphenol (12.93 mg/L) were prepared using ethanol (13%). The rest of the solutions were prepared by mixture and dilution of these stock solutions.

All parameters have been optimized using a synthetic wine solution containing concentrations of lactones. Table 1 shows those concentrations and the low and high level concentrations used for recoveries calculations.

Either individual stock standard solutions or real wine samples were prepared in 2 mL vials adding 0.77 mL of sample and 0.03 mL of internal standard solution. The vials were tightly capped with PTFE-lined cap and shaken for 10 min at 200 min\(^{-1}\).

2.2. Equipment. Regularly verified pipettes and class A volumetric flasks were used in solution preparation. A precision balance (Sartorius BP 210-S), a pH meter (WTW, pH 197-S), Milli-Q gradient A10 (Millipore), and a mechanical shaker (Selecta, Rotabit) were used in the study.

2.3. SPME Fibers. Six fibers coated with different stationary phases and various film thicknesses were purchased from Supelco (Bellefonte): polydimethylsiloxane 100 μm (PDMS/100), carboxen-polydimethylsiloxane 75 μm (CAR/PDMS), polydimethylsiloxane-divinylbenzene 65 μm (PDMS/DVB), polyacrylate 85 μm (PA), Carbownax-divinylbenzene 65 μm (CW/DVB), and divinylbenzene-carboxen-polydimethylsiloxane 50/30 μm (DVB/CAR/PDMS). All fibers were conditioned according to manufacturer recommendations.

### Table 1: Concentrations of synthetic solutions containing lactones.

| Compound                        | Low Level | High Level | Optimization |
|---------------------------------|-----------|------------|--------------|
| γ-Butyrolactone                 | 14400     | 36200      | 20300        |
| γ-Hexalactone                   | 146       | 365        | 98.9         |
| γ-Heptalactone                  | 494       |            |              |
| Whiskey-lactone I               | 48.3      | 144        | 100          |
| γ-Octalactone                   | 3.96      | 9.90       | 9.9          |
| Whiskey-lactone II              | 48.3      | 144        | 100          |
| γ-Nonalactone                   | 19.8      | 59.5       | 100          |
| γ-Decalactone                   | 3.83      | 9.56       | 9.9          |
| δ-Decalactone                   | 124       | 310        | 96.8         |
| γ-Undecalactone                 | 1.92      | 4.80       | 9.9          |
| 3,4-Dimethylphenol              |           |            | 491          |


2.4. Chromatography. The analyses were carried out on a 3800 GC gas chromatograph equipped with an 8200 Standalone autosampler, a 1079 split/splitless injector, and a mass spectrometry detector Saturn 2000 (Varian, Walnut Creek, CA, USA). Injections were performed in splitless mode, using a 0.75 mm I.D. liner which improved GC resolution. Ionization mode used was electronic impact.

Separations were performed using a DB-WAXETR capillary column (60 m, 0.25 mm I.D., 0.5 μm film thickness) (J&W Scientific) with an injector temperature of 250 °C (valid for all fibers) and an oven temperature program of 100 °C (5 min), 8 °C/min, 240 °C, and 240 °C (7.5 min). Carrier gas was helium at 2 mL/min flow. Peak identification was accomplished using retention time and experimental spectra obtained from individual standard solutions and confirmed using the NIST mass spectra database (Standard Reference Data of National Institute of Standards and Technology, USA).

The GC-MS transfer line temperature was 240 °C. The MS operated in electron impact mode at 70 eV and collected data at a rate of 1.0 scans/s over a mass range of m/z 25–350. The ion source temperature was 200 °C, the detector voltage was set to 1500 V, and the detector temperature was 300 °C.

2.5. Experimental Design. Chromatographic conditions have been set using both synthetic solutions and wine samples to ensure a good chromatographic resolution and no coelution of compounds.

Extraction time and reproducibility, extraction temperature, desorption time and temperature, and ionic strength were optimized as a need of establishing basic instrumental parameters and simultaneously for selecting the appropriate SPME fiber.

The following steps are designed to reveal and correct possible matrix effect due to specific parameters such as phenolics, sugar, pH, sulphur dioxide, and ethanol.

3. Results and Discussion

3.1. Extraction Time. Figure 1 shows an example chromatogram of a spiked wine sample for the chromatographic conditions cited above. It shows no peak coelution and the mixture is eluted in 25 min.

Table 2 shows retention time, molecular weight, and enthalpy of vaporization of analytes.

γ-Lactones show a clear direct relation between molecular weight, enthalpy of vaporization, and retention time. Compounds with higher molecular weight are less volatile and elute later. cis- and trans-Whiskey lactones elute earlier and have lower vaporization enthalpy than their C9 isomer γ-nonalactone probably due to structural differences, although δ-decalactone shows a similar behaviour as γ-decalactone.

Because of the kinetic nature of the extraction process, it is heavily influenced by fiber type and extraction time. Optimization of both parameters is the first step when building a microextraction method. Since the final aim of this work is to determine the analytes in sweet wines, which can have a high content in sugars (up to 200 g/L) and other many compounds, direct immersion mode leads to a rapid degradation of the fiber surface. To avoid this effect, all the studies were performed in headspace mode.

In order to establish optimal extraction parameters, the six fibers named above were studied. Experiences were made varying extraction time from 15 min to 90 min using a spiked synthetic wine.

Figure 2 shows normalized peak areas (absolute peak area/analyte concentration) for different analytes as a function of extraction time for the fibers studied.

CAR/PDMS fiber was immediately discarded because it offered very wide and low peaks shapes resulting in a poor peak resolution with lactone peaks overlapping between them.

As can be seen, γ-butyrolactone shows the lower peak area for all fibers studied. On the contrary, γ-undecalactone is used to show the higher peak area.
Almost all fibers show a fast initial increase in extraction during the first 15 min, and then it slows down until 45 min. After these time differences among compounds appear, some compounds like γ-heptalactone reach a saturation state shown as a horizontal graph line. On the contrary, some others like γ-octalactone, whiskey-lactone II, or δ-decalactone continue increasing with extraction time without reaching a saturation state. This is a general pattern for DVB/CAR/PDMS fiber and most compounds except γ-butyrolactone and γ-heptalactone. Finally, some analytes decrease in peak area at high extraction time probably due to competition for fiber active points with other compounds.

The extraction performance increases all over homologous series of n-γ-lactones. This is a general behaviour in all studied fibers.

PDMS fiber presents the lower extraction ability for γ-hexalactone, γ-heptalactone, γ-decalactone, and δ-decalactone; so this fiber was discarded from further optimization.

3.2. Extraction Reproducibility. A reproducibility study was made injecting five times a wine synthetic solution spiked with lactones (Table 3).

As can be observed, DVB/CAR/PDMS shows the worst RSD values for most compounds; so it was discarded for the rest of the studies.

Focusing on the rest of the three fibers, all of them present good overall reproducibility. Nevertheless, the value of 14.41 for γ-butyrolactone with PDMS/DVB fiber is bad enough for discarding this fiber.

Finally, PA and CW/DVB present a similar behaviour in terms of extraction and reproducibility; so any of them would be adequate. As CW/DVB has been discontinued by the manufacturer, PA fiber was selected as the best fiber for extracting lactones in wine samples. So, the rest of this study was done using PA fiber.

3.3. Extraction Temperature. Extraction temperature plays an important role in extraction but in two opposite ways. Increasing temperature produces desorption of molecules on the fiber decreasing sensibility. Simultaneously increasing temperature modifies liquid-gas equilibrium enriching gas phase with analytes [31, 32].

An extraction temperature, study temperature, was done using a synthetic wine spiked with lactones. Temperature was set to 60°C, 42°C, and 25°C using 45 min extraction time. Results of normalized peak area (peak area/concentration) versus temperatures are shown in Figure 3.

Figure 3 shows that increasing temperature leads to a decreasing extraction of both whiskey lactones and γ-lactones from γ-butyrolactone to γ-octalactone. The rest of the compounds show a moderate increase in extraction specially at 42°C. As lower temperatures enlarge fiber life and increasing temperature does not have a great effect, 25°C was selected as extraction temperature.
Table 3: RSD (%) (n = 5) of the relative areas of studied compounds obtained with studied fibers.

| Compound             | PA     | DVB/CAR/PDMS | PDMS/DVB | CW/DVB |
|----------------------|--------|--------------|----------|--------|
| γ-Butyrolactone      | 8.14   | 36.43        | 14.41    | 8.47   |
| γ-Hexalactone        | 8.24   | 11.76        | 6.56     | 8.94   |
| Whiskey-lactone I    | 1.26   | 31.44        | 1.47     | 4.33   |
| γ-Octalactone        | 1.72   | 26.08        | 2.45     | 2.56   |
| Whiskey-lactone II   | 2.19   | 27.04        | 3.39     | 4.27   |
| γ-Nonalactone        | 1.40   | 46.96        | 1.80     | 0.56   |
| γ-Decalactone        | 2.45   | 58.36        | 4.40     | 3.24   |
| δ-Decalactone*       | 2.91   | 16.77        | 5.23     | 3.71   |
| γ-Undecalactone      | 2.78   | 62.05        | 3.96     | 3.37   |

*3,4-Dimethylphenol as IS.

Table 4: Normalized peak areas at different temperatures and desorption time.

| Compound             | 250°C 2 min | 250°C 5 min | 250°C 10 min | 300°C 2 min | 300°C 5 min | 300°C 10 min |
|----------------------|-------------|-------------|--------------|-------------|-------------|--------------|
| γ-Butyrolactone      | 2033        | 7644        | 10782        | 3820        | 9353        | 11068        |
| γ-Hexalactone        | 20681       | 129160      | 155069       | 53359       | 143513      | 155513       |
| γ-Heptalactone (IS)  | 38894       | 185530      | 230138       | 81918       | 209111      | 230259       |
| Whiskey-lactone I    | 110605      | 364837      | 413042       | 199319      | 387667      | 413463       |
| γ-Octalactone        | 11825       | 360337      | 407674       | 197209      | 384203      | 408474       |
| Whiskey-lactone II   | 90756       | 320900      | 380063       | 183103      | 351145      | 380190       |
| γ-Nonalactone        | 119260      | 374890      | 417670       | 212936      | 389701      | 418269       |
| γ-Decalactone        | 106828      | 327823      | 363834       | 186756      | 339222      | 364786       |
| 3,4-Dimethylphenol (IS) | 444709     | 999600      | 1090969      | 490860      | 1032004     | 1092175      |
| δ-Decalactone        | 16550       | 130347      | 175598       | 49251       | 138731      | 175672       |
| γ-Undecalactone      | 135335      | 367100      | 412196       | 217489      | 381118      | 411959       |

3.4. Desorption Time and Temperature. Desorption time and temperature were also tested within the range recommended by manufacturer. Injections were made at 250°C and 300°C desorption temperatures and 2 min, 5 min, and 10 min desorption time using the rest of selected parameters. Results are shown in Table 4.

Values of normalized peak area show increasing values with time for all analytes indicating that short desorption time leads to incomplete desorption.

On the other hand, higher temperature shows higher areas until 10 min desorption time. Taking into account that fiber life is longer at lower desorption temperatures, we selected 250°C as desorption temperature and 10 min as desorption time. Blank injections showed no memory effect in desorption for any analyte.

3.5. Ionic Strength. Ionic strength affects analyte extraction, particularly those of polar character. In order to study this effect, increasing quantities of solid NaCl were added to spiked synthetic wine. Quantities of 0 mL, 80 mL, 160 mL, 200 mL, and 240 mg in 0.77 mL of sample were added to reach 0%, 10.3%, 20.7%, 25.9%, and 31.1% NaCl solutions, respectively. Results are shown in Figure 4.

Increasing ionic strength produces and increases in extraction. The best values are those obtained by saturation of NaCl and this condition is selected for further studies.

3.6. Phenolics, Sugar, pH, and Sulphur Dioxide Effect. Wine sample matrix has a wide variety of compounds that can affect extraction process. So it is necessary to study the effect of pH, phenolic compounds, sugar, sulphur dioxide, and ethanol content as influencing extraction process.

Polyphenol content presents wide variations in wines especially from white to red wine. An extraction study was made in order to test its influence in the process.

Synthetic wine spiked solution was prepared with tannins concentrations ranging from 0 g/L to 1 g/L and anthocyanins from 0 g/L to 5 g/L. Obtained results do not show tannins or anthocyanins influence in the extraction.

In the same way, sugar content varies widely from dry to sweet wine reaching even values higher than 200 g/L. Spiking synthetic wine with concentrations up to 200 g/L showed no influence. This is in coincidence with results reported for other compounds [18]. Wine pH usually ranges between 3 and 4 depending on grape variety and kind of wine; it is higher in red than in white wines. Different pH implies variation in dominant chemical species when acid-base properties are present. Solid-phase microextraction only extracts molecular components so ionized acids or bases remain unextracted. An extraction study was made varying pH from 3 to 4. Results showed no variation in extraction process for analytes studied.
Table 5: O.O, slope, $R^2$, and linear range of lactones ($n = 8$).

| Compound            | Intercept | Slope     | $R^2$ | Linear range (μg/L) |
|---------------------|-----------|-----------|-------|---------------------|
| γ-Butyrolactone     | 0.001 ± 0.001 | 0.110 ± 0.001 | 0.999 | 0.17–60.26$^*$      |
| γ-Hexalactone       | 0.003 ± 0.002 | 1.445 ± 0.009 | 0.999 | 11–609              |
| Whiskey-lactone I   | 0.003 ± 0.002 | 3.425 ± 0.003 | 0.999 | 1–401               |
| γ-Octalactone       | 0.003 ± 0.001 | 3.418 ± 0.003 | 0.999 | 1–21                |
| Whiskey-lactone II  | 0.005 ± 0.002 | 3.272 ± 0.002 | 0.999 | 1–401               |
| γ-Nonalactone       | 0.004 ± 0.001 | 3.665 ± 0.003 | 0.997 | 2–206               |
| γ-Decalactone       | 0.001 ± 0.001 | 3.294 ± 0.002 | 0.997 | 1–20                |
| δ-Decalactone       | 0.001 ± 0.001 | 0.311 ± 0.001 | 0.997 | 4–517               |
| γ-Undecalactone     | 0.004 ± 0.001 | 3.724 ± 0.003 | 0.999 | 1–15                |

Table 6: LOD, repeatability, and reproducibility of method [36–38].

| Compound            | LOD (μg/L) | LOQ (μg/L) | Odor threshold (μg/L) | Repeatability RSD (%) | Reproducibility RSD (%) |
|---------------------|------------|------------|-----------------------|-----------------------|------------------------|
| γ-Butyrolactone     | 170.97     | 569.93     | 35000                 | 0.63                  | 1.93                   |
| γ-Hexalactone       | 10.51      | 35.04      | 359000                | 2.98                  | 2.57                   |
| Whiskey-lactone I   | 0.97       | 3.24       | 790                   | 3.54                  | 4.41                   |
| γ-Octalactone       | 1.19       | 3.98       | 7                     | 4.31                  | 3.96                   |
| Whiskey-lactone II  | 0.60       | 2.02       | 67                    | 4.13                  | 5.25                   |
| γ-Nonalactone       | 2.11       | 7.05       | 30                    | 3.24                  | 2.73                   |
| γ-Decalactone       | 0.86       | 2.89       | 88                    | 2.50                  | 2.78                   |
| δ-Decalactone       | 4.17       | 13.92      | 386                   | 3.19                  | 4.56                   |
| γ-Undecalactone     | 0.63       | 2.10       | 60                    | 2.88                  | 4.45                   |

Sulphur dioxide is a commonly used additive in wine making due to its antiseptic, antioxidant, and antioxi-
dasic properties. Sulphur dioxide added to wine reacts with carbonyl compounds forming the so-called “com-bined sul-
phur,” especially with acetaldehyde, changing the expected concentration of free carbonyl compounds. Added sulphur
dioxide quantities also change from red to white wine. To study this effect, synthetic wine solutions spiked with lactones
and sodium metabisulphite ranging up to 200 mg/L were extracted. All lactones showed no influence in extraction in
the range studied.

3.7. Ethanol Effect. Behind water, ethanol is the major com-
ponent in wines. Obviously, ethanol is extracted in fiber and
effectively competes with analytes by active positions. This
effect has been previously described by several authors [2, 19, 33–38]. Alcoholic degree usually ranges from 9% to 15%,
but most of wines vary between 11% and 14%. So the ethanol
influence was studied over synthetic wine spiked solutions
with alcoholic degree in this range. Results are shown in
Figure 5.

Figure 5 shows that δ-decalactone presented a strong
decrease with increasing alcoholic degree. A similar pattern
is presented by the internal standard 3,4-dimethylphenol.

The rest of lactones including the internal standard γ-heptalactone show no variation with ethanol increase
until 13%. Higher ethanol concentrations produce a small
decrease in extraction. According to these patterns, 3,4-
dimethylphenol was chosen as internal standard for quan-tifying δ-decalactone and γ-heptalactone for the rest of analytes
[16].

Figure 6 shows the relative peak areas. Relative peak
areas were calculated, dividing individual standard peak area
between internal standard peak areas in each chromatogram.
As can be seen, relative areas appear now independent from
alcoholic degree. So, internal standard quantification was
selected using internal standards named above.

3.8. Validation. Method validation was developed in terms of
linearity, detection and quantification limits, precision, and
matrix effect influence.

Calibration curves were elaborated using eight synthetic
wine solutions spiked with lactones, internal standards, and
using the parameters selected above. Table 5 summarizes the
results. All correlation coefficients show an excellent linearity.

Detection and quantification limits were calculated as the
concentration corresponding to 3 and 10 times signal/noise,
respectively. Values are shown in Table 5. Most of analytes
present low detection and quantification limits. The highest
value is presented by γ-butyrolactone; but it is much lower
than concentrations found in wines. In every case, detection
limit is lower than odor thresholds reported.

Method repeatability and reproducibility were obtained
analyzing 5 replicates of synthetic wine spiked with lactones
Table 7: Mean (%) and RSD (%) of recoveries.

| Compound               | Low level | High Level |
|------------------------|-----------|------------|
|                        | Mean White | Mean Red | RSD | Mean White | Mean Red | RSD | Mean White | Mean Red | RSD | RSD |
| γ-Butyrolactone        | 189.1     | 191.9     | 1.45 | 2.56      | 189.4     | 198.6 | 4.23 | 0.14 | 1.46 |
| γ-Hexalactone          | 88.1      | 86.7      | 3.05 | 5.62      | 88.6      | 88.5  | 1.80 | 0.77 |
| Whiskey-lactone I      | 144.3     | 145.2     | 9.11 | 1.98      | 144.2     | 143.1 | 1.05 | 1.46 |
| γ-Octalactone          | 76.8      | 81.3      | 2.06 | 3.21      | 78.1      | 80.1  | 0.65 | 2.10 |
| Whiskey-lactone II     | 76.8      | 80.1      | 1.36 | 8.15      | 74.7      | 76.9  | 1.26 | 1.65 |
| γ-Nonalactone          | 93.2      | 101.6     | 3.73 | 1.79      | 94.2      | 107.0 | 1.26 | 1.05 |
| γ-Decalactone          | 136.5     | 142.8     | 0.96 | 3.45      | 137.5     | 147.4 | 2.33 | 0.38 |
| δ-Decalactone          | 109.3     | 108.2     | 1.79 | 4.58      | 108.5     | 109.1 | 1.27 | 2.01 |
| γ-Undecalactone        | 112.0     | 116.5     | 0.99 | 2.10      | 111.0     | 112.6 | 1.31 | 1.17 |

Figure 3: Temperature influence on the extraction of the different analytes on a PA fiber.

Figure 4: Influence of ionic strength on the extraction of the different analytes on a PA fiber.

Table 8: Mean of recoveries and RSD.

| Compound       | Mean (%) | RSD (%) |
|----------------|----------|---------|
| γ-Butyrolactone| 192.3    | 3.01    |
| γ-Hexalactone  | 88.0     | 2.98    |
| Whiskey-lactone I | 144.2 | 4.09    |
| γ-Octalactone  | 79.1     | 2.95    |
| Whiskey-lactone II | 77.1  | 4.52    |
| γ-Nonalactone  | 99.0     | 6.23    |
| γ-Decalactone  | 141.1    | 3.72    |
| δ-Decalactone  | 108.8    | 2.37    |
| γ-Undecalactone| 113.0    | 2.27    |

Wine is a complex matrix that includes hundreds of different compounds besides those studied here. So it is necessary to perform a matrix effect study to evidence the existence of extraction interferences [7, 31–33]. In order to establish these interferences, a recovery study was realized.

Three samples of white and red wine were spiked with lactones at two different concentration levels shown in Experimental section. Results are shown in Table 7.

γ-Nonalactone and δ-decalactone are free from matrix effect showing recoveries in the range of 100 ± 10%. The most affected compounds are γ-butyrolactone, whiskey-lactone I, and γ-decalactone. Matrix effect was revealed to be similar for white and red wines.
Table 9: Concentration mean and SD (µg/L).

| Compound                | White wines \(^1\) \((n = 35)\) | Rosé wines \(^2\) \((n = 8)\) | Red wines \(^3\) \((n = 29)\) | Significative differences \((P < 0.05)\) |
|------------------------|---------------------------------|---------------------------------|---------------------------------|----------------------------------------|
| \(\gamma\)-Butyrolactone | 26287 8478                     | 25940 6553                     | 32652 6403                     | 1–3, 2–3                               |
| \(\gamma\)-Hexalactone | 200 89                          | 202 72                         | 211 67                         | —                                      |
| Whiskey-lactone I      | 10.60 14.38                     | 5.44 2.20                      | 45.73 34.42                    | 1–3, 2–3                               |
| \(\gamma\)-Octalactone | 5.41 2.64                       | 5.63 2.20                      | 6.95 3.13                      | —                                      |
| Whiskey-lactone II     | 20.31 50.46                     | d-nq                           | 138.44 111.13                  | 1–3, 2–3                               |
| \(\gamma\)-Nonalactone | 14.77 7.39                      | 19.98 5.64                     | 42.09 27.45                    | 1–3, 2–3                               |
| \(\gamma\)-Decalactone | d-nq                            | 4.16 2.91                      | 3.85 2.65                      | 1–2, 1–3                               |
| \(\delta\)-Decalactone | 157 72                          | 149 37                         | 228 96                         | 1–3, 2–3                               |
| \(\gamma\)-Undecalactone| nd                              | d-nq                           | d-nq                           | —                                      |

nd: not detected; d-nq: detected not quantified.

**3.9. Analysis of Wine Samples.** Optimized method was applied to 72 wine samples including white, red, and rosé wines. Table 9 shows average concentration values (µg/L) and standard deviations.

As expected, \(\gamma\)-butyrolactone is the most abundant compound for the three kinds of wine. For the rest of analytes, \(\gamma\)-hexalactone and \(\delta\)-decalactone present the higher medium values and \(\gamma\)-octalactone, \(\gamma\)-decalactone, and \(\gamma\)-undecalactone the lower.

**3.10. Statistical Analysis.** ANOVA for these samples revealed that \(\gamma\)-hexalactone, \(\gamma\)-octalactone, and \(\gamma\)-undecalactone presented no statistically significant differences among the three kinds of wines but red wine and rosé wines presented statistically significant differences values for \(\gamma\)-butyrolactone, whiskey-lactones I and II, \(\gamma\)-nonalactone, and \(\delta\)-decalactone. Finally, red wines had contents in \(\gamma\)-decalactone significantly higher than white wines.

**4. Conclusions**

Solid-phase microextraction is a suitable technique for determining concentrations of different lactones in wine matrix. The proposed methodology covers the range of
concentrations usually found in wines with an acceptable uncertainty. The use of two internal standards corrects the influence of ethanol content. Matrix effect exists but can be corrected using both standard addition calibration and experimental correction factors, allowing the quantification of all the compounds studied using gas chromatography, mass spectrometry detection, and electronic impact.

**Conflict of Interests**

The authors declare that there is no conflict of interests regarding the publication of this paper.

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**References**

[1] J. S. Câmara, M. A. Alves, and J. C. Marques, “Changes in volatile composition of Madeira wines during their ageing age,” *Analytica Chimica Acta*, vol. 563, no. 1-2, pp. 188–197, 2006.

[2] E. Campo, V. Ferreira, A. Escudero, J. C. Marqués, and J. Cacho, “Quantitative gas chromatography-olfactometry and chemical quantitative study of the aroma of four Madeira wines,” *Analytica Chimica Acta*, vol. 563, no. 1-2, pp. 180–187, 2006.

[3] R. Perestrelo, A. S. Barros, J. S. Câmara, and S. M. Rocha, “In-depth search focused on furans, lactones, volatile phenols, and acetics as potential age markers of Madeira wines by comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry combined with solid phase microextraction,” *Journal of Agricultural and Food Chemistry*, vol. 59, no. 7, pp. 3186–3204, 2011.

[4] J. S. Câmara, M. A. Alves, and J. C. Marques, “Multivariate analysis for the classification and differentiation of Madeira wines according to main grape varieties,” *Talanta*, vol. 68, no. 5, pp. 1512–1521, 2006.

[5] B. Fernández de Simón, E. Cadahía, T. Hernández, and I. Estrella, “Evolution of oak-related volatile compounds in a Spanish red wine during 2 years bottled, after aging in barrels made of Spanish, French and American oak wood,” *Analytica Chimica Acta*, vol. 563, no. 1-2, pp. 198–203, 2006.

[6] R. Schneider, R. Baumes, C. Bayonove, and A. Razungles, “Volatile compounds involved in the aroma of sweet fortified wines (vins doux naturels) from grenache noir,” *Journal of Agricultural and Food Chemistry*, vol. 46, no. 8, pp. 3230–3237, 1998.

[7] H. Guth, “Identification of character impact odorsants of different white wine varieties,” *Journal of Agricultural and Food Chemistry*, vol. 45, no. 8, pp. 3022–3026, 1997.

[8] S. M. Rocha, F. Rodrigues, P. Coutinho, I. Delgadillo, and M. A. Coimbra, “Volatile composition of Baga red wine: assessment of the identification of the would-be impact odourants,” *Analytica Chimica Acta*, vol. 513, no. 1, pp. 257–262, 2004.

[9] S. Nakamura, E. A. Crowell, C. S. Ough, and A. Totsuka, “Quantitative analysis of γ-nonalactone in wines and its threshold determination,” *Journal of Food Science*, vol. 53, no. 4, pp. 1243–1244, 1988.

[10] A. P. Pollnitz, G. P. Jones, and M. A. Sefton, “Determination of oak lactones in barrel-aged wines and in oak extracts by stable isotope dilution analysis,” *Journal of Chromatography A*, vol. 857, no. 1-2, pp. 239–246, 1999.

[11] F. Tateo and M. Bononi, “Determination of gamma-butyrolactone (GBL) in foods by SBSE-TD/GC/MS,” *Journal of Food Composition and Analysis*, vol. 16, no. 6, pp. 721–727, 2003.

[12] H. Guth, “Quantitative and sensory studies of character impact odorsants of different white wine varieties,” *Journal of Agricultural and Food Chemistry*, vol. 45, no. 8, pp. 3027–3032, 1997.

[13] A. P. Pollnitz, K. H. Pardon, M. Sykes, and M. A. Sefton, “The effects of sample preparation and gas chromatograph injection techniques on the accuracy of measuring guaiacol, 4-methylguaiacol and other volatile oak compounds in oak extracts by stable isotope dilution analyses,” *Journal of Agricultural and Food Chemistry*, vol. 52, no. 11, pp. 3244–3252, 2004.

[14] Y. Hayasaka and E. J. Bartowsky, “Analysis of diacetyl in wine using solid-phase microextraction combined with gas chromatography-mass spectrometry,” *Journal of Agricultural and Food Chemistry*, vol. 47, no. 2, pp. 612–617, 1999.

[15] J. S. Câmara, J. C. Marques, M. A. Alves, and A. C. Silva Ferreira, “3-Hydroxy-4,5-dimethyl-2(5H)-furanone levels in fortified Madeira wines: relationship to sugar content,” *Journal of Agricultural and Food Chemistry*, vol. 52, no. 22, pp. 6765–6769, 2004.

[16] M. Mestres, C. Sala, M. P. Martí, O. Busto, and J. Guasch, “Headspace solid-phase microextraction of sulphides and disulphides using Carboxen-polydimethylsiloxane fibers in the analysis of wine aroma,” *Journal of Chromatography A*, vol. 835, no. 1-2, pp. 137–144, 1999.

[17] M. Mestres, M. P. Martí, O. Busto, and J. Guasch, “Simultaneous analysis of thiols, sulphides and disulphides in wine aroma by Headspace solid-phase microextraction-gas chromatography,” *Journal of Chromatography A*, vol. 849, no. 1, pp. 293–297, 1999.

[18] Q. Wang, J. O'Reilly, and J. Pawliszyn, “Determination of low-molecular mass aldehydes by automated headspace solid-phase microextraction with in-fibre derivatisation,” *Journal of Chromatography A*, vol. 1071, no. 1-2, pp. 147–154, 2005.

[19] M. Bueno, L. Culleré, J. Cacho, and V. Ferreira, “Chemical and sensory characterization of oxidative behavior in different wines,” *Food Research International*, vol. 43, no. 5, pp. 1423–1428, 2010.

[20] L. Armada, E. Fernández, and E. Falqué, “Influence of several enzymatic treatments on aromatic composition of white wines,” *LWT—Food Science and Technology*, vol. 43, no. 10, pp. 1517–1525, 2010.

[21] J. J. Rodríguez-Bencomo, J. E. Conde, M. A. Rodríguez-Delgado, F. García-Montelongo, and J. P. Pérez-Trujillo, “Determination of esters in dry and sweet white wines by Headspace solid-phase microextraction and gas chromatography,” *Journal of Chromatography A*, vol. 963, no. 1-2, pp. 213–223, 2002.

[22] J. J. Rodríguez-Bencomo, J. E. Conde, F. García-Montelongo, and J. P. Pérez-Trujillo, “Determination of major compounds in sweet wines by Headspace solid-phase microextraction and gas chromatography,” *Journal of Chromatography A*, vol. 991, no. 1, pp. 13–22, 2003.

[23] M. Azenha and M. T. Vasconcelos, “Headspace solid-phase micro-extraction gas chromatography-mass detection method for the determination of butyltin compounds in wines,” *Analytica Chimica Acta*, vol. 458, no. 1, pp. 231–239, 2002.
(24) D. de la Calle García, M. Reichenbächer, K. Danzer, C. Hurlbeck, C. Bartzsch, and K.-H. Feller, “Use of solid-phase microextraction-capillary-gas chromatography (SPME-CGC) for the varietal characterization of wines by means of chemometrical methods,” *Presentis* Journal of Analytical Chemistry, vol. 360, no. 7-8, pp. 784–787, 1998.

(25) J. S. Câmara, P. Herbert, J. C. Marques, and M. A. Alves, “Variatel flavour compounds of four grape varieties producing Madeira wines,” *Analytica Chimica Acta*, vol. 513, no. 1, pp. 203–207, 2004.

(26) J. S. Câmara, J. C. Marques, A. Alves, and A. C. Silva Ferreira, “Heterocyclic acetals in Madeira wines,” *Analytical and Bioanalytical Chemistry*, vol. 375, no. 8, pp. 1221–1224, 2003.

(27) Z. Zeng, H. Zhang, T. Zhang, S. Tamogami, and J. Y. Chen, “Analysis of flavor volatiles of glutinous rice during cooking by combined gas chromatography-mass spectrometry with modified headspace solid-phase microextraction method,” *Journal of Food Composition and Analysis*, vol. 22, no. 4, pp. 347–353, 2009.

(28) P. Alanis, S. Ashkan, C. Krauter, S. Campbell, and A. S. Hasson, “Emissions of volatile fatty acids from feed at dairy facilities,” *Atmospheric Environment*, vol. 44, no. 39, pp. 5084–5092, 2010.

(29) J. C. R. Demyttenaere, C. Dagher, P. Sandra, S. Kallithraka, R. Verhe, and N. de Kimpe, “Flavour analysis of Greek white wine by solid-phase microextraction-capillary gas chromatography-mass spectrometry,” *Journal of Chromatography A*, vol. 985, no. 1-2, pp. 233–246, 2003.

(30) S. Francioli, M. Guerra, E. López-Tamames, J. M. Guadayoi, and J. Caixach, “Aroma of sparkling wines by headspace/solid phase microextraction and gas chromatography/mass spectrometry,” *American Journal of Enology and Viticulture*, vol. 50, no. 4, pp. 404–408, 1999.

(31) V. N. Emel’yanenko, S. A. Kozlova, S. P. Verevkin, and G. N. Roganov, “Vapour pressures and enthalpies of vapourization of a series of the γ-lactones,” *Journal of Chemical Thermodynamics*, vol. 40, no. 6, pp. 911–916, 2008.

(32) V. N. Emel’yanenko, S. A. Kozlova, S. P. Verevkin, and G. N. Roganov, “Vapour pressures and enthalpies of vaporization of a series of δ-lactones,” *Journal of Chemical Thermodynamics*, vol. 39, no. 1, pp. 10–15, 2007.

(33) Z. Zhang and J. Pawliszyn, “Analysis of organic-compounds in environmental-samples by headspace solid-phase microextraction,” *HRC: Journal of High Resolution Chromatography*, vol. 16, no. 12, pp. 689–692, 1993.

(34) L. Urruty and M. Montury, “Influence of ethanol on pesticide extraction in aqueous solutions by solid-phase microextraction,” *Journal of Agricultural and Food Chemistry*, vol. 44, no. 12, pp. 3871–3877, 1996.

(35) C. Fischer and U. Fischer, “Analysis of cork taint in wine and cork material at olfactory subthreshold levels by solid phase microextraction,” *Journal of Agricultural and Food Chemistry*, vol. 45, no. 6, pp. 1995–1997, 1997.

(36) D. de la Calle, M. Reichenbächer, K. Danzer, C. Hurlbeck, C. Bartzsch, and K. Feller, “Analysis of wine bouquet components using headspace solid-phase microextraction-capillary gas chromatography,” *HRC: Journal of High Resolution Chromatography*, vol. 21, no. 7, pp. 373–377, 1998.

(37) M. Liu, Z. Zeng, and Y. Tian, “Elimination of matrix effects for headspace solid-phase microextraction of important volatile compounds in red wine using a novel coating,” *Analytica Chimica Acta*, vol. 540, no. 2, pp. 341–353, 2005.

(38) D. de la Calle García, M. Reichenbächer, K. Danzer, C. Hurlbeck, C. Bartzsch, and K.-H. Feller, “Investigations on