

## Occurrence of Resveratrol and Piceid in American and European Hop Cones

VESNA JERKOVIC AND SONIA COLLIN\*

Unité de Brasserie et des Industries Alimentaires, Faculté d'Ingénierie biologique, agronomique et environnementale, Université catholique de Louvain, Croix du Sud, 2 bte 7, B-1348 Louvain-la-Neuve, Belgium

The recent discovery in hops of *trans*-resveratrol and its glucoside, *trans*-piceid, both famous in wine for their potential role in the "French paradox", opens new doors to understanding hop health benefits. In the present work, *trans*-resveratrol and *trans*-piceid were quantified by HPLC-APCI-MS/MS in 40 American and European hop cone samples from harvests of 2004, 2005, and 2006. Their content varies greatly, in the range 0.5–12 mg/kg. All German varieties revealed low amounts of stilbenes. The highest concentrations were found in Cascade (2004) and Willamette (2004 and 2006), two American low- $\alpha$ -acid varieties. Yet, probably because *trans*-resveratrol is a phytoalexin, a strong influence of harvest year was also observed.

**KEYWORDS:** Hop; polyphenols; resveratrol; stilbenes; HPLC-APCI-MS/MS; piceid; phytoalexin

### INTRODUCTION

Female inflorescences of the hop plant (*Humulus lupulus* L.) are used in the brewing industry as a source of bitterness and aroma (1, 2). About 30% of the polyphenols in wort and beer also derive from hops. Most studies have focused on their contribution to wort reducing power (3), beer color, and haze (4). The recent discovery of *trans*-resveratrol and its glucosides, *trans*- and *cis*-piceid, in hops (5, 6) opens new doors to understanding hop health benefits. Resveratrol is a phytoalexin produced by a variety of plants in response to stress, injury, or fungal infection (7). In grapes, free *trans*-resveratrol levels usually range from 0.5 to 26 mg/kg (8, 9), while *trans*-piceid can reach up to 43 mg/kg (10). Much lower values of stilbenes are usually found in peanuts and pistachios, from 0.03 to 1.92 mg/kg (11, 12) and from 0.09 to 1.67 mg/kg (13), respectively. Very recently, resveratrol and piceid were also found at 0.5–1 mg/kg in cocoa and chocolate (14). The cardioprotective effects of red wine have been attributed to resveratrol (15). These effects include antiplatelet, anti-inflammatory, estrogenic, cardioprotective, antitumor, and antiviral properties (15, 16). The resveratrol glucoside, *trans*-piceid, seems to exhibit very similar biological properties (16, 17).

In hop pellets, first results indicate *trans*-stilbene concentrations from 4.8 to 9 mg/kg, *trans*-piceid being in all cases the major constituent (4–8.8 mg/kg) (6). Among the nine American hop cultivars investigated, it appeared that the lower the pellets'  $\alpha$ -acid content, the higher the resveratrol potential, except in highly oxygen sensitive varieties (6).

The aim of the present work was to investigate the *trans*-piceid and *trans*-resveratrol contents of hop cones before

pelletization. The 40 hop cone samples came from three harvest years (2004, 2005, and 2006) and three growing areas (USA, Germany, and Czech Republic).

### EXPERIMENTAL PROCEDURES

**Materials.** Willamette, Cascade, Nugget, Simcoe, Tomahawk, and Warrior varieties, all cones grown in the Yakima Valley (USA) from the harvests of 2004, 2005, and 2006, were a kind gift from the Yakima Chief Company (Louvain-la-Neuve, Belgium). Saaz, Premiant, and Sladeck varieties, all cones grown in the Zatec area (Czech Republic) from the harvests of 2005 and 2006, were a kind gift from the Hop Growers Union of the Czech Republic (Zatec, Czech Republic). German Nugget, Saphir, Smaragd, Wye Target, Hallertau Tradition, Spalter, Hallertau Taurus, Hallertau Magnum, Hallertau Mittelfrüher, and Hersbrucker Spat varieties, all cones grown in the Hallertau area (Germany) from the harvests of 2005 and 2006, were a kind gift from the Hopfenforschungszentrum (Hüll, Germany). All samples were stored at 4 °C under inert atmosphere and analyzed within two months after the harvest.

**Chemicals.** Ethanol (97%) was obtained from Belgaco (Gent, Belgium). Acetonitrile (99.99%), was supplied by Fisher Scientific (UK). Formic acid (pa) was obtained from Aldrich (Germany). Aqueous solutions were made with Milli-Q (Millipore, Bedford, MA) water. *trans*-Resveratrol (99 %) and *trans*-piceid (97%) were supplied by Sigma-Aldrich (Bornem, Belgium).

**Stilbene Extraction from Hop.** This method has been developed in our laboratory (5). All extraction steps have been done with protection against daylight, in duplicate. Hop cones were milled. Ground samples (2.5 g) were extracted, in successive 10 min steps at room temperature under gentle stirring, three times with 50 mL toluene and three times with 50 mL cyclohexane, in order to remove hydrophobic compounds. At the end of each step, the sample was centrifuged for 10 min at 3000 g. At the last step, hop powder was dried under vacuum to get rid of residual solvent. Delipidated hop powder was extracted three times with 40 mL ethanol/water (80:20, v/v), each time for 10 min under gentle

\* Author to whom correspondence should be addressed [e-mail: collin@inbr.ucl.ac.be; fax: +32-10-47 21 78].

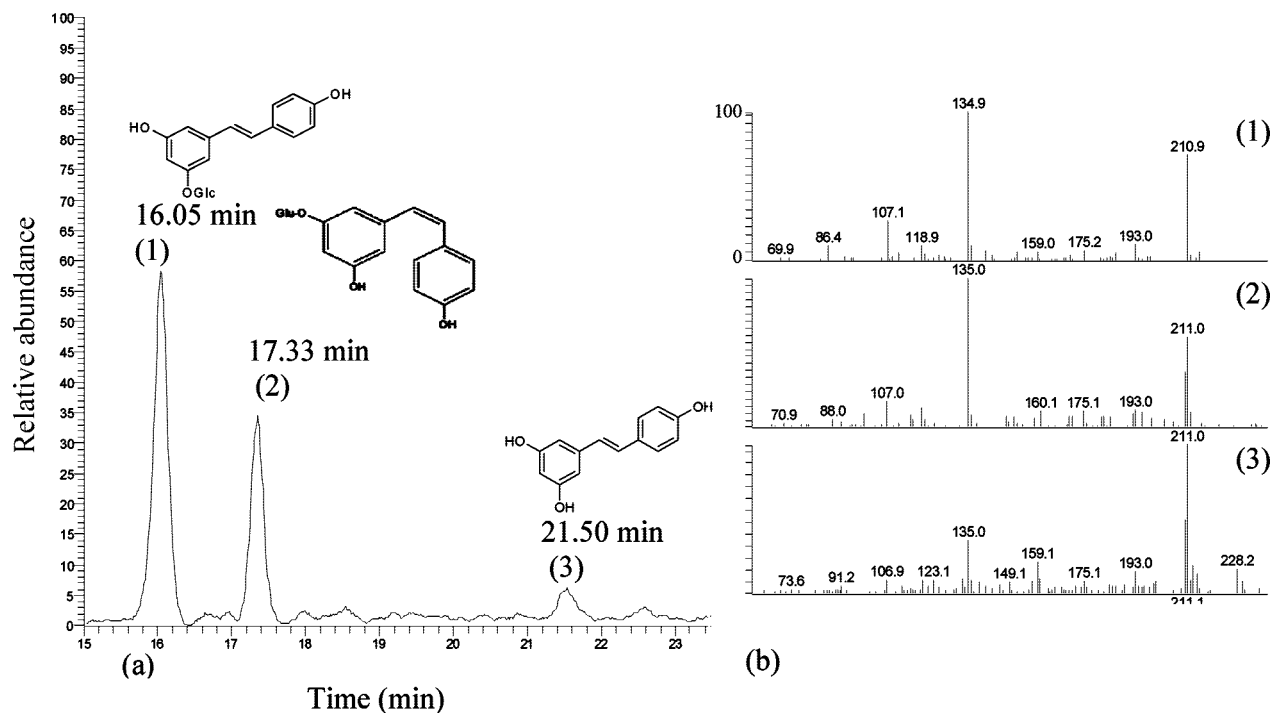


Figure 1. RP-HPLC-APCI (+)-MS/MS data for a Willamette hop cone (harvest 2005): (a) MS/MS chromatogram ( $m/z = 229$ ) and (b) experimental mass spectra for *trans*-piceid (1), *cis*-piceid (2), and *trans*-resveratrol (3).

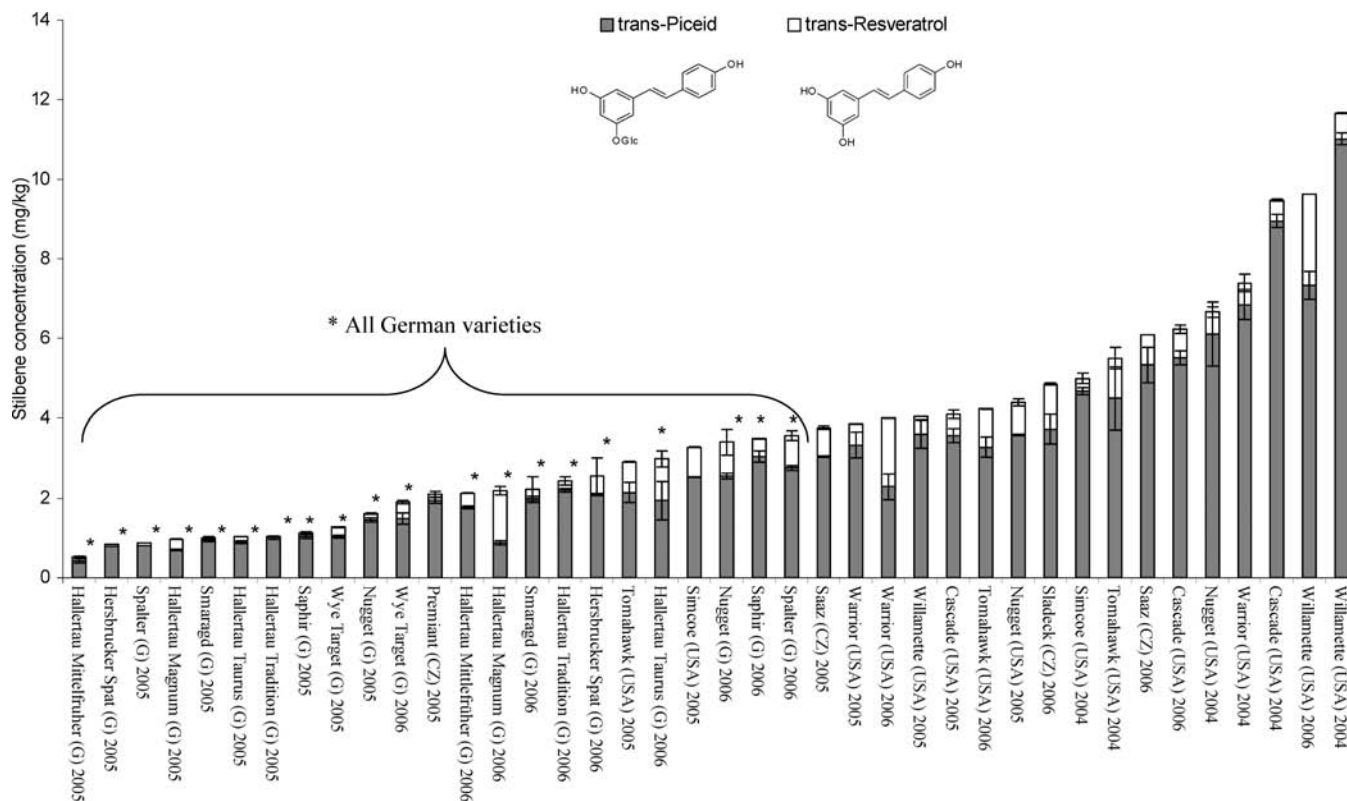


Figure 2. Concentration (mg/kg) and standard deviations of *trans*-piceid and *trans*-resveratrol in fresh American, German, and Czech hop cones from harvests 2004, 2005, and 2006. Assay in duplicate.

stirring at 60 °C. After each extraction, the sample was centrifuged for 10 min at 3000 g and the supernatant collected. After filtration to remove residual particles, the combined supernatants were concentrated by rotary evaporation (35 °C) to dryness. The residue was solubilized in 2 mL of 50:50 (v/v) mixture of ethanol/water. The extract obtained was stored at -20 °C in the dark until use. For the standard addition method, 2.5 g milled hop was spiked with increasing amounts of either

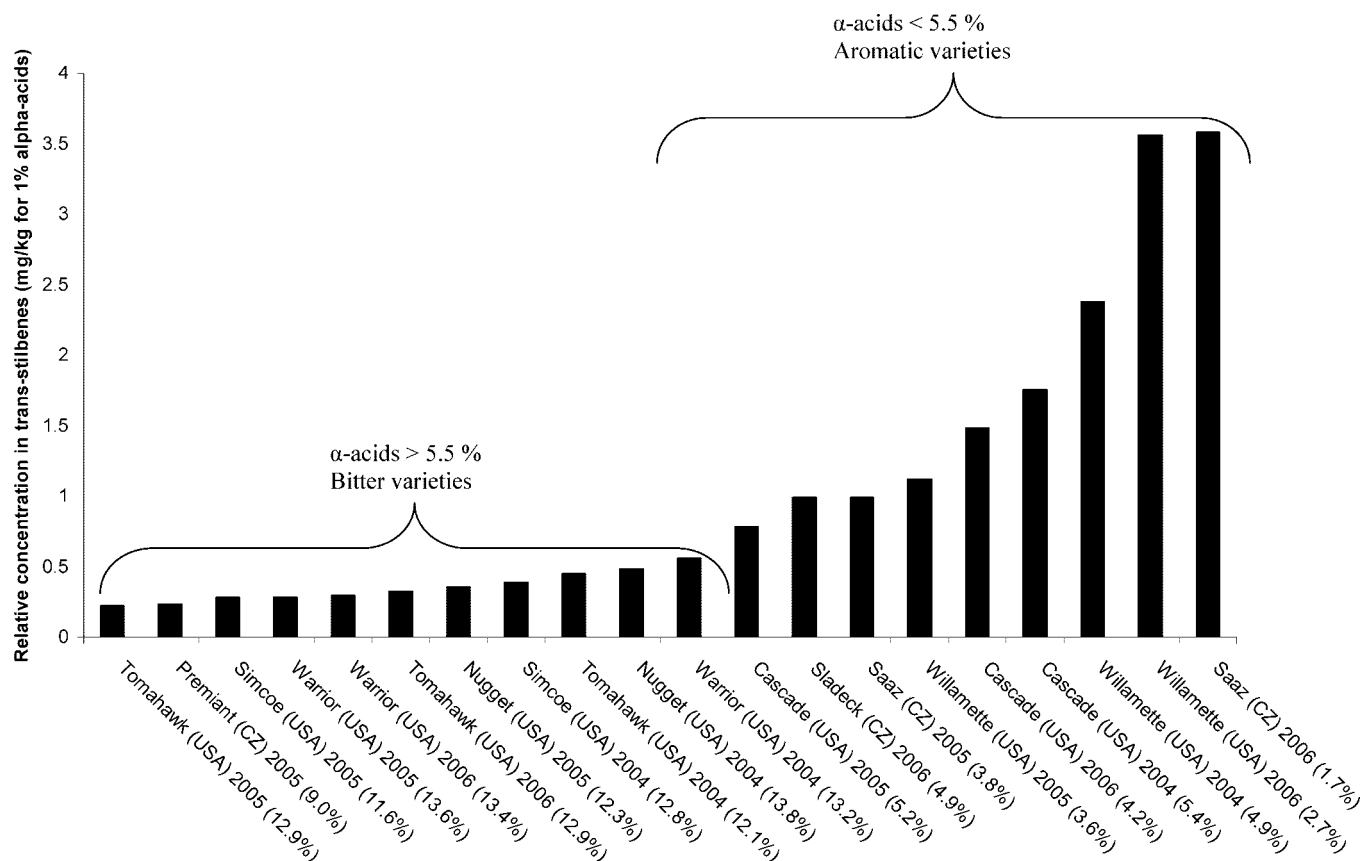
*trans*-resveratrol (0, 0.5, 1, and 5 ppm) or *trans*-piceid (0, 5, 10, and 20 ppm) before extraction. 75% of *trans*-piceid and 80% of *trans*-resveratrol have been recovered by this procedure applied on hop cones.

**RP-HPLC-APCI (+)-MS/MS Analysis of Stilbenes.** Quantifications were performed on a C18 Preval column (150 × 2.1 mm, 2 μm) (Alltech, Deerfield, IL) eluted with a linear gradient using two solvents:

**Table 1.** Bitterness and Stilbene Content of Hop Cones<sup>a</sup>

varieties	harvest 2004				harvest 2005				harvest 2006			
	$\alpha$ -acids (%)	<i>trans</i> -piceid (mg/kg)	<i>trans</i> -resveratrol (mg/kg)	total <i>trans</i> -stilbenes (mg/kg)	$\alpha$ -acids (%)	<i>trans</i> -piceid (mg/kg)	<i>trans</i> -resveratrol (mg/kg)	total <i>trans</i> -stilbenes (mg/kg)	$\alpha$ -acids (%)	<i>trans</i> -piceid (mg/kg)	<i>trans</i> -resveratrol (mg/kg)	total <i>trans</i> -stilbenes (mg/kg)
German Varieties												
Hersbrucker Spat	n.t.	n.t.	n.t.	n.t.	2.6	0.79	0.05	0.84	2.0	2.09	0.46	2.55
Spalter	n.t.	n.t.	n.t.	n.t.	3.4	0.80	0.08	0.88	2.2	2.76	0.81	3.57
Saphir	n.t.	n.t.	n.t.	n.t.	3.8	1.04	0.08	1.12	2.2	3.05	0.44	3.49
Hallertau Mittlefrüher	n.t.	n.t.	n.t.	n.t.	4.2	0.42	0.10	0.52	3.4	1.76	0.36	2.12
Smaragd	n.t.	n.t.	n.t.	n.t.	6.4	0.94	0.06	1.00	7.8	1.99	0.23	2.22
Hallertau Tradition	n.t.	n.t.	n.t.	n.t.	6.5	1.01	0.03	1.04	5.6	2.20	0.23	2.43
Wye Target	n.t.	n.t.	n.t.	n.t.	8.7	1.03	0.24	1.27	10.0	1.49	0.41	1.90
Nugget	n.t.	n.t.	n.t.	n.t.	11.5	1.45	0.16	1.61	12.3	2.55	0.85	3.40
Hallertau Magnum	n.t.	n.t.	n.t.	n.t.	13.2	0.70	0.27	0.97	11.4	0.87	1.32	2.19
Hallertau Taurus	n.t.	n.t.	n.t.	n.t.	14.9	0.89	0.15	1.04	15.0	1.94	1.05	2.99
Czech Varieties												
Saaz	n.t.	n.t.	n.t.	n.t.	3.8	3.05	0.72	3.77	1.7	5.33	0.76	6.09
Sladec	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	n.t.	4.9	3.73	1.12	4.85
Premiant	n.t.	n.t.	n.t.	n.t.	9.0	1.94	0.16	2.10	n.t.	n.t.	n.t.	n.t.
American Varieties												
Willamette	4.9	11.01	0.65	11.66	3.6	3.61	0.43	4.04	2.7	7.34	2.28	9.62
Cascade	5.4	8.95	0.52	9.47	5.2	3.57	0.52	4.09	4.2	5.51	0.72	6.23
Tomahawk	12.1	4.50	1.00	5.50	12.9	2.14	0.77	2.91	12.9	3.28	0.95	4.23
Simcoe	12.8	4.67	0.32	4.99	11.6	2.53	0.75	3.28	n.t.	n.t.	n.t.	n.t.
Warrior	13.2	6.85	0.54	7.39	13.6	3.33	0.53	3.86	13.4	2.29	1.71	4.00
Nugget	13.8	6.11	0.55	6.66	12.3	3.58	0.81	4.39	n.t.	n.t.	n.t.	n.t.

<sup>a</sup> Assay in duplicate. Coefficient of variation always under 7 %. n.t.: not tested.



**Figure 3.** Relative concentration of *trans*-stilbenes for a same  $\alpha$ -acid content (1%) in fresh American and Czech hop cones.  $\alpha$ -Acid concentrations are given in parentheses. Assay in duplicate. Coefficient of variation always below 7%.

(A) water/acetonitrile/formic acid (98.9:1:0.1, v/v) and (B) acetonitrile. Gradient elution was as follows: from 95% (A) to 55% in 23 min, 55% to 0% in 7 min, and isocratic for 10 min at a flow rate of 200  $\mu$ L/min. Ten microliter samples were injected into the column kept at 30  $^{\circ}$ C. A SpectraSystem equipped with an AS3000 autosampler and a

P4000 quaternary pump was used. The system was controlled with Xcalibur software version 1.2 (Finnigan Mat). Mass spectra were acquired using an LCQ mass spectrometer equipped with an APCI source (Finnigan Mat). The following APCI inlet conditions in positive mode were applied: vaporization temperature, 470  $^{\circ}$ C; capillary voltage,

3 V; capillary temperature, 175 °C; sheath gas, 40 psi; auxiliary gas, 7 psi; discharge current 5  $\mu$ A. After the first monitoring on the  $m/z = 229$ , collision-induced dissociation spectra were recorded at 37% relative collision energy.

**Determination of  $\alpha$ -Acids in Hop.** The content of  $\alpha$ -acids in hop cones was determined by HPLC-UV according to the Analytica EBC (18).

## RESULTS AND DISCUSSION

The stilbene contents of 40 cones from 6 American, 3 Czech, and 10 German hop cultivars were determined according to the method proposed by Callemien et al. (5) for hop pellets analysis. After removal of resins by means of several cleaning steps with toluene and cyclohexane, stilbenes were extracted with ethanol/water (80:20) at 60 °C. The method enabled us to recover 75% *trans*-resveratrol and 80% *trans*-piceid from hop cones (recovery factor determined by standard addition experiments).

The RP-HPLC-APCI (+)-MS/MS chromatogram obtained (Figure 1) enabled us to separate *trans*-piceid, *cis*-piceid, and *trans*-resveratrol in all varieties. As previously reported for hop pellets (6) and grapes (8, 10, 17, 19), *cis*-resveratrol was absent from the hop cones investigated here. In the absence of a commercial standard for *cis*-piceid and significant MS variations in the *cis/trans* relative sensitivity, only *trans*-stilbenes were quantified. As expected (5, 6, 20), fragmentation of the monoglucoside was characterized by loss of the sugar, leading in all cases to an intense aglycone M + 1 ion. Therefore, both *trans*-stilbenes were measured just by selecting  $m/z = 135$ .

As depicted in Figure 2 and Table 1, the total concentration of *trans*-piceid and *trans*-resveratrol ranged from 0.5 mg/kg for Hallertau Mittlefrüher (G) 2005 to 11.7 mg/kg for Willamette (USA) 2004. This range is larger than that previously reported by Jerkovic et al. (6) for American hop pellets (from 4.8 to 9.0 mg/kg, harvest 2003). Willamette (USA) 2006 contained the highest concentration of free *trans*-resveratrol with 2.3 mg/kg. Surprisingly, all German varieties showed very low amounts of *trans*-stilbenes (<3.6 mg/kg, see left part of Figure 2 and Table 1), regardless of their  $\alpha$ -acid content ( $r^2 = 0.13$  between *trans*-stilbenes and bitterness potentials). Only a few low- $\alpha$ -acid American varieties seemed to be able to reach total *trans*-stilbene values above 9.0 mg/kg (Willamette (USA) 2004/2006 and Cascade (USA) 2004). As the hopping rate in the boiling kettle is calculated according to the  $\alpha$ -acid content of the hops (during wort boiling, thermal isomerization of  $\alpha$ -acids into more bitter iso- $\alpha$ -acids occurs), total *trans*-stilbene contents were compared in American and Czech varieties for the same bitterness potential. When all German samples (Nugget, Saphir, Smaragd, Wye Target, Hallertau Tradition, Spalter, Hallertau Taurus, Hallertau Magnum, Hallertau Mittlefrüher, and Hersbrucker Spat) are eliminated, the nine low-bitterness cultivars clearly emerge as the most interesting (Figure 3).

However, no general rule could be drawn from one year to the other. As depicted in Table 1, a strong influence of the harvest year was observed. Such huge differences between samples have been mentioned previously for the stilbene content of grapes (10, 18, 21, 22). Stilbenes are phytoalexins; thus, growing area, climate conditions, interactions with pathogens, and many other factors are probably needed to induce this secondary metabolism (7). In the case of strong induction (e.g., American harvests 2004 and 2006), we think there might be competition in bitter varieties for acetyl CoA, the fundamental precursor required to synthesize both  $\alpha$ -acids and stilbenes ( $r^2 = 0.77$  and 0.80 for bitterness potential decrease vs *trans*-stilbenes in 2004 and 2006, respectively).

In conclusion, only American and Czech low- $\alpha$ -acid varieties are promising for enhancing the stilbene content in the boiling kettle. Yet, as stilbenes are phytoalexins, major differences can distinguish samples from different harvests.

## ACKNOWLEDGMENT

The authors thank Mr. Stéphane Meulemans from Yakima Chief Company, Dr. Elisabeth Seigner from the Hopfenforschungszentrum, and Mr. Zdenek Rosa from the Hop Growers Union of the Czech Republic for their kind collaboration.

## LITERATURE CITED

- (1) Lermusieau, G.; Bulens, M.; Collin, S. Use of GC-olfactometry to identify the hop aromatic compounds in beer. *J. Agric. Food Chem.* **2001**, *49*, 3867–3874.
- (2) Lermusieau, G.; Collin, S. Volatile sulfur compounds in hops and residual concentrations in beer. *J. Am. Soc. Brew. Chem.* **2003**, *61*, 109–113.
- (3) Lermusieau, G.; Liégeois, C.; Collin, S. Reducing power of hop cultivars and beer ageing. *Food Chem.* **2001**, *72*, 413–418.
- (4) Siebert, K. J.; Carrasco, A.; Lynn, P. Y. Formation of protein-polyphenol haze in beverages. *J. Agric. Food Chem.* **1996**, *44*, 1997–2005.
- (5) Callemien, D.; Jerkovic, V.; Rozenberg, R.; Collin, S. Hop as an interesting source of resveratrol for brewers: optimization of the extraction and quantitative study by liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. *J. Agric. Food Chem.* **2005**, *53*, 424–429.
- (6) Jerkovic, V.; Callemien, D.; Collin, S. Determination of stilbenes in hop pellets from different cultivars. *J. Agric. Food Chem.* **2005**, *53*, 4202–4206.
- (7) Jeandet, P.; Douillet-Breuil, A. C.; Bessis, R.; Debord, S.; Sbaghi, M.; Adrian, M. Phytoalexins from the vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. *J. Agric. Food Chem.* **2002**, *50*, 2731–2741.
- (8) Burns, J.; Yokota, T.; Ashihara, H.; Lean, M. E. J.; Crozier, A. Plant foods and herbal sources of resveratrol. *J. Agric. Food Chem.* **2002**, *50*, 3337–3340.
- (9) Ector, B.; Magee, J.; Hegwood, C.; Coign, M. Resveratrol concentration in muscadine berries, juice, pomace, purees, seeds and wines. *Am. J. Enol. Vitic.* **1996**, *47*, 57–62.
- (10) Romero-Pérez, A. I.; Lamuela-Raventós, R. M.; Andrés-Lacueva, C.; de la Torre-Boronat, M. C. Method for the quantification extraction of resveratrol and piceid isomers in grape berry skins. Effect of powdery mildew on the stilbene content. *J. Agric. Food Chem.* **2001**, *49*, 210–215.
- (11) Sanders, T.; McMichael, R.; Hendrix, K. Occurrence of resveratrol in edible peanuts. *J. Agric. Food Chem.* **2000**, *48*, 1243–1246.
- (12) Ibern-Gómez, M.; Roig-Pérez, S.; Lamuela-Raventós, R. M.; de la Torre-Boronat, M. C. Resveratrol and piceid levels in natural and blended peanut butters. *J. Agric. Food Chem.* **2000**, *48*, 6352–6354.
- (13) Tokusoglu, O.; Kemal Unal, M.; Yemis, F. Determination of the phytoalexin resveratrol (3,5,4'-trihydroxystilbene) in peanuts and pistachios by high-performance liquid chromatography diode array (HPLC-DAD) and gas chromatography-mass spectrometry. *J. Agric. Food Chem.* **2005**, *53*, 5003–5009.
- (14) Counet, C.; Callemien, D.; Collin, S. Chocolate and cocoa: new sources of *trans*-resveratrol and *trans*-piceid. *Food Chem.* **2006**, *98*, 649–657.
- (15) Frémont, L. Minireview: biological effects of resveratrol. *Life Sci.* **2000**, *66*, 663–673.
- (16) Aggarwal, B.; Bhardwaj, A.; Aggarwal, R.; Seerman, N.; Shishodia, S.; Takada, Y. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res.* **2004**, *24*, 2783–2848.

- (17) Romero-Pérez, A. I.; Ibern-Gómez, M.; Lamuela-Raventós, R. M.; de la Torre-Boronat, M. C. Piceid, the major resveratrol derivative in grape juices. *J. Agric. Food Chem.* **1999**, *47*, 1533–1536.
- (18) *Analytica EBC*; Fachverlag Hans Carl: Nürenberg, Germany, 2005.
- (19) Adrian, M.; Jeandet, P.; Breuil, A. C.; Levite, D.; Debord, S.; Bessis, R. Assay of resveratrol and derivative stilbenes in wines by direct injection high performance liquid chromatography. *Am. J. Enol. Vitic.* **2000**, *51*, 37–41.
- (20) Jerkovic, V.; Nguyen, F.; Nizet, S.; Collin, S. Combinatorial synthesis, reverse-phase and normal-phase high performance liquid chromatography elution data and liquid chromatography/positive atmospheric pressure chemical ionization tandem mass spectra of methoxylated and glycosylated resveratrol analogues. *Rapid Commun. Mass Spectrom.* **2007**, *21*, 2456–2466.
- (21) Burns, J.; Gardner, P. T.; Matthews, D.; Duthie, G. G.; Lean, M. E. J.; Crozier, A. Extraction of phenolics and changes in antioxidant activity of red wines during vinification. *J. Agric. Food Chem.* **2001**, *49*, 5797–5808.
- (22) Jeandet, P.; Bessis, R.; Maume, B. F.; Meunier, P.; Peyron, D.; Trollat, P. Effect of enological practices on the resveratrol isomers content of wine. *J. Agric. Food Chem.* **1995**, *43*, 316–319.

---

**Received for review June 18, 2007. Revised manuscript received August 15, 2007. Accepted August 19, 2007. Vesna Jerkovic is grateful to the Inbev-Baillet Latour Foundation (Leuven, Belgium) for financial support.**

JF071792K