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Abstract

There has been considerable public interest and a growing number of scientific studies linking certain phenolic compounds in grapes and wines, particularly trans-resveratrol (trans-3,5,4′-trihydroxystilbene, TRA), to human health benefits. Typical TRA concentrations in wine are very low. It is a polar compound with very low volatility, which makes it difficult to extract and to separate on a gas chromatography (GC) column without derivatization. In this study, a new method for trace analysis of TRA was developed using solid-phase microextraction (SPME) with on-fiber silylation derivatization. Multidimensional GC equipped with a heartcut valve and cryogenic focusing was coupled with a mass-selective detector and used for improved separations and analysis. The effects of SPME fiber selection, extraction time, temperature, and desorption time were investigated. The derivatization conditions, time/temperature and the volume of derivatization reagent were also optimized. The calibration curve was linear over the concentration range from 10 ng L−1 to 5 mg L−1, with a correlation coefficient of 0.9996. The average recovery of TRA in red wine was 83.6 ± 5.6%. The method detection limit (MDL) for TRA in ethanol:water (12.5:87.5, v/v) solution in this study was 7.08 ng L−1 whereas the MDL for TRA in pure water was 2.85 ng L−1. The new method was used to test the TRA content in six selected Iowa red wine samples. Measured concentrations varied from 12.72 to 851.9 μg L−1.

Keywords

trans-Resveratrol, cis-Resveratrol, Solid-phase microextraction, Silylation, Multidimensional gas chromatography–mass spectrometry, Heartcut, Cryotrap, Red wine

Disciplines

Biochemical and Biomolecular Engineering | Bioresource and Agricultural Engineering | Food Chemistry

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Rapid determination of *trans*-resveratrol in red wine by solid-phase microextraction with on-fiber derivatization and multidimensional gas chromatography – mass spectrometry

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Abstract

There has been considerable public interest and a growing number of scientific studies linking certain phenolic compounds in grapes and wines particularly *trans*-resveratrol (TRA) to human health benefits. Typical TRA (*trans*-3,5,4'-trihydroxystilbene) concentrations in wine are very low. It is a polar compound with very low volatility, which makes it difficult to extract and to separate on a gas chromatography (GC) column without derivatization. In this study, a new method for trace analysis of TRA was developed using solid-phase microextraction (SPME) with on-fiber silylation derivatization. Multidimensional GC equipped with a heartcut valve and cryogenic focusing was coupled with a MSD and used for improved separations and analysis. The effects of SPME fiber selection, extraction time, temperature, and desorption time were investigated. The derivatization conditions, time/temperature and the volume of derivatization reagent were also optimized. The calibration curve was linear over the concentration range from 10 ng L^{-1} to 5 mg L^{-1}, with a correlation coefficient of 0.9996 ~~0.9998~~. The average recovery of TRA in red wine was 83.6%±5.6% ~~91.7%± 7.1%.~~ The method detection limit (MDL) for TRA in 12.5% (*v/v*) ethanol:water solution in this study was 7.08 ng L^{-1} whereas the MDL for TRA in pure water was 2.85 ng L^{-1}. ~~The method detection limit for TRA was 2.85 ng L^{-1}. The new method is superior in terms of sensitivity for TRA to all previously published methods.~~ The new method was used to test the TRA content in six selected Iowa red wine samples. Measured concentrations varied from 12.72 to 851.9 µg L^{-1} ~~12.7 to 881.4 µg L^{-1}.~~

46 *Keywords*: *trans*-Resveratrol, *cis*-Resveratrol, ~~*Trans*-resveratrol, *Cis*-resveratrol,~~ Solid-
47 phase microextraction, Silylation, Multidimensional gas chromatography-mass
48 spectrometry, Heartcut, Cryotrap, Red wine
49

50 **1. Introduction**

51 Resveratrol (3, 5, 4'-trihydroxystilbene, $C_{14}H_{12}O_3$) is a phytoallexin produced by
52 plants in response to fungal infection [1] as well as to a variety of stress conditions, such
53 as vicissitudes in climates, exposure to ozone, sunlight and heavy metal ions in soil [2].
54 Recently, resveratrol has attracted considerable public and scientific attention due to its
55 beneficial effects on human health revealed by biological and clinical studies. These
56 benefits include the antioxidative and anti-inflammatory effects [3], inhibition of human
57 low-density lipoprotein oxidation ~~[4,5]~~, platelet aggregation ~~[6]~~, and the inhibition of the
58 growth of a variety of cancer cells [3].

59 Resveratrol has been detected in many plant species [4]. ~~[7].~~ The main
60 commercial source of resveratrol is the Japanese knotwood (*Fallopia japonica*) and Giant
61 or Sakhalin knotwood (*Fallopia sachalinensis*). Peanuts are another dietary source of
62 resveratrol [5]. However, grapes and grape products are considered the most important
63 dietary sources [6] ~~[8,9].~~ Resveratrol is synthesized and concentrated especially in the
64 grape skin, but not in the fruit flesh [7]. ~~[10,11].~~ Red wines are produced by fermenting
65 grapes on skins as opposed to fermentation without grape skins that it typical for white
66 wines. Thus, it is not surprising that the resveratrol content in red wines is much higher
67 than in white wines [8], ~~[12-18],~~ regardless of winemaking techniques. Resveratrol exists
68 in wine in two isomers, *trans*- and *cis*-. *trans*-Resveratrol ~~*Trans*-resveratrol~~ (TRA) has

69 been widely studied, although *cis*- isomer may also possess health-promoting properties

70 [9]. ~~[19].~~ *cis*-Resveratrol ~~*Cis*-resveratrol~~ (CRA) is not a natural constituent of grape.

71 However, since CRA has been detected in almost all wines analyzed so far, it is likely

72 that CRA is derived from its *trans*- isomer during the wine-making process, storage in the

73 bottle, or during analysis [10, 11]. ~~[20-22].~~ Resveratrol is becoming an important quality

74 indicator of red wine and dietary supplements and it is possible that the TRA

75 concentration could be used for marketing of wine and food products. Also, the

76 knowledge of the TRA concentrations in wines could aid the optimization of viticultural

77 practices and enological techniques targeting TRA level improvements [12] ~~[9,22-25].~~

78 Interest in resveratrol have led to the development of various analytical methods

79 for its measurement in wine. Methods developed for detection of resveratrol are mainly

80 suitable for the analyses of the *trans*-form. Fewer methods are applicable for

81 determination of both isomers. Sample preparation, such as liquid-liquid extraction

82 ~~[12,21,22]~~ and solid-phase extraction [~~9,25-28~~] is usually required prior to the

83 chromatographic separation due to the complex nature of the wine matrix. However,

84 these conventional sample preparation procedures are time-consuming, labor-intensive

85 and multi-stage operations and require the use of organic solvent and large sample

86 volumes. As an alternative, solid-phase microextraction (SPME) integrates sampling,

87 extraction, concentration and sample introduction into a single solvent-free step. The use

88 of SPME results in a number of advantages by simplifying sample preparation, increasing

89 reliability, selectivity, sensitivity and reducing the cost and time of analysis [13]. ~~[29].~~

90 Recently, several methods for determination of TRA in wine based on SPME

91 were developed. Luan et al. proposed the method using SPME with

92 bis(trimethylsily)trifluoroacetamide (BSTFA) on-fiber derivatization coupled with GC-

93 MS for the analysis of TRA in wine [14]. ~~[30].~~ A linear concentration range over 10 ng L^{-}

94 ^{1} to 1 mg L^{-1} with a detection limit of 5 ng L^{-1} of TRA was reported, which is about 2000-

95 times lower than that reported by Soleas et al., [12] ~~[25]~~ for the solid phase extraction

96 (SPE) method. Shao et al., [15] ~~[31]~~ developed another method combining SPME-on-

97 fiber derivatization with GC-MS and ~~the~~a comprehensive two-dimensional GC×GC –

98 flame ionization detector (FID) for the determination of TRA in wines. Shao et al., [15]

99 used two different derivatization reagents including acetic anhydride and BSTFA. The

100 linearity range of TRA of the developed method based on acetic anhydrite was 0.02 ~ 2

101 mg L^{-1} without specifically reporting MDLs. Shao et al., [15] reported TRA content of

102 five Australian red wine samples.

103 High-performance liquid chromatography (HPLC) coupled with UV detection

104 [16], ~~[15,18,32,33]~~, fluorescence detection [17], ~~[34,35],~~ electrochemical detection [18]

105 ~~[36]~~ and with mass spectrometry [19] ~~[37,38,39]~~ can also be used to quantify resveratrol

106 in wine. The lowest method detection limit for HPLC method was about 0.1 μg L^{-1}[17]

107 ~~[35]~~ without derivatization. Capillary electrophoresis (CE) has been used in several

108 investigations for the determination of resveratrol as well [20]. ~~[40-42].~~ Typical CE-

109 based methods for measuring resveratrol in wine could detect resveratrol at 45 to 228 μg

110 L^{-1} [20]. ~~[42].~~ However, high resolution and very good sensitivity make the GC method

111 with derivatization very attractive for the identification and quantification of resveratrol

112 isomers in wines. The analysis times for methods using GC are typically much shorter

113 than for those using LC. Most of GC-based methods require derivatization with BSTFA

114 prior to separation and detection by ~~on~~ a FID or a MSD [14, 15]. ~~[26,30,31,43,44].~~

115 However, compared with conventional single-column GC separations, additional

116 selectivity can be provided by multidimensional gas chromatography (MDGC)–MS.

117 To date, two main types of MDGC: conventional MDGC with a Dean's switch

118 and heartcut capability and the comprehensive two-dimensional GC×GC have been used.

119 For high-throughput applications, comprehensive GC×GC is likely to be a better choice

120 for separation since it gives a greater peak capacity and is less time consuming (i.e., one

121 run can provide a complete chromatogram of the entire sample). However, in applications

122 where a specific compound is of interest, conventional MDGC could be more useful. The

123 conventional MDGC techniques have already been used in areas such as environmental

124 analysis [21], ~~[45~~ biochemical studies [22], ~~[46-48],~~ food science [23], ~~[49,50],~~ wine

125 industry [24] ~~[51]~~ and livestock, poultry, and insect odors [25-29]. ~~[52-55].~~

126 The objective of this study was the development and validation of an analytical

127 method based on on-fiber derivatization SPME and multidimensional gas

128 chromatographic analysis for the determination of *trans*-resveratrol in selected Iowa red

129 wines.

130

131 ## 2. Materials and Methods

132 *2.1. Standard and solutions*

133 The TRA standard (*trans*-3,5,4'-trihydroxystilbene, 99% GC-grade) and BSTFA

134 (containing 1% trimethylchlorosilane) were purchased from Sigma-Aldrich (St. Louis,

135 MO, USA) and used without further purification. Ethanol ~~Methanol~~ (HPLC-grade) was

136 also obtained from Sigma-Aldrich. *cis*-Resveratrol was prepared from the *trans*- isomer

137 by UV irradiation (2 h ~~hrs~~ at 254 nm) and it was used for qualitative assessment only [43].

138 The stock solution of 3 mg mL^{-1} TRA was prepared by dissolving 0.03 g TRA standard
139 in 10 mL of ethanol in a volumetric flask. The stock solution was sealed with Parafilm,
140 covered with aluminum foil, and stored in the dark at 4 ºC until use. Standard solutions
141 used for the optimized SPME extraction conditions were prepared freshly by diluting
142 different amounts of stock solution with pure water to the required concentrations.
143 Ultrapure-grade water from a high purity water system (Culligan Water Conditioning,
144 Lexington, KY, USA) with 18 MΩ·cm resistivity was used for developing the calibration
145 curve. The external calibration standard solutions ranged from 10 ng L^{-1} to 5 mg L^{-1} and
146 were made by dilution of the stock solutions in 12.5% (*v/v*) ethanol:water solution using
147 optimized direct SPME immersion conditions.
148
149 *2.2 Multidimensional GC-MS system*
150 A multidimensional GC-MS-olfactometry (MDGC-O) system (Microanalytics,
151 Round Rock, TX, USA) built on a 6890 GC / 5973N MS platform (Agilent ~~Inc.~~,
152 Wilmington, DE, USA) was used for all analyses. This system allows for the
153 simultaneous identification and analysis of chemicals and corresponding odors. In this
154 study, we only utilized the system for the chemical analysis. The system was equipped
155 with two columns in series connected by a Dean's switch. The non-polar pre-column was
156 12 m, 0.53 mm i.d., 1 µm film thickness, with 5% phenyl methylpolysiloxane stationary
157 phase (SGE BP5). The medium-polarity analytical column was a 30 m × 0.53 mm fused
158 silica capillary column coated with 50% phenyl methylpolysiloxane stationary phase
159 (SGE BP50) with film thickness of 1 µm. The GC was operated in a constant pressure
160 mode where the mid-point pressure, i.e., pressure between pre-column and column, was

161 always at 5.8 psi and the heartcut sweep pressure was 5.0 psi. System automation and
162 data acquisition software were MultiTrax™ V. 6.00 (Microanalytics, Round Rock, TX,
163 USA) and ChemStation (Agilent, Santa Clara, CA, USA), respectively.

164 The general run parameters used were as follows: injector, 280 °C; FID, 280 °C,
165 column, 150 °C initial, 10 °C min^{-1}, 300 °C final, 10 min hold; carrier gas, GC-grade
166 helium. The FID system connected to the pre-column was maintained at 280 °C with a
167 H_2 flow rate of 35 mL/min, an airflow rate of 350 mL/min, and the makeup N_2 flow rate
168 of 10 mL/min. The FID data acquisition rate was 20 Hz. Mass to charge ratio (m/z) range
169 was set between 50 and 550. The MS was operated in the electron impact (EI) ionization
170 mode with electron energy of 70 eV. The MS ion source and mass filter temperature
171 were held at 230 and 150 ºC, respectively. Spectra were collected at 6 scans sec^{-1} and
172 electron multiplier voltage was set to 1800 V.

173 The selected ion monitoring mode (SIM) of MS was chosen for quantitative trace
174 analyses. The most abundant ion was generally monitored and quantified while the
175 specific ions were used for confirmation. Mass channels were m/z = 443, 444 and 445 for
176 the TRA derivative with 50 ms dwell times. Ion m/z = 444 was used for the
177 quantification of TRA. The MS detector was auto-tuned every day. The solvent delay
178 was set to 5 min to minimize the baseline shifting after the elution of the derivatizing
179 reagent peak. The simultaneous acquisition of full scan and SIM mode was used. This
180 allowed for analyte confirmation and identification of unknowns while retaining the
181 sensitivity and selectivity of target compound analysis by the SIM. Simultaneous SIM
182 and full scan reduced reporting of false positive results. The full-scan data were used to

183 confirm analyte identity using library search techniques and enabled complimentary low
184 level quantitative and qualitative data analysis from the same injection.
185 The MDGC equipped with a heartcut valve and cryogenic focusing extends the
186 separation power on a single GC column. The heartcut valve based on Dean's switch
187 concept was located between the pre-column and analytical column. In such a dual
188 column system, the heartcut valve and cryogenic cooling system was used to transfer and
189 focus specific pre-separated GC retention time regions with the target compounds from
190 the pre-column (and the entire sample matrix) to the analytical column. Transfer of only
191 selected compounds to the analytical column was done to improve the quality and
192 sensitivity of chemical analyses by reducing the background from the sample matrix [27].
193 [54]. The heartcut effluent was cryogenically focused onto the head of the analytical
194 column by using a spray nozzle with liquid CO_2 to provide additional peak separation.
195 The cryotrap was cooling the short section of the outside of the front of analytical column
196 and was maintained at -40 ºC when the cryotrap was activated.
197
198 *2.3 Analytical procedure*
199 The manual SPME holder and three different SPME fibers including 100 µm
200 polydimethylsiloxane (PDMS), 85 µm polyacrylate (PA) and 65 µm
201 polymethylsiloxane/divinylbenzene (PDMS/DVB) were purchased from Supelco
202 (Bellefonte, PA, USA). New fibers were conditioned before the first use according to the
203 manufacturer's instructions. Direct immersion extraction was carried out for the sampling
204 of TRA from standard solution and from wine samples. A certain volume of the standard
205 solution was added into 4 mL amber sample vials (from Supelco) sealed with a PTFE-

206 coated silicon septum, phenolic screw-caps and prefilled with a PTFE-coated stir bar
207 (12.7 mm × 3.2 mm, Fisher Scientific, Pittsburgh, PA, USA) and with 3 mL of pure
208 water.

209 The effects of several parameters on the efficiency of the microextraction was
210 investigated using spiked aliquots of working solution in pure water (at 10 µg L^{-1} level)
211 and fixing the on-fiber derivatization conditions. Resveratrol with three hydroxyl groups
212 is a very polar compound and it has a low vapor pressure (1.24·10^-9 mmHg, at 25 °C; 1
213 mmHg = 133.322 Pa ~~1.24E-09 mm Hg~~, at 25 ºC) and has good water solubility (16.9 mg
214 L^{-1} at 25 ºC) [29]. ~~[56].~~ Thus, the direct immersion sampling with SPME was carried out
215 for the entire study. Sample agitation increased the rate of resveratrol extraction onto the
216 fiber coating, and a constant rapid agitation speed of 500 rpm was applied for all the
217 experiments in this study. However, care must be taken with the direct immersion
218 extractions of red wine. Wine is an acidic aqueous ethanol solution with aroma
219 compounds where ethanol content is approximately 12.5%. Organic acids, colloids,
220 polyphenols, mineral salts and sugars constitute about 2% of wine composition [33].
221 With direct immersion extractions, the PA SPME fiber was directly exposed in the liquid
222 phase of red wine. Thus, the matrix of wine could build up sugar and colloid coating on
223 the SPME fiber causing irreversible adsorption. As a result, these potentially interfering
224 compounds could still be absorbed in the SPME fiber even after relatively long thermal
225 desorption at the GC injection port. We observed that the color of the surface of PA
226 fibers became gradually dark yellow/brown and fibers were significantly less efficient in
227 extractions after ~30 injections. Thus, the SPME fiber was replaced after 30 direct
228 extractions from red wine in this study.

229 Unless specified otherwise, all of the optimization experiments were performed in
230 triplicate. For the final, optimized extraction conditions, the 85 µm PA SPME fiber was
231 immersed in the stirred liquid sample (at 500 rpm) for 30 min at room temperature.
232
233 *2.4 Derivatizations of TRA and CRA*
234 Resveratrol is a low vapor pressure and a very polar compound with the CRA less
235 polar than the TRA. Derivatization can increase the volatility and/or reduce the polarity
236 of some of analytes and therefore can improve extraction efficiency, selectivity and
237 detection. There are three different derivatization procedures that are currently used in
238 SPME including direct derivatization, derivatization on the SPME fiber and
239 derivatization in the GC injection port [30]. ~~[57].~~ On-fiber SPME derivatization was used
240 in this research. The BSTFA was employed as derivatizing reagent for resveratrol. On-
241 fiber silylation was conducted after the direct extraction with SPME. Any residual drop
242 of water attached to the fiber needle after direct extractions was removed by a soft tissue
243 after completion of the extraction step. The SPME fiber with extracted compounds
244 including resveratrol isomers was transferred to a sealed headspace of 4 mL vial where it
245 was exposed to the derivatizing reagent in the vapor phase. The 4 mL vial was prefilled
246 with 5 µL BSTFA. Resveratrol absorbed on the SPME fiber was then derivatized with
247 the BSTFA vapor that was at equilibrium in the vial. After 20 min of derivatization, the
248 fiber was retracted into the needle, pulled out from the vial and immediately inserted into
249 the GC injection port at 280 ºC for 10 min. A new vial containing a fresh aliquot of
250 BSTFA was used in each experiment. Wine samples were analyzed with the similar
251 procedure described above.

252 Compared to conventional SPE, the SPME with on-fiber derivatization has
253 several advantages [31]. [58]. First, the relatively hydrophobic SPME fiber resists polar
254 matrix interferences found in wine better than the silica-phase extraction. Also, on-fiber
255 derivatization was conducted in the vapor of the derivatizing reagent instead of the pure
256 liquid or a solution, which should favor desirable kinetics and regioselectivity for the
257 derivatizing reaction. Finally, SPME with on-fiber derivatization eliminated the removal
258 of the derivatizing reagent step that is needed with SPE. This, in turn, reduced a possible
259 source of sample loss.
260

261 *2.5 Linearity, repeatability and the method detection limit of the analytical method*

262 The new method repeatability was estimated at seven different TRA
263 concentrations prepared in 12.5% (*v/v*) ethanol:water solution: 10 and 100 ng L^{-1}, 1, 10,
264 and 100 μg L^{-1} and 1 and 5 mg L^{-1}. All tests were conducted using 3 replicates. The
265 exception was the lowest concentration of 10 ng L^{-1} in 12.5% (*v/v*) ethanol:water solution
266 that was analyzed in 7 10 replicates for the estimation of method detection limits (MDLs).
267 The MDL was also estimated at 10 ng L^{-1} in pure water with 10 replicates. Data were
268 analyzed and compared using means and relative standard deviations (RSDs). All
269 analyses were based on manual SPME injections.
270 The US U.S. Environmental Protection Agency (USEPA) methodology for
271 estimation of MDLs was used [32]. [59]. The MDLs were defined as the minimum
272 concentration of a substance that can be measured and reported with 99% confidence
273 when the analyte concentration is greater than zero and is determined from analysis of a

274 sample in a given matrix containing the analyte [32]. ~~[59].~~ The MDLs for TRA was

275 estimated using equation 1:

276 MDL= $s \times t_{(n-1,\ 1-\alpha)}$ (1)

277 where:

278 n = number of replicate spike determinations at 1 to 5 times greater than the estimated

279 MDL,

280 s = standard deviation of measured concentrations of n spike determinations,

281 t = Student's t-value at n-1 degree of freedom and 1-α (equal to 99%) confidence level.

282 When n = 7 and 10, α (defined as the level of significance) = 0.01, then t = 2.821 for 7

283 replicates and t=3.14 for 10 replicates.

284

285 *2.6 Wine samples*

286 Six Iowa red wine samples were obtained from the cooperating local wineries. All

287 of the collected samples were in the original marketed bottles and were refrigerated until

288 the time of analysis. All wines were from 2006 vintage. The ethanol content was

289 measured for selected Iowa red wines and the average ethanol content was 12.5% (*v/v*).

290

291 *2.7 Method recovery assays*

292 The recovery experiments were performed using spiked standard resveratrol

293 solutions in red wine samples at three different concentrations, i.e., 1000 μg L^{-1}, 60 μg L^{-}

294 ^{1} and 10 μg L^{-1}. The spiked wine samples were then analyzed by following the optimized

295 extraction method described above.

296

297 ## 3. Results and Discussion

298 *3.1 Isolation of resveratrol isomers with MDGC-MS system*

299 It is well-know that wine is a very complex matrix where more than 680
300 constituents [33] ~~[60]~~ have been found belonging to different chemical groups of
301 compounds with different polarities. Resveratrol is a very polar compound and it exists as
302 *trans*- and *cis*- isomers, both present in wines. The polarity poses challenges for the
303 separation and quantification of resveratrol isomers in the complex wine matrix.
304 Figures 1 and 2 illustrate the use of multidimensional GC-MS for the separation and
305 quantification of resveratrol in red wine. Figure 1 shows a typical FID chromatogram of
306 resveratrol-BSTFA derivatives separated from red wine using only the first GC column.
307 The entire sample was separated on the pre-column without heartcut or cryotrapping.
308 However, the TRA-BSTFA-derivative was not completely separated from baseline and
309 eluted tightly with the adjacent peaks and with relatively high background. Then, the
310 multidimensional GC mode with the heartcut valve and cryogenic cooling was used for
311 the transfer and focusing of the resveratrol-BSTFA derivatives from the pre-column to
312 the analytical column for improved isolation and separation. Heartcuts were
313 cryogenically focused at the front of the second column, which resulted in enhanced
314 sensitivity and narrow peak widths (Figures 2A and 2B). The rest of the sample (i.e.,
315 prior and post heartcuts) were sent from the Dean's switch to the FID (Figure 2A). In this
316 context, the ability to obtain cleaner mass spectrum and higher quality peaks for the
317 selected region cut from the complex wine matrix was the goal. This approach is more
318 'mechanistic' (peaks are visually separated) compared with the comprehensive GCxGC
319 where sophisticated software is needed to deconvolute separate peaks eluting from a

320 shorter second column and resulting from faster run-times. This intrinsic difference
321 between separations is the key for achieving such low MDL for *trans*-resveratrol in this
322 research. The same objective is more challenging to achieve with a shorter second
323 column and faster run-times used in comprehensive GC×GC for the whole sample.
324 Compared with comprehensive GC×GC [34], [61], the MDGC could obtain a cleaner
325 mass spectrum for TRA and achieved lower method detection limits. Furthermore,
326 selected ion monitoring mode (SIM) coupled with MDGC was used in this study to
327 provide additional 'mass-based separation' of target analyte. This resulted in improved
328 quantitative results compared with those obtained by Shao et al., (2003) where the
329 derivatized analyte could have been interfered with by co-eluting peaks by using a FID
330 [15]. This study is a novel application of heartcut two-dimensional GC-GC/MS in wine.
331 It also illustrated the advantages of MDGC over comprehensive GC×GC relative to
332 focusing on the quantification of specific compound in a very complex matrix. Similar
333 applications were successfully used in our previous work with insect volatiles [27] as
334 well as other work with essential oil, wine, beverage and fragrance products [34-36].
335 Figures 2B and 2C show the synchronous total ion chromatogram (TIC) and the
336 SIM chromatogram, respectively, of two heartcuts of CRA- and TRA-BSTFA derivatives
337 from red wine samples. Precise heartcut times of two resveratrol isomers were
338 determined by injecting reference standards and ensuring that only the CRA- and TRA-
339 BSTFA derivatives and co-eluting matrix were heartcut to the analytical column. As a
340 result, there were no interference peaks and the target analytes had a clean mass spectrum
341 and low background, especially for the SIM mode (Figure 2C). Thus, the

342 multidimensional GC significantly improved the separation of target compounds and was
343 used for the methods development.
344 The simultaneous acquisition of full scan (TIC) and SIM data illustrated in
345 Figures 2B and 2C allowed for identifications of unknown compounds while retaining the
346 sensitivity and selectivity of target compound analysis by SIM. ~~Figures 3A, B, C and D~~
347 ~~show the comparison of total ion mass spectrum and selected ion mass spectrums of~~
348 ~~CRA- and TRA-BSTFA derivatives from red wine.~~ Both of CRA- and TRA-BSTFA
349 derivatives were confirmed with pure standards. The c*is*-isomer derivative eluted
350 approximately 3 min earlier than the *trans*- resveratrol derivative. ~~It is evident that~~ The
351 mass spectrum of CRA-BSTFA derivative is identical with the same molecular ions and
352 characteristic abundance ratio ~~(Figures 3B and 3D)~~ as that of TRA-BSTFA. The
353 molecular ion (m/z = 444) was in both cases the major ion with a relative abundance of
354 100%. Therefore, m/z = 444 ion was used for quantitative analysis in SIM mode. The M-
355 H (m/z = 443) and the C-isotope (m/z = 445) ions were used as qualifiers.
356
357 *3.2 Optimization of SPME extraction conditions*
358 Selection of a suitable SPME coating is one of the most important steps in the
359 development of a SPME method. Two kinds of fibers including (a) absorptive 100 µm
360 PDMS and 85 µm PA and (b) adsorptive 65 µm PDMS-DVB were tested. The 100 µm
361 PDMS fiber coating is non-polar and was found to be less efficient for the resveratrol-
362 BSTFA derivatives than polar 85 µm PA fiber coating. Furthermore, the PDMS coating
363 was found to swell in the vapor of BSTFA, which eventually damaged the fiber coating.
364 This observation is consistent with Shao et al., [15]. ~~[31].~~ The PDMS-DVB fiber coating

365 was also considered because of the benzyl group in DVB polymer, which might favor the
366 extraction of resveratrol with two benzyl rings due to the π-π interactions. However, the
367 results showed the extraction efficiency for 85 µm PA fiber was still approximately 10
368 times greater than that of 65 µm PDMS/DVB. Therefore, the polar 85 µm PA was used
369 for subsequent studies. Caution must be taken when the PA fiber is used for direct
370 extractions and on-fiber silylation. It was found that the coating of the new fiber was
371 unstable for the first one or two derivatizations. A significant decrease of extraction
372 efficiency after approximately 30 extraction-derivatization cycles was also observed.

373 An extraction time profile for TRA-BSTFA at a fixed concentration of 10 µg L^{-1}
374 is shown in Figure 3. ~~4.~~ The extraction time varied from 5 min to 90 min for
375 determination of the optimum extraction time. All the extractions were conducted at
376 room temperature. It was consistently observed that the extraction of TRA-BSTFA
377 reached equilibrium at 30 min. Hence, 30 min extraction time was used for the
378 subsequent experiments.

379 The effect of temperature on TRA-BSTFA extractions is summarized in Figure 4.
380 ~~5.~~ Extraction temperature varied from room temperature (22 ºC) to 60 ºC. The sensitivity
381 decreased proportionally with the increase in temperature. This was due to the decrease
382 of fiber-sample partitioning coefficient with the increasing temperature. The apparent
383 decrease of TRA-BSTFA with increasing extraction temperature was -0.0148 $ºC^{-1}$. The
384 sample recovery was also investigated between room temperature and 60 ºC. The
385 recovery of TRA-BSTFA derivatives was greater at higher temperature (50 ºC).
386 However, slight losses were observed at 60 ºC and even greater at 70 ºC. This may due
387 to the derivatives, which are generally more volatile and could be desorbed from the fiber

388 at high temperatures [31]. [58]. Few researchers have reported that the PA fiber coating

389 may be damaged by high derivatization temperatures [14]. [30]. Thus, the optimum

390 temperature of room temperature was used for the subsequent experiments.

391 Ethanol is one of the major constituents of wine and concentration typically varies

392 from 10 to 15%. The effect of ethanol on the extraction efficiency was investigated in this

393 study. Standard 10 μg L^{-1} TRA was prepared with 10%, 20% and 50% ethanol in pure

394 water, respectively. Figure 5 6 indicates that the extraction efficiency of TRA-BSTFA

395 decreased proportionally with increasing ethanol content. The apparent rate of extracted

396 TRA-BSTFA decrease with % ethanol increase was -0.0127 % $ethanol^{-1}$. However, the

397 apparent decrease of the extraction efficiency for the ethanol content < 20% was not

398 significant ~~rather insignificant~~. This finding is consistent with Luan et al. [14]. ~~[30].~~ In

399 summary, the optimized SPME extraction conditions used in this study were: 85 μm PA

400 fiber, 30 min direct extraction time from wine at room temperature (22 ºC) and 500 rpm

401 stirring, 10 min desorption time at 280 ºC.

402

403 *3.3 Optimization of the on-fiber silylation*

404 Two additional factors that affect to the performance of the on-fiber derivatization

405 were investigated including derivatization time, temperature and the dose of BSTFA.

406 Figure 6 ~~7~~ shows the effect of derivatization time on the derivatization of TRA. Based on

407 this experiment, it was determined that 20 min derivatization was adequate and was used

408 for all experiments in this study. Longer derivatization times did not yield more of the

409 TRA-BSTFA derivative. The apparent reaction rate for the 0 to 20 min derivatization

410 range was 0.0323 min^{-1}. The effect of BSTFA dose was also tested. Various volumes of

411 derivatization reagent from 1 μL to 100 μL were used to explore the effects on the

412 derivatization efficiency. The greatest derivatization efficiency was obtained with 5 μL of

413 BSTFA in equilibrium with 4 mL vial headspace at room temperature for 20 min.

414

415 *3.4 Method validation*

416

417 The optimized procedure was applied for the validation of the developed

418 analytical method including linearity, detection limit, repeatability and recovery. The

419 linearity of the method was evaluated by preparing calibration standards with seven

420 different TRA concentrations in 12.5% (*v/v*) ethanol:water solution. Each concentration

421 was conducted in triplicate. The calibration curve was linear over the concentration range

422 of 10 ng L^{-1} to 5 mg L^{-1}, with R^2 = 0.9996. The linear regression equation was as follows:

423 y = 55131x - 1E+06, ~~y = 52973x-727040,~~

424 where y and x are the peak area counts and concentrations (μg L^{-1}) of standard

425 TRA solutions, respectively.

426 The method detection limits (MDLs) were estimated based on the experiment

427 with 7 ~~10~~ replicate direct SPME extractions of standard TRA in 12.5% (*v/v*)

428 ethanol:water solution at the lowest concentration (10 ng L^{-1}) using equation 1. The MDL

429 for TRA in ethanol:water solution in this study was 7.08 ng L^{-1} whereas the MDL for

430 TRA in pure water was 2.85 ng L^{-1}. ~~Thus, SPME coupled with heartcut MDGC/MS~~

431 ~~method presented in this study is superior in terms of sensitivity for TRA to all~~

432 ~~previously published methods.~~ Such a low MDL is likely due to the reduction of

433 interferences with the introduction of narrow heartcuts and cryotrapping in

434 multidimensional mode. The analytical column was only separating a very small portion

435 of the total sample at one time. The separation based on MDGC of the specific region
436 enabled isolation of the target compounds from the interference background and resulted
437 in a cleaner mass spectrum and furthermore, it improved the MDL for the target
438 compound. In order to further assess the new method and to estimate the MDL, the
439 recovery of 10 ng L^{-1} TRA was investigated by spiking the TRA standard solution in
440 12.5% (*v/v*) ethanol:water solution. The RSD of 7 replicates was 15% and the average
441 recovery of 10 ng L^{-1} TRA in 12.5% (*v/v*) ethanol:water solution was 78.2%.

442 The repeatability of the optimized direct SPME on fiber silylation and MDGC-
443 MS method for TRA in 12.5% ethanol:water solution, expressed as relative standard
444 deviation (RSD, %, n = 3), ranged from 3.5 to 8.9% ~~1.3 to 6.7%~~ at seven different
445 concentrations including 10, 100 ng L^{-1}, 1, 10, 100 µg L^{-1} and 1, 5 mg L^{-1}.

446

447 *3.5 Analysis of wine samples and the recovery assay for* trans-*resveratrol in selected*
448 *Iowa red wines.*

449 Six selected Iowa red wine samples were analyzed using the optimized analytical
450 method developed in this research (Table 1). These wines were from three winemakers
451 and represented five different varieties with the same 2006 vintage. Reported RSD (%)
452 are for three replicate samples from the same bottle of wine. The average amount of TRA
453 was 206.70 µg L^{-1}. Lamuela-Raventos et al., (1997) reported resveratrol content in red
454 US wines below 1 mg L^{-1} [37] ~~[62]~~ and being significantly lower compared to Italian,
455 French and Spanish wines [38]. ~~[16].~~ The highest average level of TRA was found in
456 wines made from Pinot Noir grown in France (5.4 ±1.2 mg L^{-1}) [8]. ~~[63].~~ For the selected
457 few wines analyzed in this work the amount of TRA varied greatly from one wine variety

458 to another, i.e., from 12.72 to 851.9 μg L^{-1} ~~12.7 to 881.4 μg L^{-1}~~. It is interesting to note

459 that the amount of TRA varied from 181.7 to 12.72 μg L^{-1} ~~202.67 to 12.7 μg L^{-1}~~ for the

460 same wine variety originating from a different winemaker, e.g., Frontenac from winery B

461 and winery A, respectively. ~~This finding suggests that wine making techniques, such as~~

462 ~~increased temperature, high levels of SO_2 and/or decreased pH results in higher levels of~~

463 ~~TRA in red wine [64].~~ This finding is consistent with McMurtey ~~(1997)~~ [39] ~~[65]~~ who

464 also reported that a number of factors such as climate, geographical area of cultivation,

465 growing conditions, wine-making techniques and storage conditions affect resveratrol

466 content in wines within the same grape variety.

467 The ratios of *trans*- to *cis*-resveratrol from the selected red wines were also

468 investigated in this study (Table 1). The peak area count of *trans*- and *cis*- isomers were

469 used for the calculation of the ratio. The average ratio of *trans*- to *cis*- found in the six

470 selected Iowa red wines was about 3.0. *Trans*- isomer content was greater for almost all

471 of the selected wines except for Marechal Foch from winery C, for which the ratio of

472 *trans*-/*cis*- was 0.66 ~~0.22~~. The generally high ratio of *trans*- to *cis*- isomers supports the

473 notion that *cis*-isomers could arise from light exposure of wine during the winemaking

474 process or possibly from the light exposure of wine bottles during storage [40]. ~~[66].~~

475 The recovery of the overall method was determined for the overall assay by

476 analysis of three wine samples with low, medium and high concentrations of TRA

477 supplemented with known concentrations of standard TRA. The summary of TRA

478 recoveries for each concentration is presented in Table 2. The new method showed very

479 good recoveries (from 72.7% to 94.7% ~~81.8% to106.5%~~) with a RSD < 7.1%. The mean

480 recovery was 83.6% ~~91.7%.~~

4. Conclusions

The following conclusions were drawn from this study:

(1) Heartcut-based multidimensional GC-MS is a powerful approach to improve the separation of *trans*- and *cis*- resveratrol-BSTFA derivatives.

(2) ~~The SPME coupled with MDGC-MS method is superior in terms of sensitivity of resveratrol detection to all previously published methods.~~ The MDL for TRA in ethanol:water solution in this study was 7.08 ng L^{-1} whereas the MDL for TRA in water was 2.85 ng L^{-1} with heartcut and cryotrap and SIM. ~~The MDL was as low as 2.85 ng L^{-1} with heartcut and cryotrap.~~ The linearity was excellent between 10 ng L^{-1} to 5 mg L^{-1}. The average recovery for *trans*-resveratrol in wine was 83.6% ~~91.7%.~~

(3) ~~The new method can be used to determine both *trans*- and *cis*-resveratrol. This study focused on the more interesting *trans*- form. However,~~ The *trans*- to *cis*- ratio was investigated in this study. The average ratio of *trans*- to *cis*- found in the six selected Iowa red wines was approximately 3.0. The *trans*-isomer ~~*Trans*-isomer~~ content was predominant for five out of the six selected wines. The new method could also be used for resveratrol analyses in grape juice, jams and jellies, and other related products.

~~(4) There was a considerable variability in *trans*-resveratrol concentrations even in wines produced from the same grape variety, which is not unexpected since a number of factors such as climate, geographical area of cultivation, growing~~

~~conditions, wine-making techniques and storage conditions affect *trans*-resveratrol content of wines.~~

~~(5) Winemaking techniques may have important effects on the resveratrol content of wine. Further studies are needed to investigate the contribution of winemaking procedures to resveratrol content in wine.~~

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629

630 ## Figure captions

631 **Figure 1** Separation of *cis*- and *trans*-resveratrol-BSTFA derivatives in GC-FID mode
632 with no heartcut. Chromatogram (FID) of the red wine sample collected with 85
633 μm PA SPME fiber for 30 min direct extraction at room temperature.

634 **Figure 2** Separations in multidimensional GC-MS mode with cryotrap and heartcut
635 between pre-column and analytical column: comparison of the FID
636 chromatogram (part A), total ion chromatogram (part B) and selected ion
637 chromatogram (part C) isolating *trans*- and *cis*-resveratrol-BSTFA derivatives
638 from red wine samples with direct-SPME-MDGC-MS. Cryotrap range: 10.1
639 min -10.9 min; 12.7 min -13.4 min. Heartcut range: 10.2 min -10.9 min; 12.8
640 min - 13.4 min.

641 ~~**Figure 3** Comparison of total ion mass spectrum and selected ion mass spectrum for *cis*-~~
642 ~~and *trans*- resveratrol-BSTFA derivatives from red wine sample.~~

643 **Figure 3** ~~**4**~~ Extraction time profiles using 85 μm PA fiber. Direct-SPME extraction at
644 room temperature and 500 rpm stirring for the standard *trans*-resveratrol (at 10
645 μg L^{-1}) -BSTFA derivatives.

646 **Figure 4** ~~**5**~~ Effect of the extraction temperature on extractions of *trans*-resveratrol-
647 BSTFA derivatives using 85 μm PA fiber and direct-SPME for 30 min and 500
648 rpm stirring.

649 **Figure 5** ~~**6**~~ Effect of ethanol content on the 30 min direct SPME extraction efficiency of
650 *trans*-resveratrol (at 10 μg L^{-1}) -BSTFA derivatives at room temperature and
651 500 rpm stirring.

652 **Figure 6** ~~**7**~~ Effect of reaction time on derivatization of *trans*-resveratrol (at 10 μg L^{-1})
653 with BSTFA at room temperature.

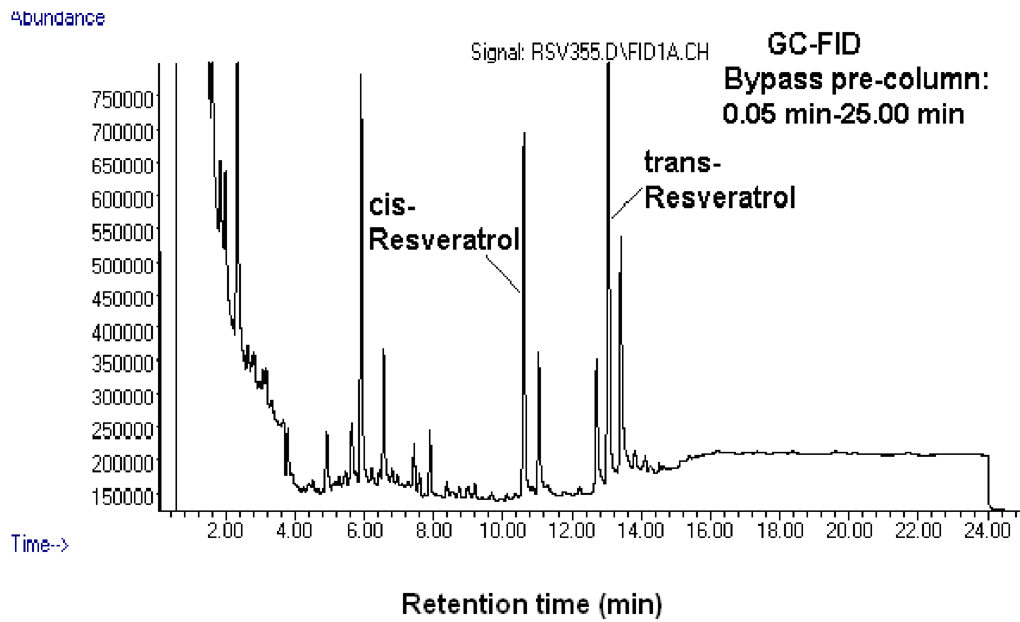

Figure 1 Cai et al.

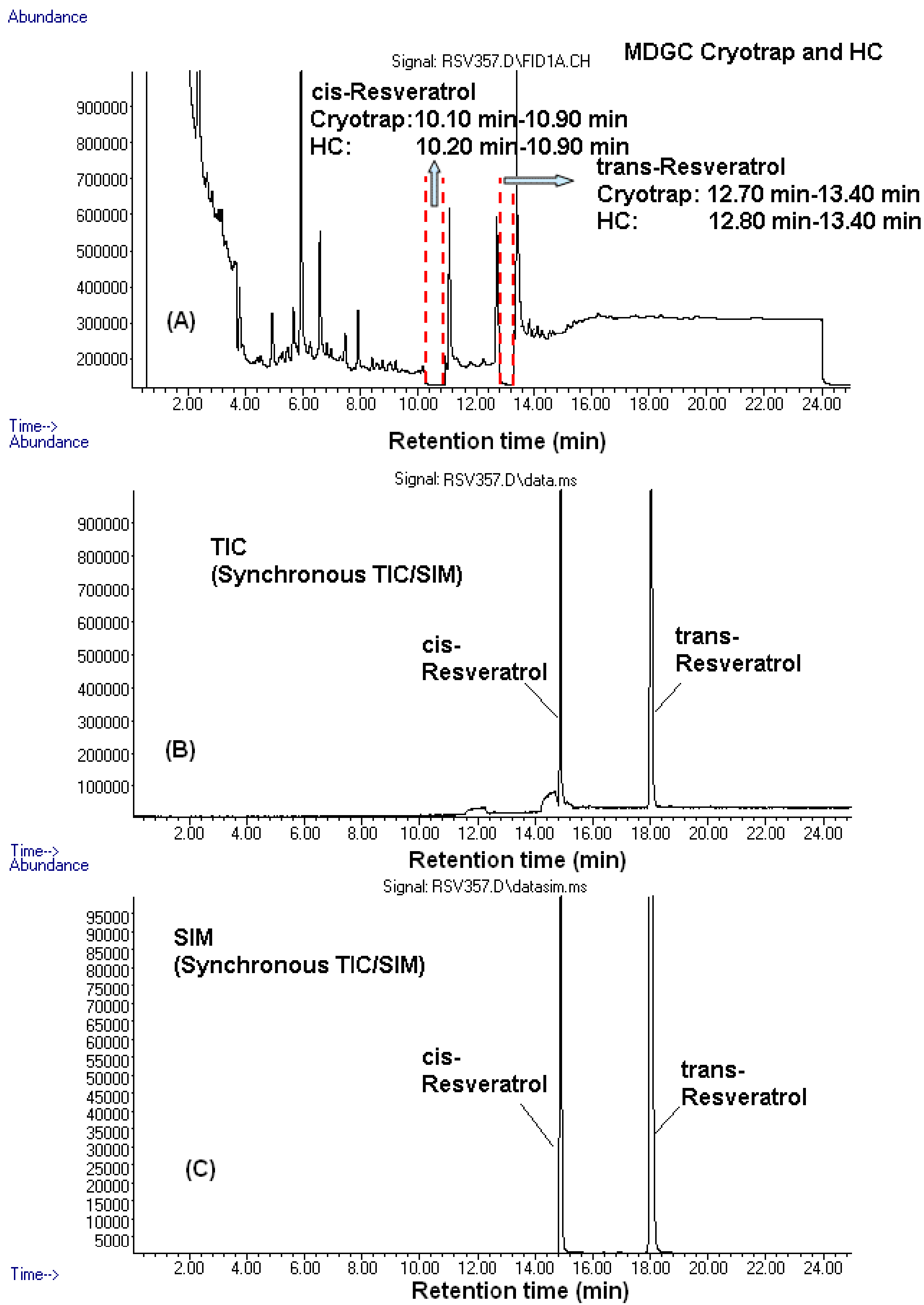

Figure 2 Cai et al.

~~Figure 3 Cai et al.~~

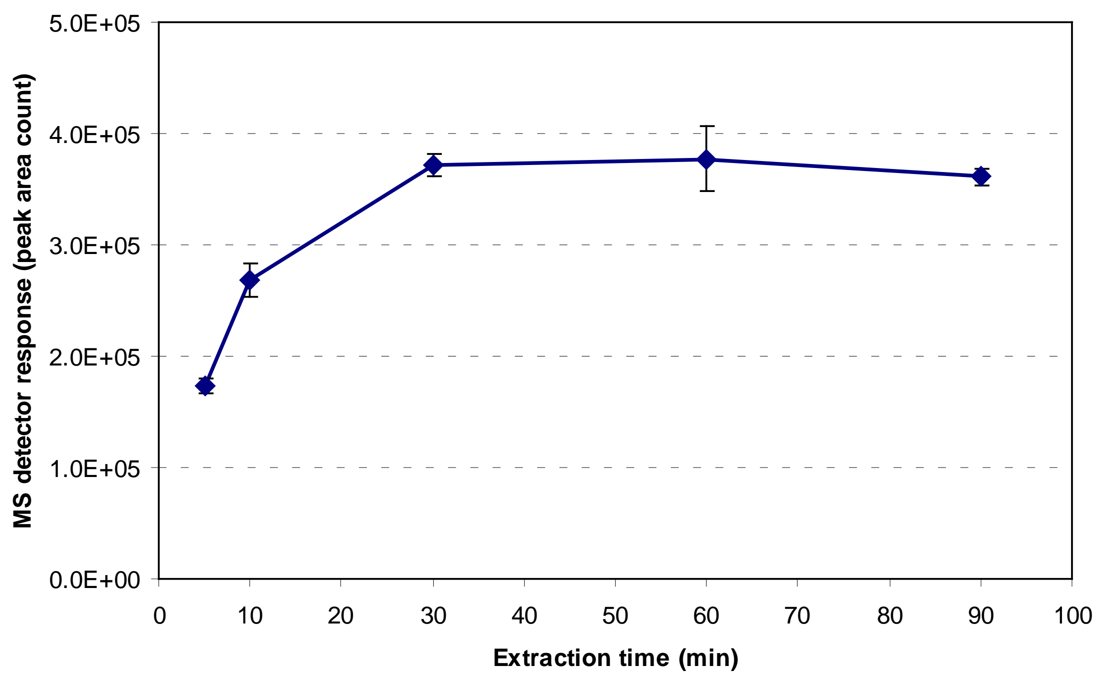

Figure 3 Cai et al.

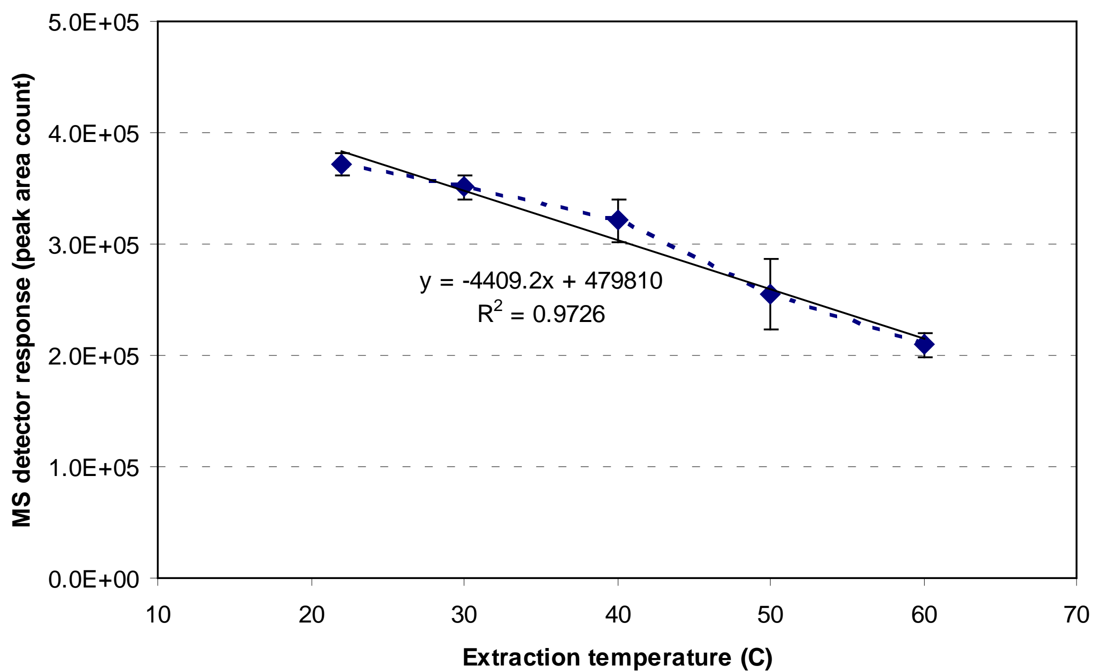

Figure 4 ~~5~~ Cai et al.

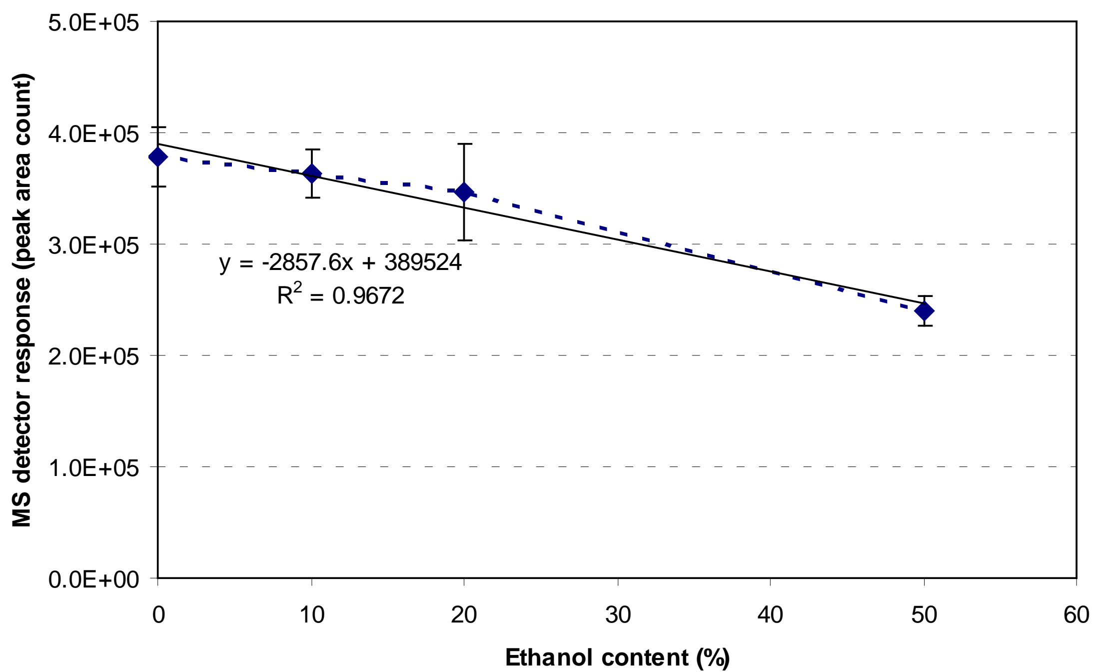

Figure 5 ~~6~~ Cai et al.

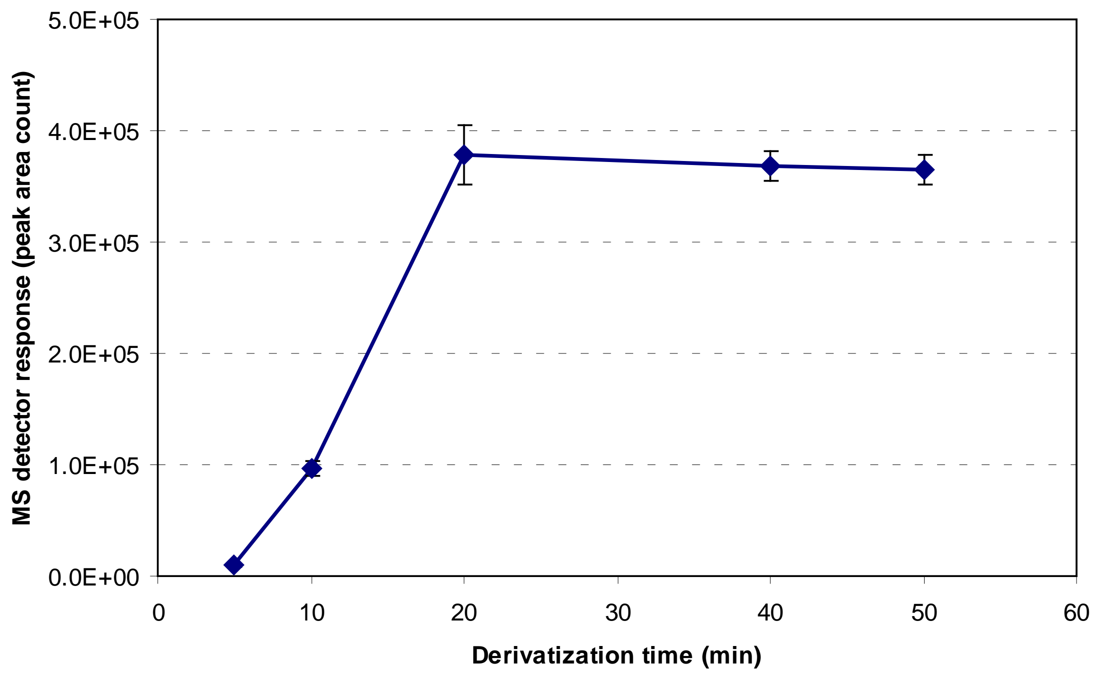

Figure 6 7 Cai et al.

Table 1 Measured concentrations of *trans*-resveratrol in six selected Iowa red wines.

Sample number	Winery	Variety	Vintage	trans-/cis-	RSD (%, n=3)	trans-Resveratrol (μg L^{-1})	RSD (%, n=3)
1	A	FOCH	2006	6.67	8.8	53.34	3.0
2	A	St. Croix	2006	4.35	3	19.41	6.0
3	A	Frontenac	2006	1.56	7.8	12.72	9.2
4	B	Vincent	2006	2.63	10.0	851.9	5.2
5	B	Frontenac	2006	2.22	1.3	181.7	5.8
6	C	Marechal Foch	2006	0.66	1.7	59.10	6.3

Sample number	Winery	Variety	Vintage	Ratio *trans- / cis-*	RSD % (n = 3)	*trans*-resveratrol (μg L^{-1})	RSD % (n = 3)
1	A	Foch	2006	6.67	8.8	58.40	3.0
2	A	St. Croix	2006	4.35	3	20.23	6.0
3	A	Frontenac	2006	1.56	7.8	12.70	9.2
4	B	Vincent	2006	2.63	10.0	881.40	5.2
5	B	Frontenac	2006	2.22	1.3	202.67	5.8
6	C	Marechal Foch	2006	0.66	1.7	64.85	6.3

Table 2 Recovery of the spiked *trans*-resveratrol from wine samples.

Winery	Wine sample	Found before spiking (μg L^{-1})	Spiked (μg L^{-1})	Total found after spiking (μg L^{-1})	Recovery (%)	RSD (%, n=3)
A	Foch	53.34	60	96.97	72.7	5.1
A	Frontenac	12.72	10	22.19	94.7	4.7
B	Vincent	851.9	1000	1685.5	83.4	7.1

~~Winery~~	~~Wine sample~~	~~Found (μg L^{-1})~~	~~Spiked (μg L^{-1})~~	~~Total found (μg L^{-1})~~	~~Recovery (%)~~	~~RSD(%, n = 3)~~
~~A~~	~~Foch~~	~~58.40~~	~~60~~	~~107.40~~	~~81.8~~	~~5.1~~
~~A~~	~~Frontenac~~	~~12.70~~	~~10~~	~~23.40~~	~~106.5~~	~~4.7~~
~~B~~	~~Vincent~~	~~881.40~~	~~1000~~	~~1749.10~~	~~86.8~~	~~7.1~~